THE EFFECT OF THE CO-ADMINISTRATION OF THE H_2-RECEPTOR ANTAGONISTS ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF THE H_1-RECEPTOR ANTAGONISTS IN RABBITS

BY

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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In the name of God, the Beneficent, the Merciful

Abstract

The effect of the co-administration of the H_2-receptor antagonists cimetidine, famotidine and ranitidine, and the antifungal agent ketoconazole, on the pharmacokinetics and pharmacodynamics of the H_1-receptor antagonist hydroxyzine and its active metabolite cetirizine in rabbits was evaluated by measuring hydroxyzine and cetirizine serum concentrations by HPLC and using suppression of the histamine-induced wheal to evaluate the peripheral antihistaminic effects of these compounds.

The co-administration of cimetidine inhibited the elimination of hydroxyzine and cetirizine so that increased serum concentrations of these H_1-receptor antagonists were achieved, compared to serum concentrations observed when hydroxyzine was administered alone. Co-administration of cimetidine resulted in enhanced suppression of the histamine-induced wheal formation. This effect may be due both to increased serum H_1-antagonist concentrations and to the direct inhibition of cimetidine on the H_2-receptors of the skin vasculature.

The co-administration of famotidine and ranitidine had no effect on the pharmacokinetics of hydroxyzine and cetirizine, resulting in serum concentrations similar to those obtained when hydroxyzine was administered alone. However, the co-administration of both famotidine and ranitidine resulted in enhanced suppression of the histamine-induced wheal formation. This effect may be due to the direct inhibition of famotidine and ranitidine on the H_2-receptors on the skin vasculature.

The co-administration of ketoconazole, an antifungal agent which has an inhibitory effect on the hepatic cytochrome P-450 enzyme system, delayed the elimination of both hydroxyzine and cetirizine, yielding increased serum concentrations compared to those obtained when hydroxyzine was administered alone. The enhanced suppression of the histamine-induced wheal formation following ketoconazole co-administration was probably due to the increased serum H_1-antagonist concentrations, since ketoconazole has no effect on H_1- or H_2-receptors.

The elimination of cetirizine from patients with primary biliary cirrhosis (PBC) was prolonged but renal clearance of unmetabolized drug was reduced when compared to results obtained in healthy volunteers. The suppression of the histamine-induced wheals and flares was also enhanced and prolonged. Doses of cetirizine may need to be adjusted in patients with PBC.

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Table of contents

List of figures

List of tables

Glossary

$AUC_{0-\infty}$: the area under the concentration versus time curve from zero to infinity.

AUC_{0-t}: the area under the concentration versus time curve from zero to time t.

C.V.: the coefficient of variation.

R: the ratio of cetirizine $AUC_{0-\infty}$ to hydroxyzine $AUC_{0-\infty}$.

S.D.: standard deviation.

$T_{1/2}$: the elimination half life.

T_{max}: the time to reach the maximum serum concentration.

$CP_{5\ min}$: the serum concentration at 5 minutes.

VD: apparent volume of distribution.

Cp_{max}: maximum serum concentration of hydroxyzine or cetirizine.

CNS: Central nervous system.

PBC: Primary biliary cirrhosis.

Cls : the systemic clearance.

Clr : the renal clearance.

q.12.h: Every 12 hours.

q.24.h: Every 24 hours.

P026 : Cetirizine metabolite.

P_{265} : Ethoxy derivative of cetirizine.

CHAPTER I

INTRODUCTION

1.1 Histamine

1.1.1 Background

The history of histamine closely parallels that of acetylcholine. Both compounds were discovered when they were synthesized extemporaneously as chemical curiosities, rather than being isolated from biological sources following identification of their pharmacological properties (1).

In 1910-1911 Dale and Laidlaw discovered that histamine stimulates smooth muscle and has vasopressor activity (2,3). In a series of experiments they determined that histamine induced a shock-like syndrome when injected into frogs and mammals. In 1927 Best *et al.* (4) isolated histamine from fresh samples of liver and lung, and established that histamine is a natural constituent of the body. Its effect was evaluated in the tissue and skin (4,5). Its name comes from the Greek word "histos" which means tissue.

In 1945 Loew *et al.* synthesized the histamine antagonist diphenylhydramine (6), while Yonkman and his colleagues (7) developed tripelennamine, both of which are still in clinical use. In the following years, scores of additional potent antihistaminic agents were developed (8).

In 1952, Riley discovered that the mast cell was the major source of histamine (9). Later, in 1966 Ash and Schild (10) proposed the name H_1 for receptors blocked by known antihistamines, now to be called H_1-receptor antagonists. In 1972, Black and co-workers (11) confirmed the existence of the H_2- receptors by synthesizing a group of drugs that specifically blocked them while having minimal or no effect on the H_1-receptors.

1.1.2 Chemistry

Histamine, 2-(4-imidazole)ethyl amine or ß-amino ethyl imidazole (Figure 1), is formed following decarboxylation of the amino acid histidine by the enzyme L-histidine decarboxylase (Figure 2). It is a hydrophilic molecule containing an imidazole ring to which an amino group is connected by two methylene groups. It has a strong capacity for

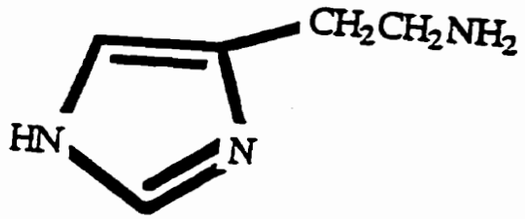


Figure 1. Chemical structure of histamine.

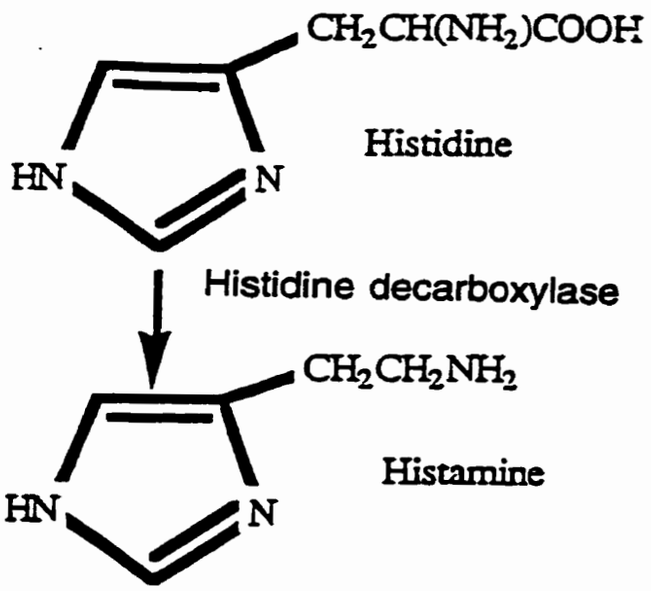


Figure 2. The biosynthesis of histamine.

hydrogen bonding in which both the imidazolium and the amino moieties can act as hydrogen donors, while the uncharged ring can act both as hydrogen donor and acceptor (12).

1.1.3 Source.

Histamine is stored in granules in tissue mast cells and in blood basophils. In these granules histamine is synthesized by decarboxylation of the amino acid histidine, and stored as an inactive complex called histamine-glycosamino glycan (heparin) complex (8).

In animals and humans, histamine is detected in high concentrations in the skin and the mucosa of the bronchial tree. It is also found in the lining of the stomach wall due to the presence of a large number of mast cells in the gastrointestinal tract, and also due to the presence of histamine in histaminocytes in the stomach (1,13). Non-mast cells involved in histamine formation and storage include cells of the human epidermis, neurons in the central nervous system and cells in rapidly growing tissues.

During allergic reactions, histamine is released from its storage sites as the active form by a secretory process. Release is initiated when

an antigen combines with, and bridges adjacent molecules of the reaginic anti-bodies (IgE) on the mast cell surface (Figure 3). A series of reactions showing a critical requirement for calcium and metabolic energy results in the extrusion of the contents of the secretory granules by the process of exocytosis. Among the reactions involved in this process are : activation of proteases, methylation of phospholipids, opening of membrane calcium channels, mobilization of calcium ions, activation of phospholipase A_2 and arachidonate metabolism, reduction in the synthesis of cyclic AMP and enhancement of protein phosphorylation (14).

Non-allergic release of histamine can be induced by opiates and peptides and surfactants such as compound 40/80 which cause the direct release of histamine from its storage sites (14).

1.1.4 Absorption, fate and excretion.

Histamine is readily absorbed after parenteral administration and acts rapidly following subcutaneous or intramuscular injection. Its action is short-lived because it diffuses readily into tissues where it is rapidly metabolized. Very large doses of histamine can be given orally without

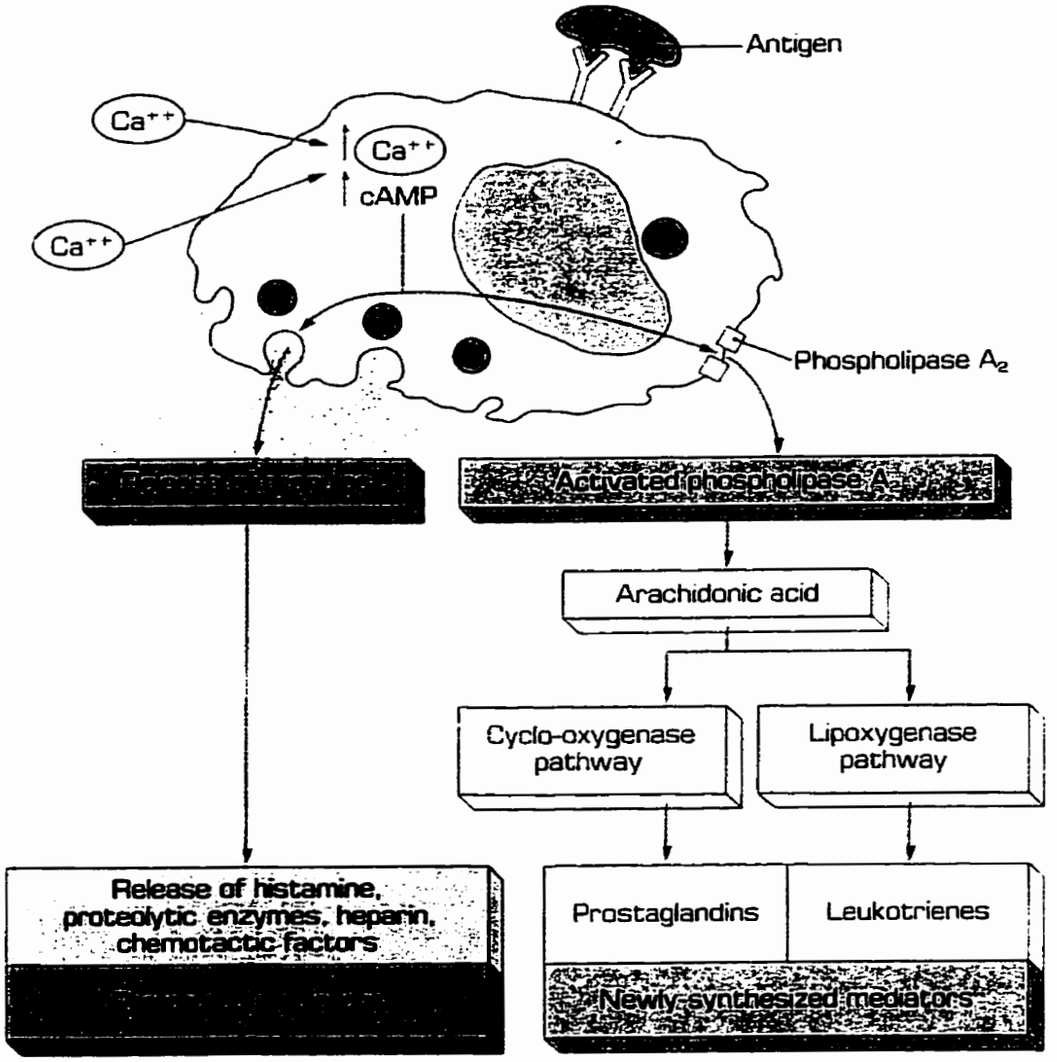


Figure 3. Antigen antibody interaction.

any noticable effect, due to the rapid conversion by intestinal bacteria to the inactive N-acetyl histamine. Any histamine which is absorbed is inactivated to methyl imidazolyl acetic acid or imidazolyl acetic acid due to ring methylation by the N-methyl transferase enzyme as it circulates through the liver.

There are two major pathways of histamine metabolism (Figure 4). One, involving ring methylation, is catalysed by the enzyme histamine-N-methyl transferase. This is followed by conversion through the action of the enzyme mono-amine-oxidase (MAO) to N-methyl imidazole acetic acid. The other pathway involves the enzyme diamine-oxidase (DAO), yielding the product imidazole acetic acid. Both of these metabolites show minimal pharmacological activity, and are excreted unchanged in the urine (1).

1.1.5 Pharmacological effects.

1.1.5.1 Cardiovascular effects.

Histamine has a variety of actions in the cardiovascular system. In the vascular tree, both H_1- and H_2-receptor stimulation cause vasodilation. H_1-receptors have high affinity for histamine and a short

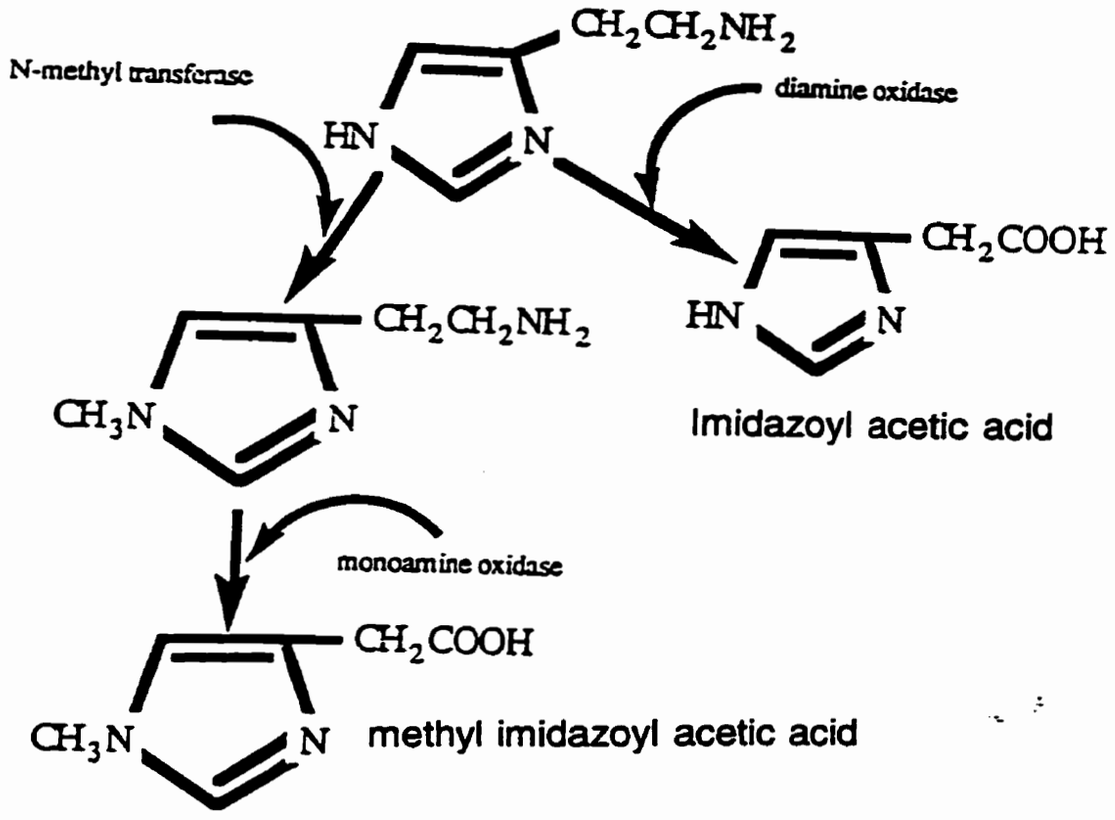


Figure 4. The metabolic pathways of histamine.

duration of action. H_2-receptors induce dilation of blood vessels that develops more slowly, but is sustained. In capillaries, H_1-receptors promote vessel permeability while the role of H_2-receptors is slight. In the heart, histamine effects are due to stimulation of H_2-receptors which causes an increase in the force of contraction and speeds the heart rate. However, the net sum of all these actions results in a fall in blood pressure.

The classical effect of histamine on the capillaries results in outward passage of plasma protein and fluid into the extracellular spaces, an increase in the flow of the lymph and its protein contents and formation of edema, the so-called triple response (8). This response can also be reproduced by intradermally injected histamine. The initial response is the appearance of a localized red spot, which extends for a few millimeters around the site of injection. It appears within a few seconds after injection and reaches the maximum size in about one minute. The second response is the brighter red flush or flare which extends about 1 cm or so beyond the original red spot and develops more slowly. A wheal that is discernible and palpible and develops in 1 to 2 minutes, occupying the same area as the original red spot at the site of

injection, is the final response. The initial red spot results from a direct vasodilatory effect of histamine, the flush is due to histamine-induced stimulation of axon reflexes that causes vasodilatation indirectly, and the wheal reflects histamine's capacity to cause edema (1).

Histamine also regulates the microcirculation within tissues which is mediated by interaction between blood-borne substances and nervous stimuli. It was proposed by Schayer (15) that some histamine is synthesized continually in the small blood vessels, called nascent histamine. Its accumulation and washout could account for the spontaneous opening and closing of the vessels in the vascular bed (8).

1.1.5.2 Regulation of gastric secretion.

In 1938 MacIntosh discovered that histamine is released from the stomach by vagal stimulation, but the significance of this effect was confirmed only with the development of H_2-receptor agonists and antagonists (1,11). Histamine acts on parietal cells through H_2-receptors which are linked to adenylate cyclase to activate gastric acid release.

1.1.5.3 Role of histamine in inflammation and allergy.

Histamine is released from mast cells or basophils in humans by conditions such as allergies or respiratory infections. Mast cells or peripheral blood basophil leucocytes are passively synthesized by plasma cells following stimulation by appropriate allergens. The binding of IgE molecules to the mast cells involves the Fc portion of the IgE molecules and a receptor on the cell surface. On subsequent challenge this same allergen combines with its specific IgE antibody at the cell membrane of the sensitized mast cell. This combination of allergen and antibody causes calcium influx which is essential for degranulation and release of histamine (14).

Mast cells are the chief source of histamine in tissues, and basophils are the chief source of histamine among circulating cells. Histamine is located in the granules in a form ionically bound to acidic groups in a heparin-protease matrix. Upon release of the granule contents by antigen stimulation, the histamine becomes freely soluble (8).

1.1.6 Histamine receptors.

There are two main types of histamine receptor called H_1-and H_2-receptors, whose classification is determined by the use of highly specific and competitive agonists and antagonists. A third type of histamine receptor called the H_3-receptor, has been described which may be involved in the feed-back control of histamine release and synthesis (1,16). There is also evidence of a low affinity, non-H_1, non-H_2 and non-H_3 receptor through which histamine may act as an intracellular messenger (8).

H_1- and H_2- receptors are considered to be part of the contents of the cell membrane structure. The two main classes of histamine receptors may be identified by differential responses to various histamine-like agonists; 2-methyl histamine preferentially induces responses mediated by H_1-receptors, whereas 4-methyl histamine provides the effects mediated through H_2- receptors. These compounds are representatives of two classes of histamine-like drugs, the H_1- and H_2- receptor agonists.

It was reported by Yamashita et al. 1991 (17) that the histamine H_1-receptor is very similar to other G protein-coupled receptors. Amino acid residues that are conserved in G protein-coupled receptors were also seen in the H_1-receptors. These consist of two cysteines (cys-101 and cys-181), an aspartate residue (asp-74) present in the second transmembrane domain, an anionic and cationic amino acid pair (asp-125 and arg-126) at the cytoplasmic border of the third transmembrane domain, and a conservative sequence of 10 amino acids (leu-460-pro-469) observed in the seventh transmembrane domain (17).

The H_2-receptors of rats and dogs were cloned by Ruat *et al.* 1991 (18). They determined that these two putative H_2-receptors have a short, 30 amino acid third intracellular loop and a long, 71 amino acid C-terminal cytoplasmic tail. The encoded amino acid in the fifth transmembrane domain were an aspartate and threonine residue, asp-185 and thr-189 for the rat, or an asp-186 and thr-190 for the dog which were postulated to be responsible for hydrogen bonding with the nitrogen atoms of the imidazole ring of histamine (18).

The sequence homology of transmembrane domains between H_1 and H_2 receptors is 40.7% (19).

The gene for human H_1-receptor has now been cloned, using the sequence information of the bovine H_1-receptor gene (20).

The gene encoding the H_2-receptor has also been cloned in humans, using the information from the canine gene(20). The various genes show a considerable homology of 80-90% (18,19).

1.2 Histamine antagonists.

Blocking of histamine activity was first accomplished in 1937 by Bovet and Staub using a series of studies of compounds formed of amines with phenolic ethers. The substance, 2-isopropyl-5-methyl-phenoxy-ethyl-diethyl-amine protected guinea pigs against lethal doses of histamine, antagonised the spasm caused by histamine and reduced the symptoms of anaphylactic shock. Unfortunately this substance was too toxic for clinical use. In 1940 pyrilamine maleate was discovered by Bovet and his colleagues (1). These developments took place in France during World War II. The discovery of diphenhydramine and tripelenamine followed in the United States in 1950 (1).

In the following years many histamine blockers were discovered and brought to market for use by physicians. These compounds did not

block all the effects of histamine, especially gastric acidity, so in 1966, Ash *et al.* (21) ascribed these effects to H_1-receptors. The gastric response remained of great interest and was finally resolved in 1972 when Black and his colleagues (11) designed a new class of drugs which selectively blocked the gastric acid secretion induced by histamine. This discovery established the existence of the second population of histamine receptors, the H_2-receptors (1).

1.2.1 H_1-receptor antagonists.

Among their many uses, H_1-receptor antagonists are often prescribed for symptomatic relief of itching in skin disorders. It is also known that H_1-receptor antagonists have a greater antipruritic effect in allergic skin disorders than in other types of pruritic skin disorders (8). Also in patients with allergic conjunctivitis, H_1-receptor antagonists are useful for the relief of itching, tearing and erythema. they are also effective in the treatment of allergic rhinitis and in the adjunctive treatment of bronchial asthma as weak bronchiodilators (8).

1.2.1.1 Structure and pharmacology.

Most of the H_1- antagonists have a substituted ethyl amine moiety similar to histamine. However, unlike histamine these antagonists have a tertiary amino group linked by a two or three- carbon atom chain to a double aromatic substituent (1). The general formula of the H_1- receptor antagonists is shown in Figure 5.

Ar_1 could be aryl e.g. phenyl or substituted phenyl or hetero aryl e.g. pyridyl.

Ar_2 could be an aryl methyl or another aryl group. Sometimes the two aromatic rings are bridged as in tricyclic derivatives like the phenothiazines.

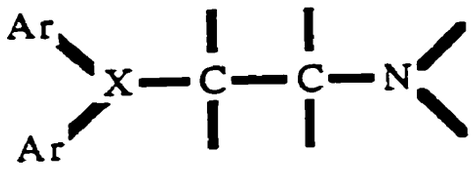
X is a nitrogen or carbon atom or -C-O- ether linkage to the β-amino ethyl side chain (1).

H_1- receptor antagonists can be divided into six groups (Figure 5).

1.Ethers or ethanolamine derivatives e.g. diphenhydramine or carbinoxamine.

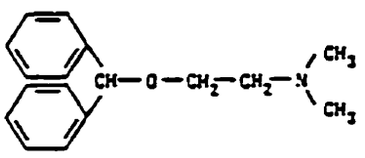
2. Piperazine derivatives e.g. cyclizine.

3. Phenothiazine derivatives e.g. promethazine.



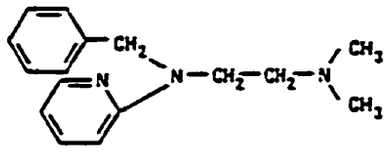
General structure

Ethers or ethanolamine derivatives



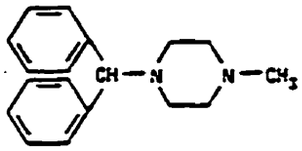
Diphenhydramine or dimenhydrinate

Ethylenediamine derivatives



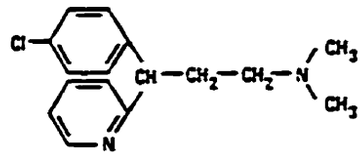
Tripelennamine

Piperazine derivatives



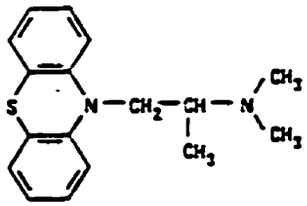
Cyclizine

Alkylamine derivatives



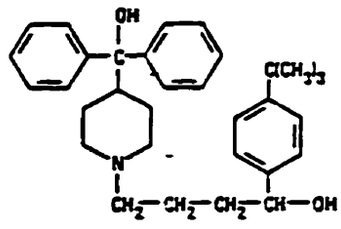
Chlorpheniramine

Phenothiazine derivatives



Promethazine

Piperidine derivatives



Terfenadine

Figure 5. General structure of H_1-antagonist drugs and examples of major subgroups.

4. Ethylene diamine derivatives e.g. tripelennamine.
5. Alkyl amine derivatives e.g. chloropheniramine.
6. Piperidine derivatives e.g., terfenadine, astemizole, loratadine.
7. Other new agents e.g. azelastine.

However, the above traditional classification of H_1-receptor antagonists according to chemical structure is not useful in determining the degree of efficacy and the extent of adverse effects. Compounds such as terfenadine and astemizole are in the same piperidine chemical class as the older sedating agents such as cyproheptadine. Furthermore, with the newer compounds the chemical structure is sometimes far removed from having a substituted alkylamine side chain. Therefore, instead of classifying these agents on the basis of their chemical structure, it is also useful to classify them as first generation sedating H_1-receptor antagonists such as chlorpheniramine, diphenhydramine,cyproheptadine and hydroxyzine and second generation non-sedating H_1-receptor antagonists such as terfenadine, astemizole, loratidine and cetirizine (8,88).

Most antihistamines are chemically stable weak bases used as water soluble acidic salts. At low concentrations, H_1-receptor antagonists

are reversible competitive inhibitors of the actions of histamine, blocking histamine from reacting with its receptors. They antagonise the action of histamine on the capillaries, leading to reduced capillary permeability, edema and wheal formation. H_1-receptor antagonists do not inhibit gastric secretion which is the function of H_2-receptor antagonists.

Many of the newer H_1-receptor antagonists have been shown to prevent the release of inflammatory mediators from sensitized mast cells and basophils. Some such as cetirizine also exhibit anti-inflammatory activity. Some first-generation H_1-receptor antagonists such as hydroxyzine may have weak anticholinergic effects and suppress salivary and lacrimal secretions. Other first-generation H_1-receptor antagonists such as promethazine possess α-adrenergic blocking ability. At high concentrations, certain H_1-receptor antagonists possess local anesthetic effects.

First-generation H_1-receptor antagonists such as dimenhydrinate and diphenhydramine cross the blood-brain barrier and cause central nervous effects such as sedation but are also able to prevent the symptoms of motion sickness. Second-generation H_1-receptor antagonists such as cetirizine, terfenadine, astimazole and loratidine are

lipophobic and they do not cross blood-brain barrier to any great extent and consequently have no effect on motion sickness (8).

1.2.1.2 Pharmacokinetics of H_1-receptor antagonists.

In general, H_1-receptor antagonists are well-absorbed when given orally. The peak serum concentrations are achieved within 2 hours following administration. All the first-generation H_1-receptor antagonists and most of the second-generation are metabolized by the hepatic cytochrome P-450 series of enzymes.

The serum elimination half-life values range from 24 hours or less for chlorpheniramine, diphenhydramine, hydroxyzine, terfenadine and loratidine to 9.5 days for astemizole and its active metabolites (8). The volumes of distribution are large and usually not corrected for bioavailability because intravenous formulations are not available for comparison with oral formulations for most compounds.

The elimination half-life values of most H_1-receptor antagonists increases with increase in age of patients, and they are also prolonged in patients with hepatic dysfunction (22).

The maximum peripheral antihistaminic effects of H_1-receptor antagonists occur several hours after peak serum concentrations have passed, and persist even when serum concentrations of the parent compound have declined to the lowest limits of analytical detection. This prolonged effect could be due to the persistant tissue concentrations of unchanged drug or the presence of active metabolites (8).

1.2.1.3 H_1-receptor antagonists used in this study.

1.2.1.3.1 Hydroxyzine

1.2.1.3.1.1 Chemistry

Hydroxyzine hydrochloride, 2-[2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]-ethanol dihydrochloride, is a piperazine-class, first generation antihistamine (Figure 6). Hydroxyzine is commercially available as the dihydrochloride salt. It occurs as a white, odorless powder and is very soluble in water and freely soluble in alcohol (23).

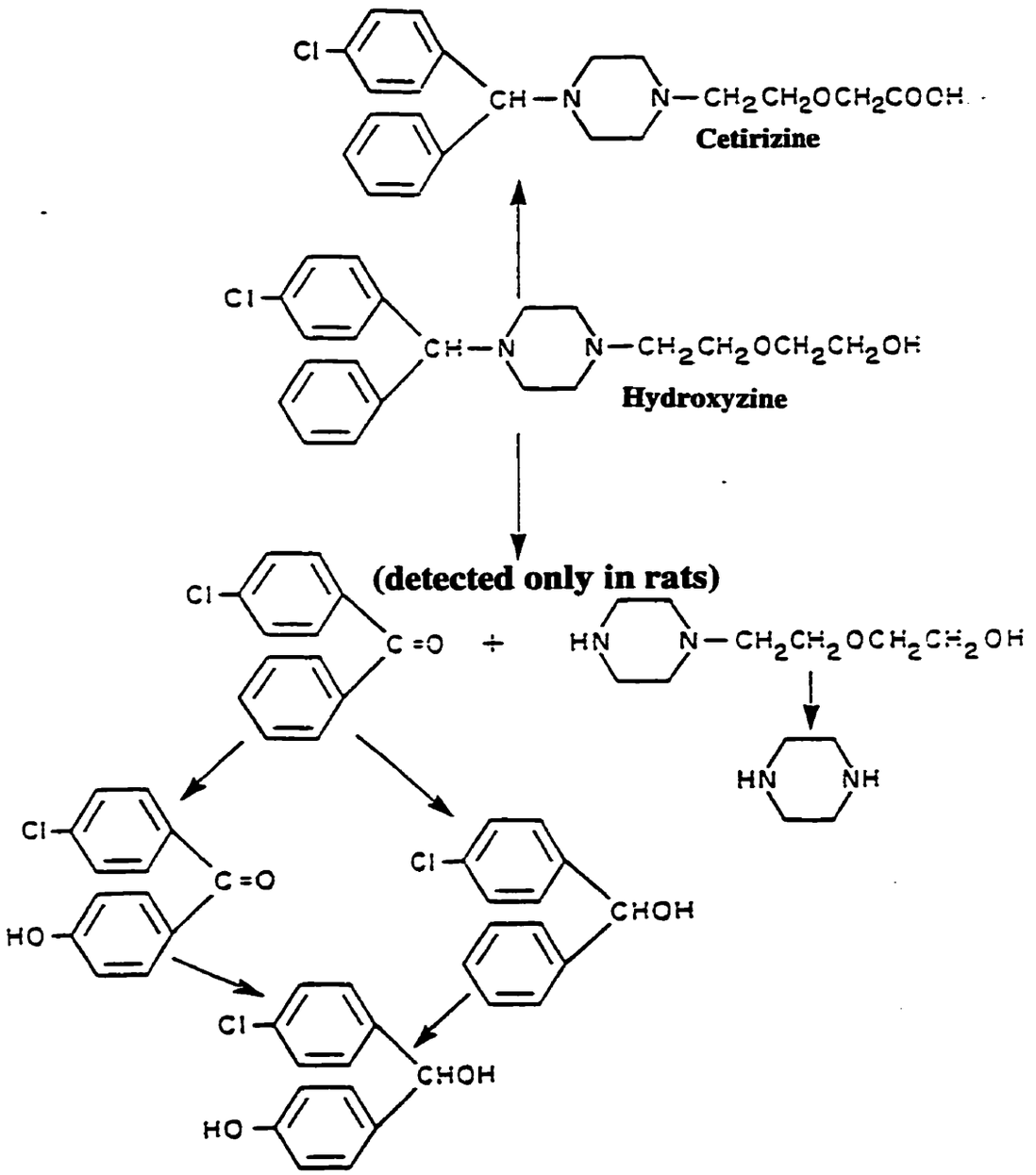


Figure 6. The metabolic pathways of hydroxyzine.

1.2.1.3.1.2 Pharmacology.

Hydroxyzine has a range of pharmacological properties. It has primary skeletal muscle relaxant activity. Hydroxyzine also exhibits analgesic activity, an effect which may be related to its sedative activity. It has CNS depressant, anticholinergic, antispasmodic and local anesthetic activity in addition to its H_1-receptor antagonist effects. The sedative and tranquilizing effects of hydroxyzine are thought to be as a result of the suppression of the activity at the subcortical level of the CNS. Since hydroxyzine does not have cortical depressant activity, the actual mechanism of its anti-emetic and anti-motion sickness activities are unknown, but appear to result, at least in part, from its central anticholinergic and CNS depressant properties (23).

The antispasmodic effect of hydroxyzine is mediated through interference with the mechanism that responds to spasmogenic agents such as acetylcholine (23).

1.2.1.3.1.3 Pharmacokinetics.

Although H_1-receptor antagonists have been used clinically for more than five decades, there is still minimal information about the pharmacokinetics of most of the first generation H_1-receptor antagonists. Pharmacokinetics is the discipline that concerns the study of characterization of the time course of drug absorption, distribution, metabolism, and excretion, as well as the relationship of these processes to the intensity and time course of therapeutic and toxicologic effects of drugs (87).

From the absorption site, the drug diffuses to the blood stream where it may bind to plasma proteins. The transfer of the drug from one compartment to another is associated with a specific rate constant for that particular transfer (87). The transfer rate of the drug is described by first order kinetics such that the transfer rate of a drug from plasma to the elimination sites is directly proportional to the drug concentration in the plasma (87).

An oral dose of hydroxyzine 0.7 mg/kg given to seven healthy adult volunteers produced a mean peak serum concentration of 72.5 ±

11.1 ng/ml at a mean time of 2.1 ± 0.4 hours (24). The mean elimination half life was 20 ± 4.1 hours and the mean clearance rate was 9.78 ± 3.25 ml/min/kg. The mean apparent volume of distribution was 16.0 ± 3.0 L/kg (24).

In another study hydroxyzine was given in doses of 1.7-1.4 mg/kg orally. The drug was well absorbed with a peak serum concentration of 80 ng/mL within 3 hours after administration (25).

In elderly subjects, hydroxyzine 0.7 mg/kg given orally appeared to be rapidly absorbed with a peak serum concentration of 77 ± 17 ng/mL occuring at 2.3 ± 1.5 hours (26). These values were not significantly different from studies in healthy young adults. The elimination half life of hydroxyzine in the elderly was 29.3 ± 10.1 hours, significantly longer than the serum elimination values of young adults (24). The volume of distribution measured in elderly subjects was found to be greater, 22.5 ± 6.3 L/kg compared to younger adult subjects, 16.0 ± 3.0 L/kg (26).

During normal aging, adipose tissues tends to increase as a fraction of total body weight while the lean body mass decreases. Hydroxyzine is a very lipophilic compound which could be expected to

distribute widely in adipose tissues. The large volume of distribution found in elderly subjects also may be due to the increase in the elimination half life, because the oral clearance rate of the drug was 9.6 ± 3.2 mL/min/kg in elderly subjects, not different from that of young subjects, 9.78 ± 3.25 mL/min/kg (24). It was not possible to determine the absolute bioavailability because intravenous formulations are not available for comparison with oral formulations (26). In all of these oral studies the fraction of drug absorbed is assumed to be 1.00 (26).

Hydroxyzine was studied for the relief of pruritus in patients with primary biliary cirrhosis. When administered as a single oral dose of 0.7 mg/kg, a mean peak serum concentration of 116.5 ± 60.6 ng/mL occured at 2.3 ± 0.7 hours. The elimination half life of hydroxyzine in this study was 36.6 ± 13.1 hours which was much longer compared to adult and elderly subjects (22, 24, 26). The mean hydroxyzine clearance rate was 8.65 ± 7.46 mL/min/kg with a volume of distribution of 22.7 ± 13.3 L/kg (22).

1.2.1.3.1.4 Pharmacodynamics.

The histamine-induced wheal and flare is a safe, well-studied, quantitative bioassay to monitor peripheral H_1-receptor antagonist activity (27). Hydroxyzine results in more lasting suppression of the histamine-induced wheal and flare than diphenhydramine, tripelennamine, chlorpheniramine or promethazine (28). The potency of hydroxyzine in suppression of antigen-induced wheal and flare response is superior to that of diphenhydramine, tripelennamine, chlorpheniramine and promethazine (29). The suppression of histamine-induced wheals and flares in the skin after administration of a single oral dose of 0.7 mg/kg hydroxyzine to healthy adult subjects was significantly long from 1-36 hours ($p<0.05$)(24). The maximum suppression of wheals was 80 % and of flares was 92 %. The effects persisted for 36 and 60 hours, respectively (24).

The wheal and flare response to histamine persists even in old age. In the elderly, the persistence of significant suppression of the wheal and flare at times when serum hydroxyzine concentrations are extremely low supports the pharmacodynamic hypothesis which explains that a given

drug concentration at receptor sites yields a greater pharmacodynamic response in an elderly subject than a younger one (30). A single dose of hydroxyzine syrup 0.7 mg/kg produced a significant suppression of wheal and flare in elderly subjects from 1-144 hours (26). Hydroxyzine, chlorpheniramine and terfenadine have long elimination half lives and large apparent volumes of distribution in the elderly (8,26) suggesting the possibility of enhanced H_1-receptor activity with these drugs in old age (26).

In patients with primary biliary cirrhosis, a single dose of hydroxyzine had an extremely long duration of action, with a significant suppression of wheal and flare from 120-144 hours, respectively (22). Maximal suppression of wheals and flares occured from 2-48 hours and from 3-24 hours, respectively.

1.2.1.3.2 Cetirizine.

1.2.1.3.2.1 Chemistry.

Cetirizine, 2-[2-(4((4-chlorophenyl)phenylmethyl)-1-piperazinyl) ethoxyl]- acetic acid dihydrochloride) is one of the second-generation, non-sedating H_1-receptor antagonists.

Cetirizine, a piperazine derivative and primary metabolite of hydroxyzine produced by carboxylation of the alcohol side chain (Figure 6), is a potent H_1-receptor antagonist with reduced access to the CNS and high selectivity for the H_1-receptor (31).

Commercially it occurs as a dihydrochloride salt which is soluble in water and methanol, and insoluble in acetone and chloroform (32).

1.2.1.3.2.2 Pharmacology.

Cetirizine has been shown to be selective for H_1-receptors (33) and provide greater than 50% mean suppression of wheals 24 hours after oral administration of 10 mg (34).

Oral doses of cetirizine were shown to produce antihistaminic activity similar to that of hydroxyzine with little or no CNS effect. The presence of the carboxylic acid group makes cetirizine less lipophilic than hydroxyzine and inhibits its passage through the blood-brain barrier, yielding the low incidence of adverse sedative effects (32).

Cetirizine demonstrates affinity similar to that of terfenadine for <u>in vitro</u> binding to peripheral histamine H_1-receptors. However, it is highly selective for histamine H_1-receptors, possessing less affinity than

terfenadine or hydroxyzine for calcium channel receptors, α-adrenergic, dopamine D_2, serotonin $5HT_2$, and muscarinic receptors (35).

The tests of antihistaminic activity in animals have shown cetirizine to have greater potency on a weight-to-weight basis than other antihistamines such as clemastine, mepyramine, terfenadine and hydroxyzine. Cetirizine 10 mg had a similar potency to diphenhydramine 50 mg, hydroxyzine 25 mg and terfenadine 180 mg, but was more potent than terfenadine 60 mg, with a more rapid onset and longer duration of action. It was more potent than loratidine 10 mg , chlorpheniramine 6 mg or mequitazine 5 mg, and more rapid in onset of action than astemizole 10 mg (35).

Cetirizine 5-20 mg also provided a dose-dependent protective effect from bronchiospasm induced by inhaled histamine in asthmatics, with the 20 mg dose superior in activity to hydroxyzine 25 mg.

Cetirizine had little effect on stabilizing mast cells and the release of mediators of immediate hypersensitivity (35). However, cetirizine appears to have some modulating effects on the late phase inflammatory reactions which involves the migration of inflammatory cells, eosinophils, neutrophils, basophils, and monocytes, to the reactive

site with subsequent release of inflammatory mediators. Cetirizine 10 mg demonstrated a significant inhibition of skin oesinophil recruitment during skin challenge compared with loratidine 10 mg (36,37).

1.2.1.3.2.3 Pharmacokinetics.

The most important pharmacokinetic property of cetirizine is its rapid and predictable absorption from the gut, and its direct pharmacologic action that does not require biotransformation (38). Following oral administration, peak serum concentrations are observed within 1 hour. Unlike the other non-sedating antihistamines, this drug is not metabolized by the liver. Food may decrease the rate but not the extent of absorption (31).

Lefebvre *et al.* 1988 studied the pharmacokinetics of cetirizine for 48 hours after a single intake of 10 mg in 10 elderly (60-90 years) and in young healthy volunteers (21-29 years). In young volunteers about 60% of an oral dose of cetirizine was excreted in the urine in unchanged form. Pharmacokinetic parameters were slightly higher in the elderly subjects compared to the young adults; C_{max} 362 ng/mL vs 337 ng/mL, T_{max}

1.30 h vs 1.12 h, elimination half-life 11.8 h vs 10.6 h and AUC 4316 ng-h/mL vs 3721 ng-h/mL (39).

In a double-blind study, cetirizine 5 or 10 mg was ingested once daily by children. It was rapidly absorbed with mean peak cetirizine concentrations of 427.6 ± 144.2 ng/mL, at T_{max} of 1.4 ± 1.2 hour after 5 mg dose, and 978.4 ± 340.6 ng/mL with T_{max} of 0.8 ± 0.4 hour after a 10 mg dose. The serum elimination half-life of cetirizine was 7.1 ± 1.6 hour after 5 mg dose, and 6.9 ± 1.6 hour after a 10 mg dose. Urinary excretion of unchanged cetirizine during 24 hours after the initial dose of 5 mg was 40 ± 15%, and after a 10 mg dose was 39 ± 14% (40).

Matzke *et al.* 1987 studied the pharmacokinetic of cetirizine in elderly subjects and in patients with renal insufficiency. They found that the elimination half-life of cetirizine was prolonged in patients with mild and moderate renal insufficiency, compared with age-matched individuals with normal renal function, 19.0 ± 3.3 and 20.9 ± 4.4 hours, versus 7.4 ± 3.0 hours respectively. The apparent volumes of distribution did not differ significantly between the groups, ranging from 0.41 to 0.47 L/kg. The renal clearance of the drug was significantly slower in patients with renal insufficiency (41). In the elderly subjects, the $t_{1/2}$ of

cetirizine was significantly prolonged compared with younger adults, and the body clearance rate was reduced (41).

Cetirizine and loratidine have a comparable serum half life of 9h, while the astemizole half-life is considerably longer (10-20 days) (42). Sixty to seventy percent of cetirizine is excreted unchanged in the urine within 24 hours (35).

It was suggested by many studies that cetirizine may contribute significantly to the antihistaminic actions of hydroxyzine. After oral administration of hydroxyzine, 45-60% of the dose is metabolized by oxidation of the side chain to cetirizine (43). A single dose of 25 mg hydroxyzine produces peak plasma concentrations of 145 ± 35 ng/mL cetirizine at four hours (31).

1.2.1.3.2.4 Pharmacodynamics.

De Vos *et al.* 1987 reported that the wheal and the flare response to intracutaneous injection of histamine was significantly inhibited by oral doses of cetirizine in 4 species including dogs, mice, rats and guinea pigs. In rats, cetirizine was similar in potency to clemastine and 20 times more potent than mepyramine. In guinea pigs, it was 2.5 times more

potent than clemastine and 15-20 time more potent than mepyramine, hydroxyzine, and terfenadine. In mice, it was 10 times more potent than hydroxyzine and 5 times more potent than terfenadine (44).

In human studies, the wheal response to intracutaneously injected histamine phosphate 0.1-10.0 mg, is suppressed by cetirizine. In several studies, the wheal response was inhibited as early as two hours after administration of 10 mg with peak effects at 4-8 hours after administration and a duration of action of up to 24 hours (45).

When 10 mg cetirizine was administered for 4 days to atopic volunteers, the wheal and flare responses were inhibited by 75-80% (46). In 19 children, administration of 5 or 10 mg cetirizine for 5 weeks inhibited wheal and flare response for the duration of the study (40).

Cetirizine 10 mg was more rapid in onset and longer lasting in its effect than terfenadine 60 mg and loratidine 10 mg. It produced greater maximal suppression in wheal size 73-94% with cetirizine vs 49-79% with terfenadine, and 94% with cetirizine versus 49% with loratidine (34). Cetirizine 10 mg produced greater suppression of wheal and flare than did astemizole 10 mg (38).

Pretreatment with cetirizine 10 mg caused reduction in occular redness and itching which is induced by intraconjunctival application of pollen extract to atopic patients, while pretreatment of cetirizine 20 mg to 10 asthmatic patients produced no protection against allergen-induced bronchiospasm (47,48).

1.2.1.4 Adverse effects of H_1-receptor antagonists.

H_1-receptor antagonists are considered to be safe drugs with no severe toxic effects. First-generation H_1-receptor antagonists cause diminished alertness, sedation, confusion, dizziness, anticholinergic side effects such as dry mouth, blurred vision, and urinary retention. H_1-receptor antagonists such as tripelennamine cause gastrointestinal tract side effects such as anorexia, nausea, vomiting, diarrhea, and constipation. Cyproheptadine and astemizole cause appetite stimulation and weight gain. Overdosing with terfenadine or astemizole can cause cardiac arrest. Patients with hepatic dysfunction and elderly subjects are particularly susceptable to these side effects (8).

1.2.2 H_2-receptor antagonists.

The development of H_2-receptor antagonists began in 1972 and resulted in a new classification of the effects of histamine agonists and antagonists. The frequency of peptic ulcer disease and other related gastrointestinal diseases stimulated the great interest shown in the therapeutic potential of the H_2- receptor antagonists.

H_2-receptor antagonists have a proven value in the treatment of duodenal ulcer especially in patients who have signs of hyper- secretion of gastric acid. They lower the basal and nocturnal secretion of gastric acid which is stimulated by foods and other factors. Use of H_2-receptor antagonists reduces the daytime and night time pains and also the consumption of antacids. The incidence of healing promoted by cimetidine in a 4-6 week period, is more than twice that of patients taking placebo (1). Following 8 weeks of therapy, 85-90% of duodenal ulcers are healed. In the treatment of gastric ulcers, healing rate is accelerated by H_2-receptor antagonists such as cimetidine at the beginning of the therapy and is about 50% at the end of 8 weeks therapy (1).

In Zollinger-Ellison syndrome, gastric secretion is produced by a tumour. The use of H_2-receptor antagonists has provided a valuable treatment, but high doses of these agents may be needed resulting in a high incidence of adverse effects, particularly with cimetidine (59).

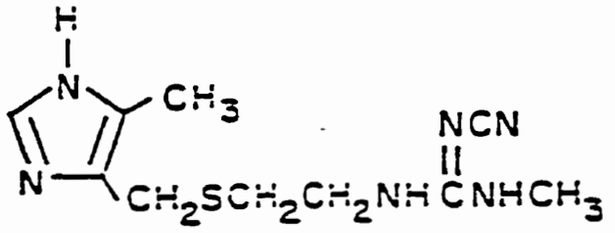
The first two H_2-receptor antagonists discovered were burimamide and metiamide, which are imidazole compounds with long side chains containing a thiourea group, but these two compounds were not used in humans because of their toxicity.

The four H_2-receptor antagonists which are currently available in the U.S.A. are cimetidine, ranitidine, famotidine, and nizatidine (1).

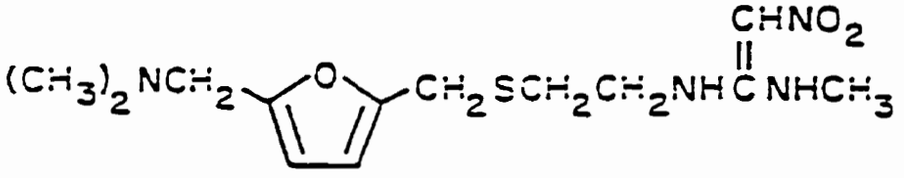
1.2.2.1 Structure and pharmacology.

The structures of the H_2- receptor antagonists are shown in Figure 7 (83). H_2-receptor antagonists compete reversibly with histamine at H_2-receptor sites. This action is selective because H_1-receptors are not affected by H_2-receptor antagonists (13).

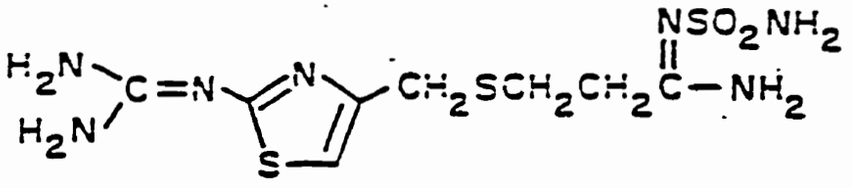
The synthesis of H_2-receptor antagonists was developed by modifying the histamine molecule which subsequently produced compounds that were well absorbed orally, and had an acceptable level



Cimetidine



Ranitidine



Famotidine

Figure 7. The chemical structures of cimetidine, ranitidine and famotidine.

of toxicity. Cimetidine was the first H_2-receptor antagonist for general clinical use. It had widespread acceptance for treatment of duodenal ulcer and other gastric hypersecretory conditions, and soon became one of the most widely prescribed of all drugs.

H_2-receptor antagonists such as cimetidine and ranitidine are polar hydrophillic molecules. Cimitidine is an imidazole derivative like histamine. However, the ring structure does not appear to be critical for pharmacologic activity because ranitidine possesses a substituted furan ring (Fig. 7) and alternative ring structures appear in other highly effective agents (1).

H_2-receptor antagonists are competitive and reversible antagonists for histamine activity at H_2-receptor sites. They are highly selective in their blocking of H_2-receptors, but not H_1-receptors or receptors of other drugs. The most prominent effect of H_2-receptor antagonists is the inhibition of gastric acid secretion which is stimulated by histamine through H_2-receptors. Although H_2-receptors are widely distributed throughout the body, the interference of H_2-receptor antagonists with physiological functions is minimal, indicating that the extragastric H_2-receptors are of minor physiological importance (1).

H_2-receptor antagonists inhibit gastric secretion that is stimulated by histamine or other H_2-receptor agonists in a dose dependent competitive manner. The degree of the inhibition parallels the plasma concentration of the drug over a wide range. H_2-receptor antagonists also inhibit the gastric secretion caused by muscarinic agonists or gastrin. This breadth of inhibition is due to the specific action of these drugs at the receptors for those other secretagogues. This interaction indicates that both these classes of secretagogues utilize histamine as final mediator.

H_2-receptor antagonists reduce both the volume of gastric fluid secreted and its hydrogen ion concentration. The out put of pepsin, which is secreted by gastric glands under cholinergic control also falls with the reduction of gastric fluid volume (1).

1.2.2.2 Pharmacokinetics of H_2-receptor antagonists.

1.2.2.2.1 Cimetidine.

Cimetidine is rapidly and completely absorbed after oral administration, and there is little impairment of absorption by food or antacids. Peak concentrations in plasma are achieved after one to two hours. Cimetidine undergoes an hepatic first pass metabolism which leads to an absolute bioavailability of 60%. The elimination half life for cimetidine is about 2-3 hours. About 60% of a dose of cimetidine is excreted unchanged in the urine by the kidneys (8).

1.2.2.2.2 Ranitidine.

Peak plasma concentrations of ranitidine are usually attained up to 3 hours after ingestion. The mean bioavailability of oral ranitidine is 50%. The systemic bioavailability of 50% was determined by comparing the area under the plasma concentration-time curve (AUC) after oral doses to that after intravenous doses. Bioavailability and hepatic clearance values suggest significant first pass metabolism after oral administration (83).

The apparent volume of distribution after intravenous administration has usually been reported to be 1.16 to 1.87 L/kg. The mean total volume of distribution after repeated intravenous bolus and infusion was 98 and 106 L respectively.

The products of ranitidine which were excreted in the urine within 24 hours are unchanged ranitidine, ranitidine-N-oxide, ranitidine-S-oxide and desmethyl-ranitidine. The majority of the dose was excreted during the first 6 hours after administration. The total plasma clearance of ranitidine after intravenous administration was found to range between 568 and 709 mL/min. Hepatic clearance is about 30% of total clearance after intravenous administration, whereas after oral administration it is 73% of total clearance. The elimination half life of an intravenously administered single dose of ranitidine is 1.6 to 2.1 hours, but after oral dosing in the same patients, the half life is increased to 2.1 to 3.1 hours (49).

1.2.2.2.3 Famotidine.

The bioavailability of famotidine is relatively low, being 37% after doses of 20 and 40 mg. In an intravenous versus oral cross over study,

bioavailability was found to be 20-66% indicating that absorption from the gastrointestinal tract was not complete. After oral administration, the time of plasma peak concentration was between 1-4 hours (50,51,52). The effect of food on the maximum plasma concentration, and on the area under the plasma concentration-time curve was minimal (52).

After intravenous administration, the volume of distribution at steady state was 1.13 to 1.42 L/kg (50,51). However, there are few studies on the tissue distribution of famotidine in humans. It was detected in cerebrospinal fluids (CSF) in 10 patients who were receiving 40 mg for seven days and the ratio of CSF to plasma concentrations ranged from 0.06 to 0.12 (53). The protein binding in human plasma is very low (50,51).

The renal excretion of famotidine is not dose dependent (54,55). It is rapidly excreted in urine unchanged so that the renal clearance of famotidine is three times greater than glomerular filtration (50). The urinary excretion of unchanged famotidine was 20-30% of orally administered doses, and 65-85% following intravenous administration (50), while 2% of the bioavailable dose was recovered as an oxidized metabolite (sulphoxide) in urine (51). The terminal half life of

famotidine after oral or intravenous doses was reported to be 2.5 and 4 hours in subjects with normal renal function (52,56).

1.2.2.3 Adverse reaction and side effects.

The incidence of adverse reactions to H_2-receptor antagonists is low. This may be attributed to the relative absence of physiologically important mechanisms which utilize H_2-receptors in organs other than stomach. Also, it may reflect the inability or poor ability of these medications to cross the blood brain barrier.

The side effects associated with cimetidine are headache, dizziness, malaise, myalgia, nausea, pruritus, loss of libido and impotence. Cimetidine also binds to androgen receptors which may lead to sexual dysfunction and to gynaecomastia. When cimetidine is given intravenously it stimulates the secretion of prolactin and elevates the concentration of this hormone during chronic oral treatment.

Cimetidine also binds to cytochrome P-450 which leads to inhibition of the activity of the hepatic microsomal mixed-function oxidase. This leads to the accumulation of other therapeutic agents which are metabolized by their enzymes during concomitant treatment with

cimetidine. Drugs whose metabolism is inhibited by cimetidine include: warfarin, phenytoin, theophylline, phenobarbital, diazepam, propranolol and imipramine (58). Cimetidine has also been reported to reduce hepatic blood flow which in turn inhibits the clearance of drugs whose elimination is blood flow dependent such as lidocaine, but this effect has not been seen by all investigators (58).

CNS disturbances such as slurred speech, somnolence, lethargy, restlessness, confusion, disorientation and hallucination have been documented as cimetidine side effects. It was noted that cimetidine causes some cell-mediated immune responses especially in immunologically depressed individuals. When it is given intravenously it leads to bradycardia and some cardio-toxic effects (62).

Usual therapeutic doses of famotidine do not interfere with the elimination of drugs which are metabolized by the mixed function oxidase system in the liver. Famotidine is therefore, unlikely to cause clinically significant drug interactions when it is administered concurrently with drugs eliminated through the liver by oxidative biotransformation such as warfarin, diazepam, phenytoin, and propranolol (57).

1.3 Effect of disease states on drug disposition.

1.3.1 Hepatic dysfunction (primary billiary cirrhosis).

Patients with more than one disease such as diabetes and various gastrointestinal diseases may respond differently to drugs used to treat other disorders, so that the patient may be more likely to develop adverse drug reactions.

Biliary cirrhosis refers to a disorder characterized by clinical and chemical signs of chronic impairment of bile excretion and morphologic evidence of progressive liver destruction centered about the interhepatic bile ducts. Major clinical symptoms of impaired bile excretion include protracted itching, progressive and prolonged jaundice and marked elevations of serum alkaline phosphatase, cholesterol and other lipid fractions. Most forms of biliary cirrhosis evolve from chronic inflammatory lesions of interlobular ducts, bile ductules, and periportal liver cells.

Biliary cirrhosis can be primary, in which case the process is related to chronic inflammation and fibrosis of the intrahepatic bile

ductules, or secondary to obstruction of the common bile ducts or its large branches (60).

The cause of primary biliary cirrhosis is unknown. The observation that about 90 % of cases occur in midle-aged women strongly suggests an endocrine contribution. Although no drug has produced a typical picture of progressive biliary cirrhosis, the occasional appearance of many elements of the syndrome in patients treated with phenothiazine suggests that drug hypersensitivity may be one etiologic factor (60).

The earliest recognizable lesion of primary biliary cirrhosis might be termed "chronic-non suppurative destructive cholengitis". This is characterized by destruction of medium and small bile duct cells. Progression of this process over a period of months to years (usually 3-5 years) leads to loss of liver cells, expansion of periportal fibrosis into a network of connective tissues scars with apparent loss of interlobular ducts, and development of cirrhosis (60).

Many patients with primary biliary cirrhosis are asymptomatic, and the disease is initially detected by the finding of a raised serum alkaline phosphatase on screening examination. The disease may remain

clinically silent for many years. The patient typically is a middle-aged woman who develops persistent generalized itching, followed by dark urine, pale stools, and jaundice with some darkening (melanosis) of the exposed areas of skin (60).

Physical examination may be entirely normal in the early stages of the disease. Later there may be jaundice of varying intensity, hyper pigmentation of exposed skin areas, clubing of the fingers. Fever and chills are rare and usually indicate mechanical biliary obstruction or other associated disease (60). Biliary cirrhosis should be considered in any patient where symptoms, signs, and laboratory evidence of protracted obstruction of bile flow are evident. Liver biopsy may be required to confirm the diagnosis of primary biliary cirrhosis.

There is no known medical therapy which alters the slowly progressive course of primary biliary cirrhosis. Therapeutic efforts are therefore directed at the many symptoms or complications of the disease as they develop.

Itching may be helped by antihistamine such as cetirizine, while systemic corticosteroids may decrease itching but are accompanied by

serious side effects such as osteopenia. Bile salt-sequestering resin, cholestyramine, also usually relieves itching (60).

1.3.2 Renal failure.

Renal failure is broadly defined as a rapid deterioration in renal function sufficient to result in accumulation of nitrogenous wastes in the body (60). The cause of such deterioration includes renal hypoperfusion. Sixty percent of all cases of renal failure are related to surgury or trauma. 40% occur in a medical setting, and 1-2 % are related to pregnancy. The most common general cause of renal failure is renal ischemia (60).

Renal failure becomes critical with patients taking medication excreted primarily by the kidney. A medicine like cetirizine for which 80 % is excreted unchanged in urine should be administered with precaution in renal failure. Matzke *et al.* 1987 (41) found that the elimination half-life of cetirizine was prolonged in patients with mild and moderate renal insufficiency, 19.0 ± 3.3 and 20.9 ± 4.4 hours, compared to age-matched individuals with normal renal function, 7.4 ± 3.0 hours.

1.4 Pharmacokinetic interaction of cimetidine.

Cimetidine, the H_2-receptor antagonist, is now the seventh most frequently used drug in the United States, in the treatment of peptic ulcer, Zollinger-Ellison syndrome, multiple endocrine adenoma syndrome, systemic mastocytosis and as a prophylactic for stress ulceration (62,63,64) with a low incidence of side effects such as bone marrow depression, bradycardia, mental confusion and hepatotoxicity (65). The most important adverse effect which should be noted in the use of cimetidine is its interaction with the absorption and clearance of other drugs leading to modification of their biological effects. The co-administration of cimetidine to humans taking an anticoagulant produces clinically significant drug interactions such as potentiation of anticoagulant action as a consequence of impaired anticoagulant metabolism.

1.4.1 The effect of cimetidine on absorption.

Cimetidine can increase the intragastric pH, so that it alters the dissolution of other drugs especially those which are weak bases. This

effect is of good clinical use in those patients who fail to respond to the therapy with ketoconazole (66). In addition to its interaction with ketoconazole, cimetidine interferes with the gastrointestinal absorption of penicillin, noted in one patient after sodium benzylpenicillin was given with cimetidine (67). Cimetidine delays the time of peak plasma concentration of some drugs such as mexiletine, ethanol, chromthiazole, and tolbutamide after oral administration, yielding a change in the gastrointestinal rate of absorption, although the extent of absorption is not affected (68).

1.4.2 The effect of cimetidine on renal excretion.

Glomerular filtration and active secretion by the proximal tubules are important mechanisms for drug excretion by the kidney. There appear to be two separate active transport processes, one for weak acids such as furosemide, and the other for weak bases such as cimetidine. Cimetidine reduces the renal clearance of procainamide and its active metabolite, N-acetyl procainamide. It was proposed that the mechanism is competition for the basic active tubular secretion pathway. This has been verified using an isolated rabbit proximal tubule model (69).

1.4.3 The effect of cimetidine on hepatic metabolism.

Metabolic reactions mainly take place in the hepatocyte and are facilitated by the microsomal enzymes of the oxidation system. These enzymes are located in the smooth endoplasmic reticulum of most cells, but the most important site of drug oxidation is the liver. The cytochrome P-450 enzyme is a haem protein enzyme that binds and metabolizes drugs and some endogenous substances.

Cytochrome P-450 is the term used to describe components of the hemoprotein super family of enzymes which catalyse the oxidative biotransformation of lipophilic substrates to more polar metabolites. These enzymes are found in the endoplasmic reticulum or mitochondrial fractions of many tissues. They function in either catabolic pathways or in the biosynthesis of molecules of physiological importance.

In the microsomal fraction of the endoplasmic reticulum, the cytochrome P-450 activity depends on a flavoprotein and reduced nicotinamide adenine dinucleotide phosphate (NADPH)-P-450 reductase which functions in the transfer of electrons from NADPH to the P-450-substrate complex. Another important component is a non-haem iron

protein that facilitates electron transfer. The cytochrome P-450 system catalyses the insertion of an active oxygen species into substrate molecules (61).

Cytochrome P-450 hemoprotein consists of two components, an iron protoporphyrin IX haem moiety and a single polypeptide chain or apoprotein. The iron atom of the haem group is located at the centre of the protoprophyrin ring, and it is bonded to the ring system by its four pyrrolic nitrogens, the fifth ligand being the sulphur atom from the cysteinyl residue of the apoprotein (54).

Chemicals such as thiosulfur have been well documented for their ability to inhibit mixed function oxidase activity in vitro and in vivo, through the covalent attachment of atomic sulphur to P-450 active sites, and also through the formation of haem protein adducts (54).

Cimetidine, like other imidazole-containing molecules, appears to interact with the ferric P-450 at its sixth axial ligand position. This results in a type 2 optical difference spectrum which is associated with a shift in the spin equilibrium away from the penta coordinate high spin state, a slower rate of electron transfer from NADPH and the inhibition of drug metabolism (61). It has been suggested that the high affinity

binding site of P-450 is associated with the inhibition of drug metabolism. Some non-imidazole H_2-receptor antagonists such as ranitidine have generally been found to have either no, or weak inhibitor activity toward the mixed function oxidase system. This is due to the fact that the furan ring replaces the imidazole in the structure of ranitidine and the nucleophilicity of the furan oxygen atom is not sufficient to interact effectively with the P-450 haem iron atom to prevent efficient electron transfer from occurring. Other agents such as nizatidine and famotidine have been found to be ineffective as inhibitors of oxidative drug metabolism either due to the low hydrophobic character of the molecule or due to the lack of an imidazole ring (61).

The imidazole nucleus of cimetidine and the cyano portion of the side chain bind to the haem protein of cytochrome P-450 and prevent it from binding with other drugs which are metabolized by cytochrome P-450 (71). Cimetidine binds to two distinct and independent classes of binding sites on cytochrome P-450 with micromolar dissociation constants of 8.3 and 100 showing a high affinity class of binding site compatible with therapeutic plasma cimetidine concentrations (68).

The binding of cimetidine with P-450 may reduce the substrate interaction with other drugs towards the enzyme, causing inhibition of the metabolism of these drugs (72). The following list will show the drugs whose hepatic elimination is inhibited by cimetidine (73): warfarin, acenocumarol, phenandion, phenytoin, carbamazepine diazepam, desmethyl-diazepam, chlordiazepoxide, alprazolam, triazolam, theophylline, caffeine, propranolol, labetalol metopralol, penbutolol, lidocaine, chlormethiazol, imipramine, and ethanol.

Some of these drugs are metabolized by specific isozymes such as cytochrome P1A2 (CYP 1A2) which is responsible for the metabolism of theophylline and caffeine. The isozyme CYP2D6 for the metabolism of propranolol. CYP2C9 for the metabolism of phenytoin, S-warfarin, diazepam and desmethyldiazepam, and CYP2E1 for paracetamol and ethanol (90). The isozyme CYP3A4 was found to be inhibited by ketoconazole which prevents terfenadine biotransformation (88).

1.5 Drug interactions of H_2-receptor antagonists; famotidine and ranitidine.

With respect to the effect of the H_2-receptor antagonists on hepatic elimination, it is important to know whether structural differences affect their inhibitory potential in terms of drug metabolism.

Famotidine has a little potential for interaction with other drugs. It can be considered as a very weak inhibitor for drug metabolism due to its low affinity for binding to the metabolizing enzyme cytochrome P-450 (78). The elimination of intravenous theophylline was not affected by famotidine, nor was the disposition of antipyrine (79,80). Famotidine did not impair the metabolism of antipyrine and no pharmacokinetic parameters of diazepam or desmethyl-diazepam were affected due to concomitant treatment with famotidine. It can be concluded that the H_2-receptor antagonist famotidine does not inhibit hepatic microsomal drug metabolism as evaluated by studies with diazepam, and theophylline (82,83,90).

Ranitidine is the H_2-receptor antagonist with which a number of controlled studies in humans have been conducted. Indications are that it

does not alter drug metabolism at usual therapeutic doses, but such an effect might be expressed at higher doses. Rolly *et al.* 1986 (84) found that ranitidine does not affect the metabolism of theophylline, at doses up to 4200 mg/day, or 14 times the usual recomended dose for the treatment of peptic ulcer. Powell *et al* . 1984 (57) also did not find any effect of ranitidine on the pharmacokinetics of theophylline. The relative effects of ranitidine on the elimination of cytochrome P-450 metabolized drugs such as theophylline indicate an advantage of ranitidine over cimetidine.

A comparison of the interaction potential of the different H_2-receptor antagonists was investigated by Pasanen and his colleagues 1987 (78) . They studied the effects of structural difference of five different H_2-receptor antagonists, famotidine, nizatidine, cimetidine, ranitidine and oxmetidine on the inhibitory potential in terms of drug metabolism.

Cimetidine exhibited its significant inhibitor potency in all systems studied. It impairs hepatic elimination of diazepam in man, binds strongly to human cytochrome P-450 and inhibits the rat microsomal marker enzyme systems studied. Ranitidine, famotidine and nizatidine

appear to be very weak inhibitors for in vitro drug metabolism. These results agree with their low binding affinities to cytochrome P-450 and to their lack of effect on the hepatic elimination of the probe drug diazepam in man. For oxmetidine, the binding and the metabolic studies indicate considerable inhibition of the microsomal metabolism in vitro, which was even more pronounced than the effects seen with cimetidine. However, the pharmacokinetic parameters of diazepam disposition, principally hepatic metabolism in vivo, were not significantly changed. This could be attributed to the fact that diazepam is a drug showing wide variation in the determination of its pharmacokinetic parameters, so that the impairment was not detected in this small group of subjects. However, since the pharmacokinetics of other drugs such as theophylline and propranolol were also not changed by oxmetidine (73,78), then the negative results in man could be due to the fact that the concentration of oxmetidine in blood not being sufficiently high to cause hepatic enzyme inhibition. This could be due to the low bioavailability of oxmetidine.

These studies suggested that the molecular structure is very important for the hepatic interaction potential of these H_2-receptor antagonists. In the case of cimetidine and oxmetidine, the imidazole ring

exhibits significant affinity for cytochrome P-450. The side chain -CN group also plays an important role in the binding characteristics of cimetidine. Oxmetidine does not possess a cyano group in the side chain, which may account for the lack of in vivo effect. On the other hand, oxmetidine is similar to cimetidine in the in vitro experiments. The lack of agreement in the results of the two drugs in in vivo studies could be due to the difference in the side chains of the two molecules.

1.6 Structure-activity relationship.

The structure of cimetidine differs from famotidine and ranitidine as shown in Figure 7. Pendic *et al.* 1979 (34) found that both the imidazole ring and the cyano group on the side chain are responsible for cimetidine's interaction with P-450. The imidazole ring interacts with the iron of P-450 and causes the inhibition of oxidative metabolism as described by Klutz *et al.* 1984 (50). These investigators found that cimetidine binds to the ferri-hemoprotein moiety with a dissociation constant of 70 μM, which indicates that the competition for the binding site that would exist between cimetidine and other ligands or drugs could result in the inhibition of monooxygenation reactions. However,

oxmetidine which does not inhibit the hepatic elimination of diazepam, theophylline, and antipyrine (61) and cimetidine sulphoxide, which has only a low affinity for cytochrome P-450 also possess an imidazole ring. Therefore, the side chain of the molecule may play a role in the binding properties of the substituted imidazole-containing compounds.

Ranitidine, in which the imidazole ring is replaced with a furan ring results in a compound which is unable to bind to P-450 because of the unsufficient nucleophilicity of the furan oxygen atom to interact with the P-450 iron atom.

The other drug, famotidine, possesses a thiazole ring which contains a sulfur atom. If this drug is classified as sulfur-containing therapeutic agent and the sulfur atom may have an important role in the behaviour of the compound.

1.7 The hypothesis.

The allergic dermatites such as eczema, pruritus and urticaria are commonly treated with H_1-blockers such as hydroxyzine, mainly to relieve wheals, flares and itching. When H_2-blockers such as cimetidine are given in combination with hydroxyzine, there is an improvement in the result of the combination in contrast to treatment by the H_1-receptor antagonist alone. Harvey *et al.* 1980 (55) proved the improvement in suppression of histamine induced wheal by the combination of both H_1 and H_2 blockers, hydroxyzine and cimetidine respectively. The mean suppression was 75% with hydroxyzine alone, but 85% when cimetidine was given in combination with hydroxyzine.

Bleehen *et al.* 1987 (74) demonstrated that the combined therapy of chlorpheniramine and cimetidine may be useful in the treatment of chronic idiopathic urticaria, especially in patients who are very sensitive to side effects of chlorpheniramine such as drowsiness.

Paul and Podker 1986 (75) had shown that itching from chronic urticaria was significantly reduced by patients receiving the H_1-blocker terfenadine and the H_2-blocker ranitidine compared to those who were

given terfenadine alone, Ranitidine administered alone had no significant effect on itching.

In all of the above studies, the interactions were explained and investigated by histamine-induced cutaneous response. In no case were the serum levels of the H_1-receptor antagonists measured until 1986 when Salo and his friends (76) reported the combination of hydroxyzine and cimetidine to be more effective than hydroxyzine alone in chronic urticaria. The plasma concentration of hydroxyzine was studied in seven patients treated both with hydroxyzine and the combination of hydroxyzine and cimetidine. Hydroxyzine plasma concentrations were higher during the combination treatment than during hydroxyzine alone. Then in 1990 Chen and Simons (77) demonstrated that the combined administration of hydroxyzine and cimetidine caused improvement in the suppression of the wheal induced by histamine in rabbits. The pharmacokinetic studies indicated that the enhanced therapeutic efficacy of hydroxyzine and cimetidine was probably due to the inhibition of hydroxyzine metabolism by cimetidine and not due only to the involvement of H_2-receptors in the skin.

We hypothesized that the increased antihistaminic effects following co-administration of an H_2-antagonist with an H_1-antagonist is due to the elevated H_1- antagonist serum concentrations resulting from inhibition of the H_1-antagonist elimination by the H_2- antagonist, and not due to antagonism of the H_2-receptors which may be involved in the total antihistaminic effects.

We tested this hypothesis in an animal model using the H_1-antagonist hydroxyzine, and its active metabolite cetirizine, and the H_2-antagonists cimetidine, ranitidine and famotidine. Serum H_1-antagonist concentrations were measured in order to calculate the pharmacokinetics of the H_1-antagonists. Suppression of the histamine-induced wheal was used to measure the peripheral antihistaminic effects of these compounds. The co-administration of ketoconazole on the elimination of the H_1-antagonists was also evaluated as a positive control, since ketoconazole is known to inhibit the metabolism of the cytochrome P-450 oxygenase enzyme, but it has no H_2-receptor effect. Ketoconazole was used to try and differentiate between the pharmacokinetic and the pharmacodynamic effects of cimetidine on hydroxyzine.

The model H_1-antagonist drug chosen is hydroxyzine since its pharmacokinetics and pharmacodynamics are well known in our laboratory (86,24). The other reason is that the major metabolite of hydroxyzine is cetirizine which is pharmacologically active and has also been evaluated. The HPLC assay for determining cetirizine was established in our laboratory (32).

Rabbits are chosen as the laboratory animal since they are of sufficient size to permit the withdrawal of the number of blood samples necessary for pharmacokinetic studies. Preliminary studies in our laboratory confirmed that rabbits metabolize hydroxyzine to cetirizine as do human subjects.

The other factor studied was the effects of disease on the pharmacokinetics and pharmacodynamics of cetirizine in patients with primary billiary cirrhosis. Chen et al. 1990 (77) studied the effect of primary billiary cirrhosis on the pharmacokinetics and pharmacodynamics of hydroxyzine in humans. It was found that the pharmacokinetic parameters of hydroxyzine were changed in patients with primary billiary cirrhosis compared to healthy young adults. It was also expected that the area under the serum concentration versus time

curve for cetirizine would be reduced, since the metabolism of the parent compound was reduced due to the disease. However, the cetirizine area under serum concentration versus time curve was found to be higher in patients with PBC (77). To try and explain these results, the pharmacokinetics and pharmacodynamics of cetirizine were studied as a *denovo* compound in patients with primary billiary cirrhosis.

CHAPTER II
MATERIAL AND EXPERIMENTAL

2.1 Chemicals and equipments.

2.1.1 Chemicals.

1. Hydroxyzine: Sigma Chemical Company, St.Louis, Mo. USA.
2. Antazoline sulphate: CIBA Company Limited, Dorval, Quebec.
3. Cetirizine: UCB S.A.Pharmaceutical Sector-Research & Development Chemin du Foriest, Belgium.
4. P_{265}: Pfizer Canada Inc., Kirkland, Quebec.
5. Evans-blue: Fisher Scientific Co., Fair Lawn, New Jersey, USA.
6. Potassium hydroxide: Fisher Scientific Co., Fair Lawn, New Jersey, USA.
7. Ammonium phosphate mono basic: Mallinckrodt Canada Inc., Pointe-Claire, Quebec.
8. Sodium citrate: Fisher Scientific Co., Fair Lawn, New Jersey, USA.
9. Citric acid: Mallinckrodt Canada Inc. Pointe-Claire, Quebec.
10. Phosphoric acid: Fisher Scientific Co., Fair Lawn, New Jersy, USA.
11. Sodium decanesulfonate: Sigma Chemical Co., St.Loius, MO. USA.

2.1.2 Solvents.

1. Diethyl ether anhydrous: Mallinckrodt Canada Inc., Pointe-Claire, Quebec.
2. Ethyl acetate: Baxter Health Care Corporation, Burdick & Jackson Division, Muskegon, MI., USA.
3. Acetonitrile (HPLC): Mallinckrodt Speciality Chemicals Canada Inc., Mississauga, Ont.
4. Acetone: Anachemia, Rouses Point, NY, USA.

2.1.3 Equipment.

1. Centrifuge: International Equipment Company Needham, Mass., USA.
2. pH meter: Beckman Zeromatic SS-3.
3. Milli-Q Water system: Millipore corporation, Bedford, Maryland, USA.
4. Balance: Mettler instrument AG CH-8608 Greifensee-Zurich.
5. Vortex mixer: Thermolyne Maxi, Fisher Scientific Co., Dubuque, Iowa, USA.

6. Meyer N-evap Analytical Evaporator,Organomation Associates Inc., Northborough, MA.

7. High performance liquid chromatography (HPLC): The HPLC was comprised of a 6000-A high pressure pump model U6K injector for the analysis of hydroxyzine, and a 710 B automatic injector for the analysis of cetirizine. A Lambda-max model 480 LC variable wavelength spectrophotometer was used for detection at 229 nm wavelength for both hydroxyzine and cetirizine, with a 720 data module for plotting and peak identification. A Radial Pak liquid chromatography cartridge 5 mm was used for the separation of hydroxyzine, cetirizine and their internal standards. All of the system is from Waters Associates Inc. Millford, MA., USA.

2.1.4. Supplies.

1. Filter unit (0.2 μm) Nalgene, Nalge Company, Rochester, NY. USA.
2. Sure-Sep II: Serum plasma separator, Organon Teknika Corporation, Durham, North Carolina, USA.
3. I.V. catheter placement unit (22 G and 24 G): Critikon, Inc., Tampa, FL. USA.

4. Male adapter plug-short with intermittent injection site: ABBOTT hospitals, Inc., N. Chicago, IL. USA.
5. Syringes (5cc& 1cc): Becton Dickinson and company Franklin Lakes, New Jersey, USA.
6. Needles (23 G, 25 G, & 26 G): Terumo Medical Corporation, Elkton, MD, USA.
7. Disposable test tubes (16×100): Baxter Healthcare Corporation, McGaw Park, IL. USA.

2.1.5 Dosage forms.

1. Cimetidine hydrochloride I.V. injection: (Tagamet 150 mg/mL) SmithKline Beecham Pharma Inc. Oakville, Ont.
2. Famotidine I.V. injection: (Pepcid 10 mg/mL), MSD, Merck Sharp and Dohme Canada Inc. Kirkland, Quebec.
3. Ranitidine I.V. injection: (Zantac 25 mg/mL), Glaxo, Canada Inc. Toronto, Ont.
4. Hydroxyzine injection: a 2.5 mg/mL solution freshly prepared, sterilized by filtering through 0.2 μm filter unit.

5. Cetirizine tablets: (Reactine 15 mg), Pfizer Canada Inc., Kirkland, Quebec.
6. Histamine phosphate injection: (USP 1mg/mL), Allen and Hanbury, Glaxo Canada Limited Company, Toronto, Ont.
7. Evans-blue injection: 100 mg/mL solution freshly prepared, sterilised by filtering through 0.2 μm filter unit.
8. Heparin sodium injection BP: (Hep-Rinse 100 I.U. /mL) Leo Laboratories Canada Ltd, Ajax, Ont.
9. Sodium chloride injection: Isotonic vehicle 0.9 %, Astra Pharma. Inc., Mississauga, Ont.
10. Ketoconazole tablets: (Ketoconazole 200 mg), Janssen Pharmaceutical Inc., Mississauga, Ont.

2.2 Experimental.

2.2.1 Pharmacokinetic and pharmacodynamic studies of hydroxyzine and cetirizine in rabbits: The effect of cimetidine, famotidine, ranitidine and ketoconazole.

2.2.1.1 Extraction procedure for hydroxyzine.

The method used for extracting hydroxyzine from rabbit serum was that of Simons *et al.* (24,86).

Twenty five microlitres of antazoline solution (1μg/mL), the internal standard, were added to 0.5 mL of serum sample along with 250 μl of 10 % KOH solution and 5 mL of freshly distilled ether. Extraction was achieved by mixing on a vortex mixer for 30 seconds followed by centrifuging for 5 minutes at 2000 rpm. The aqueous portion was frozen in a dry ice/acetone bath and the ether layer was transfered to a clean dry 16×100 mm test tube. One hundred microlitres of 0.05 % H_3PO_4 solution were added, followed by mixing on a vortex mixer for 30 seconds and centrifuging for 5 minutes at 2000 rpm. The aqueous layer was again frozen in a dry ice/acetone bath and the ether layer was discarded. The aqueous portion was exposed to a stream

of dry nitrogen to remove remaining traces of ether. All of the remaining aqueous solution was then taken up in a syringe and injected onto the column.

2.2.1.2 Extraction procedure for cetirizine.

To 0.5 mL serum, 50 µl of P_{265} (3 µg/mL) the internal standard, were added together with 1 mL sodium citrate buffer (1M, pH 5.0) and 3 mL of ethyl acetate. The sample was mixed for 1 minute on a vortex mixer and centrifuged for 15 minutes at 2000 rpm. The organic layer was transfered to a clean test tube. To the remaining serum, another 3 mL ethyl acetate were added. The sample was again mixed for 1 minute and centrifuged for 15 minutes at 2000 rpm. The ethyl acetate was mixed with that from the previous extraction. Two hundred microlitres of 1.7% H_3PO_4 were added, and the sample was mixed for 1 minute and centrifuged for 5 minutes at 2000 rpm. The aqueous portion was removed and the organic layer was discarded. The aqueous layer was then exposed to a stream of dry nitrogen to remove remaining traces of ethyl acetate. The aqueous layer was transfered to the sample tube of the automatic injector, and 150 µl of the solution were injected on to the column.

2.2.1.3 Chromatographic separation and quantitation of hydroxyzine and cetirizine.

The mobile phases used were acetonitrile:phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.5), 35:65, v/v, for the separation of hydroxyzine, and acetonitrile:phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.9, with 0.02M sodium 1-decanesulfonate), 41:59, for the separation of cetirizine. The flow rate for separating and quantifying hydroxyzine was set at 0.8 mL/min, while the flow rate for cetirizine was set at 1.1 mL/min. The effluent from the column was monitored by UV absorption at 229 nm with 0.5 to 0.005 a.u.f.s. sensitivity setting. The chart speed for the data module was 0.25 cm/min. Peak height ratios of hydroxyzine to antazoline and cetirizine to P_{265} versus hydroxyzine and cetirizine concentrations in ng/mL respectively were used for quantitation to produce calibration curves. The retention times were 5.0 and 11 minutes for antazoline and hydroxyzine, and 5.5 and 8.0 minutes for cetirizine and P_{265} respectively. The calibration curves were prepared from the results of assays on blank serum samples supplemented with known quantities of hydroxyzine and cetirizine and the corresponding internal standards, antazoline and P_{265}. All chromatographic separations were carried

out at ambient temperature. The serum concentrations of hydroxyzine and cetirizine were expressed in terms of free base.

2.2.1.4 Pharmacokinetic and pharmacodynamic studies of hydroxyzine in rabbits.

All pharmacokinetic studies on rabbits were approved by the University Animal Care Committee and Housing, and study procedures were carried out using the Guidelines of the Canadian Council of Animal Care.

Five male New Zealand white rabbits obtained from the Central Animal Care Services, University of Manitoba were used in this study. The animals were housed individually in metal cages fitted with wire floors to reduce coprophagia; food and water were supplied,*ad libitum*.

The day before each study, the rabbit's back was shaved. On the morning of the study, the back was treated with a depilatory to remove all hair.

The catheter, with male adapter filled with heparin, was set up in the ear artery of the rabbit. An amount of the blood was withdrawn immediately to insure that the catheter was functioned. Each time blood was withdrawn, it

was substituted by 0.9 % saline. Heparin was added each time after saline to prevent clotting of blood in the catheter.

After setting up the catheter, 0.1 mL of Evans Blue, 100 mg/mL was injected intravenously to facilitate the identification of thewheal border, then the control blood sample was withdrawn, usually 2-2.5 mL, discarding the first 0.5 mL. The control histamine generation was also done. All five rabbits received an I.V. bolus injection of 2.5 mg hydroxyzine hydrochloride (0.2 mL) through the ear vein. Two to three millilitres of blood were withdrawn from the ear vein using an infusion set prior to and at 5, 15, 30, 60, 90 minutes and at 2, 3 , 4 , 4.5 , 5, 5.5 and 6 hours after the injection. All blood samples were allowed to stand over night in the refrigerator. The second day the blood samples were centrifuged to obtain the serum which was kept in the normal freezer until analyzed.

The same five rabbits were subsequently used to study the interaction between the co-administered drug and hydroxyzine one week later to allow for recovery. The rabbits were given the co-administered drug for one week. On the seventh day, each rabbit received an intravenous bolus dose of 2.5 mg hydroxyzine hydrochloride after the dose of the co-administered drug. Blood

samples were obtained prior to and at 5, 15, 30, 60, 90 minutes and at 2, 3, 4, 4.5, 5, 5.5, 6, 8, 10 and 12 hours after injection.

2.2.1.4.1 The effect of cimetidine.

The H_2-receptor antagonist was co-administered in a dose of 100 mg/kg intravenously twice a day for one week.

2.2.1.4.2 The effect of famotidine.

Famotidine, also an H_2-receptor antagonist, was co-administered in a dose of 5 mg/kg intravenously once a day for one week.

2.2.1.4.3 The effect of ranitidine.

Ranitidine was co-administered as H_2-receptor antagonist in a dose of 1 mg/kg intravenously once a day for one week.

2.2.1.4.4 The effect of ketoconazole.

Ketoconazole is not an H_2-receptor antagonist, but a metabolic inhibitor. It was co-administered in a dose of 200 mg orally once a day for one week.

2.2.1.5 The efficacy test of hydroxyzine in rabbits.

The back of the animal was shaved the day before sampling. The depilitory cream was applied to the shaved back, as well as the ears, on the morning of sampling day.

The intradermal test was performed with 0.05 mL of solution containing histamine phosphate, 1.0 mg/mL. A different site on the depilated back of rabbit was used for each test. 0.1 mL of Evans Blue was injected prior to the first test to facilitate the identification of the wheal border. The wheal circumference was traced 10 minutes after each histamine injection and transfered to a transparent paper using a felt-tipped pen. The wheal areas were measured with an IBM-XT compatible digitizer, and stereometric measurement software (Sigma-Scan™,version 3.10, Jandel Scientific, Sausalito, California,U.S.A.).

2.2.2 Pharmacokinetic and pharmacodynamic studies of cetirizine in humans.

2.2.2.1 Extraction procedure of cetirizine.

To 1 mL serum, 50 µl of P_{265} (3 µg/mL), which acts as internal standard were added, together with 1 mL sodium citrate buffer (1M, pH 5.0) and 3 mL of ethyl acetate. The sample was mixed on a vortex mixer for 1 minute and centrifuged for 15 minutes at 2000 rpm. The organic layer was transfered to a clean test tube. To the remaining serum, another 3 mL ethyl acetate were added. The sample was again mixed for 1 minute and centrifuged for 15 minutes at 2000 rpm. The ethyl acetate was mixed with that from the previous extraction. Two hundred microlitres of 1.7% phosphoric acid were added, and the sample was mixed for 1 minute and centrifuged for 5 minutes at 2000 rpm. The aqueous portion was separated from the organic layer which was discarded. The aqueous layer was then exposed to a stream of a dry nitrogen to remove remaining traces of ethyl acetate. The aqueous layer was transfered to the sample tube of the automatic injector, and 150 µl of the solution were injected onto the column.

The extraction procedure for cetirizine from urine was the same as described above, except that 100 µl P265 (3 µg/mL) was added to the urine sample at the beginning of the extraction.

2.2.2.2 Chromatographic separation and quantitation of cetirizine.

The mobile phase used was acetonitrile:phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.9, 0.02M Sodium 1-decanesulfonate), 41:59. The flow rate for separating and quantifying cetirizine was set at 1.1 mL/min. The effluent from the column was monitored by UV absorption at 229 nm with 0.2 to 0.005 a.u.f.s. sensitivity setting. The chart speed for the data module was 0.5 cm/min. Peak height ratio of cetirizine to P_{265} was used for the quantitation to produce a calibration curve. The retention times were 5.5 and 8.0 minutes for cetirizine and P_{265} respectively.

The calibration curves were prepared from the results of assays on blank serum samples to which known quantities of cetirizine and corresponding internal standard P_{265} was added. All chromatographic separations were carried out at ambient temperature. The serum concentrations of cetirizine were expressed in terms of the free base.

The same procedure was used for the determination of cetirizine in urine as for measuring serum cetirizine concentrations, except that the calibration curve was prepared over a much wider range of concentrations.

2.2.2.3 Pharmacokinetic and pharmacodynamic studies of cetirizine in patients with primary biliary cirrhosis.

This study was approved by the Faculty Committee on the Use of Human Subjects in Research of the University of Manitoba. Written, informed consent was obtained from all patients before study entry.

Patients were eligible for study if they had symptomatic, biopsy-compatible primary billiary cirrhosis, with abnormal liver biochemistry tests, defined as alanine aminotransferase at least twice normal, and/or alkaline phosphatase at least 1.5 times normal, and abnormal 6-glutamyl transferase on at least two occasions over the previous 6 months. They were required to be non-smokers, within 10-15% of normal body weight for height, and to have a life expectancy of at least one year. Patients were excluded from the study if they had hepatic failure, ascites, encephalopathy; associated bowel disease, which might impair cetirizine absorption, or known hypersensitivity to any H_1-receptor antagonist. Patients were also excluded if they had taken a

resin-binding agents, such as cholestyramine, within 72 hours, or any other medication within one week, including central nervous system-active medications such as tricyclic antidepressants or H_1-receptor antagonists. Patients were excluded if they had ever taken the long acting H_1-receptor antagonist astemizole.

Before entering the study, the patients had a complete history and physical examination. Assessment of hepatic function (direct and total bilirubin, alanine aminotransferase, alkaline phosphatase, aspartate amino-transferase, 6-glutamyltransferase, lactate dehydrogenase, serum protein, prothrombin time), assessment of renal function (blood urea nitrogen, serum creatinine, creatinine clearance, and urinalysis), and a complete blood count were performed.

On the first day of the study, each patient reported to the Health Sciences Clinical Research Centre at 0800 hours after an 8 hour fast. An indwelling venous catheter with "heparin lock" was inserted. Blood samples of 5 mL for measurement of serum cetirizine concentrations were collected via the "heparin lock" before and at 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours, then by venipuncture at 24, 48, 72, and 96 hours, after administration of a single tablet of cetirizine dihydrochloride, 10 mg, with 250 mL water.

From 0-12 hours post-dose, after blood samples were obtained from the heparin lock, the lock was rinsed with 1.5 mL of 0.9% normal saline, followed by 0.5 mL of heparin lock solution (10 units of heparin/mL saline). The first one mL of blood obtained via the heparin lock was discarded.

Within 2 hours of collection, all blood samples were centrifuged at room temperature at 3700 rpm for 10 minute. The serum was transferred to plastic tubes, which were capped and stored at –20° C.

From 0-96 hours after the cetirizine dose, all urine was collected in 12-hour aliquots. After the volume of each timed aliquot of urine was recorded, each sample was thoroughly mixed, and 10 mL were frozen at – 20° C.

Serum and urine cetirizine concentrations were analyzed using high performance liquid chromatography methods developed previously in our laboratory. The lower limit of sensitivity of HPLC assay for cetirizine was 2.0 ng/mL, with a coefficient of variation of less than 15 % over 6 months.

2.2.2.4 The efficacy test of cetirizine in human subjects.

In order to assess the antihistaminic effect of cetirizine, after each blood sample was collected, an intradermal test with 0.01 mL histamine phosphate, 0.1 mg/mL was performed. A different site on the volar surface of

the forearm was used for each test, and the sequence of testing was identical in all patients. Ten minutes after histamine injection, the wheal and flare circumference was traced with a felt-tip pen and transfered to transparent paper. All injections and tracings were made by the same investigator. Wheal and flare areas were measured using an IBM-PC and digitizer; areas were traced on the digitizer and the areas were calculated directly by stereometric measurement software. Pruritus at the site of histamine injection was scored using the scale: 0 = no itching; 1 = mild, questionable itching; 2 = definite itching; 3 = severe itching.

After each blood sample was obtained, the patients were questioned about any adverse effects; specifically, sleepiness, light-headedness, feelings of disorientation or dizziness, confusion, dry mouth, blurred vision, or difficulty in urinating. If patients fell asleep, the duration of sleep was recorded.

2.3 Data analysis.

2.3.1 Pharmacokinetic data analysis.

Data from the rabbits and human pharmacokinetic studies were analyzed using PKCALC (97) on an IBM-XT compatible program which

performs standard statistical and pharmacokinetic analysis of multisubject data sets, including means, standard deviations, standard errors of variation, half-lives of absorption and elimination, area under the concentration versus time curves, and mean residence time.

PKCALC is linked to an augumented copy of ESTRIP (98),which can strip serum concentration versus time data automatically using different poly exponential equations. Data from each animal and human subject were fitted independently, and models giving the least sum of squared deviations and the best coefficient of determination were selected as the optimal fit. In addition, each fitting was visually inspected to make sure that at least three data points were used in calculating the slope of the terminal linear portion.

In PKCALC, the elimination half-life was calculated using the following equation

$$T_{1/2} = 0.693/\beta$$

where β is the slope of the terminal linear portion in the concentration versus time curve.

The area under the concentration versus time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule from the time 0 to time t of the last sample and extrapolated to time infinity according to the formula:

$$AUC_{0-\infty} = AUC_{0-t} + Ct/\beta$$

where Ct is the concentration of hydroxyzine (cetirizine) in the last sample at time t. Results are expressed in terms of ng.h/mL.

The systemic clearance (Cls) was calculated as the following:

$$Cls = Dose / AUC_{0-\infty}$$

Results were expressed as mL/min.

The renal clearance of cetirizine was calculated by applying the following equation:

$$Clr = (Xu)_{0-24} / AUC_{0-24}$$

where $(Xu)_{0-24}$ is the amount of cetirizine excreted unchanged in urine from 0 to 24 hours, and AUC_{0-24} is the area under the cetirizine concentration versus time curve from 0 to 24 hours. Results were expressed as mL/min/kg.

The apparent volume of distribution for the central compartment, V_B, was calculated as the following:

$$V_B = Cls/\beta$$

Results were expressed as L.

The ratio of cetirizine $AUC_{0-\infty}$ to hydroxyzine $AUC_{0-\infty}$ was calculated as:

R = cetirizine $AUC_{0-\infty}$ / hydroxyzine $AUC_{0-\infty}$.

2.3.2 Pharmacodynamic data analysis.

The histamine-induced wheal and flare areas were analyzed as absolute values. The two-way ANOVA using subject and sample time, or subject and age-related study as the criteria of classification. Differences were considered significant at $p<0.05$.

CHAPTER III

RESULTS

3.1 Pharmacokinetic and pharmacodynamic studies of hydroxyzine in rabbits: the effect of cimetidine.

3.1.1 HPLC assay for hydroxyzine.

Representative HPLC chromatograms for hydroxyzine and its internal standard antazoline are shown in Figure 8. The retention times of hydroxyzine and antazoline are 10.5 and 5.5 minutes respectively. There were no interfering peaks in blank serum with the retention times of hydroxyzine and cetirizine.

3.1.2 Calibration curves for hydroxyzine.

The calibration curves for hydroxyzine were constructed by plotting peak height ratios of hydroxyzine to antazoline versus concentrations of hydroxyzine (ng/mL). Calibration curves were analyzed periodically during the entire study period using concentrations of hydroxyzine from 5 ng/mL to 200 ng/mL. The variability in the calibration curves over a period of 12

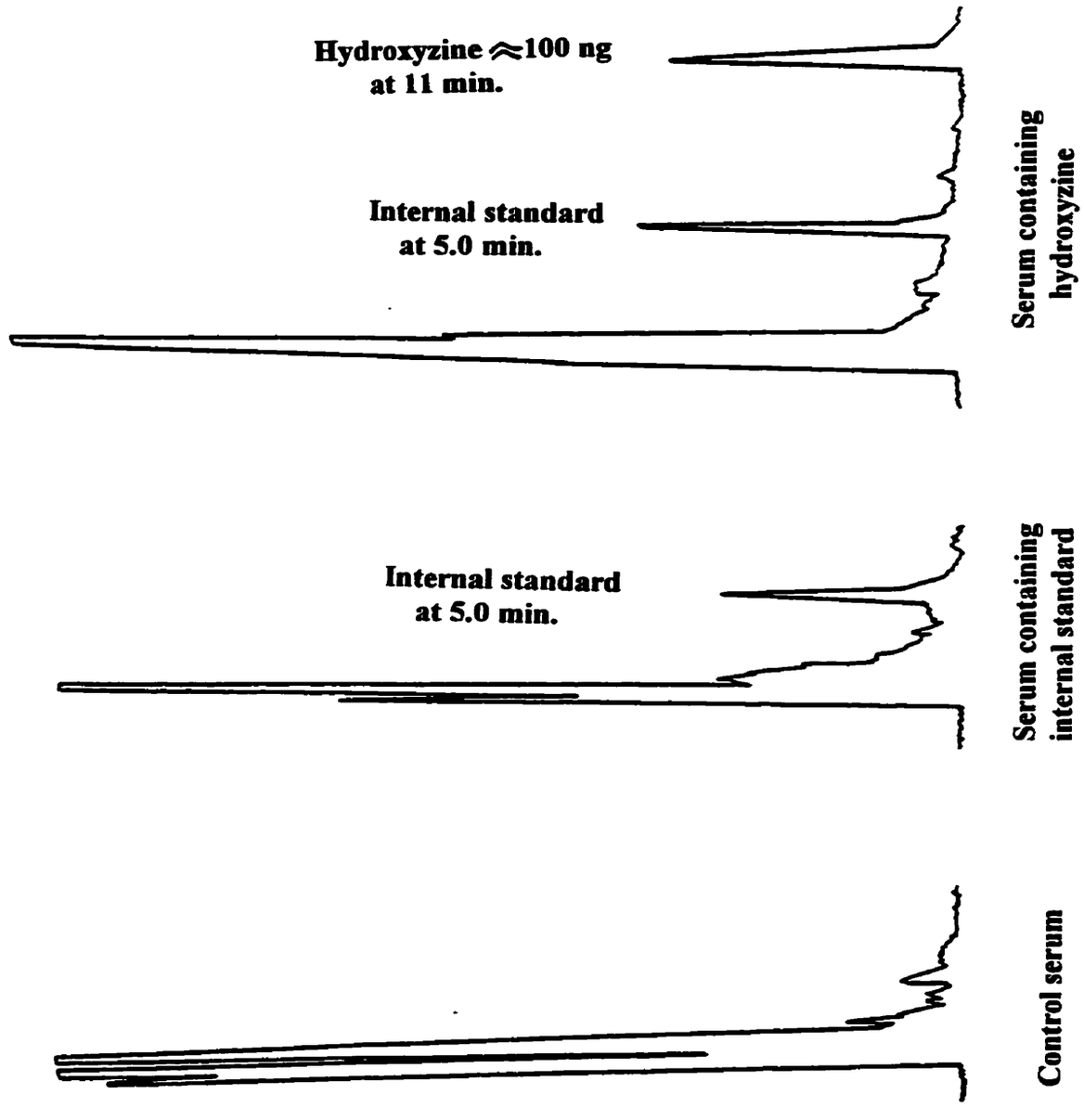


Figure 8. The HPLC chromatograms of serum hydroxyzine.

months was calculated from 9 calibration curves, each having 4 samples at every concentration. The variability is expressed as the coefficient of variation which is shown in Table 1 and the calibration curve is shown in Figure 9.

3.1.3 Serum concentration of hydroxyzine and cetirizine.

Serum concentration and pharmacokinetic parameters of hydroxyzine and cetirizine following intravenous bolus dose of 2.5 mg hydroxyzine two weeks before cimetidine and with cimetidine are listed in Tables 2 to 9. The serum concentrations of hydroxyzine are listed in Tables 2 and 3, the pharmacokinetic parameters of hydroxyzine are listed in Tables 4 and 5, the serum concentrations of cetirizine are listed in Tables 6 and 7, the pharmacokinetic parameters of cetirizine are listed in Tables 8 and 9. The mean serum concentrations are plotted versus time in Figures 10 and 11 respectively.

Table 1. Variability in HPLC calibration curves for serum hydroxyzine.

Concentration ng/mL	Peak height ratio ± SD	C.V. %
5	0.06 ± 0.01	9.1
10	0.10 ± 0.01	6.1
25	0.22 ± 0.01	1.6
50	0.41 ± 0.02	4.2
100	0.85 ± 0.05	5.9
200	1.66 ± 0.04	2.5

Figure 9

CALIBRATION CURVE FOR SERUM HYDROXYZINE

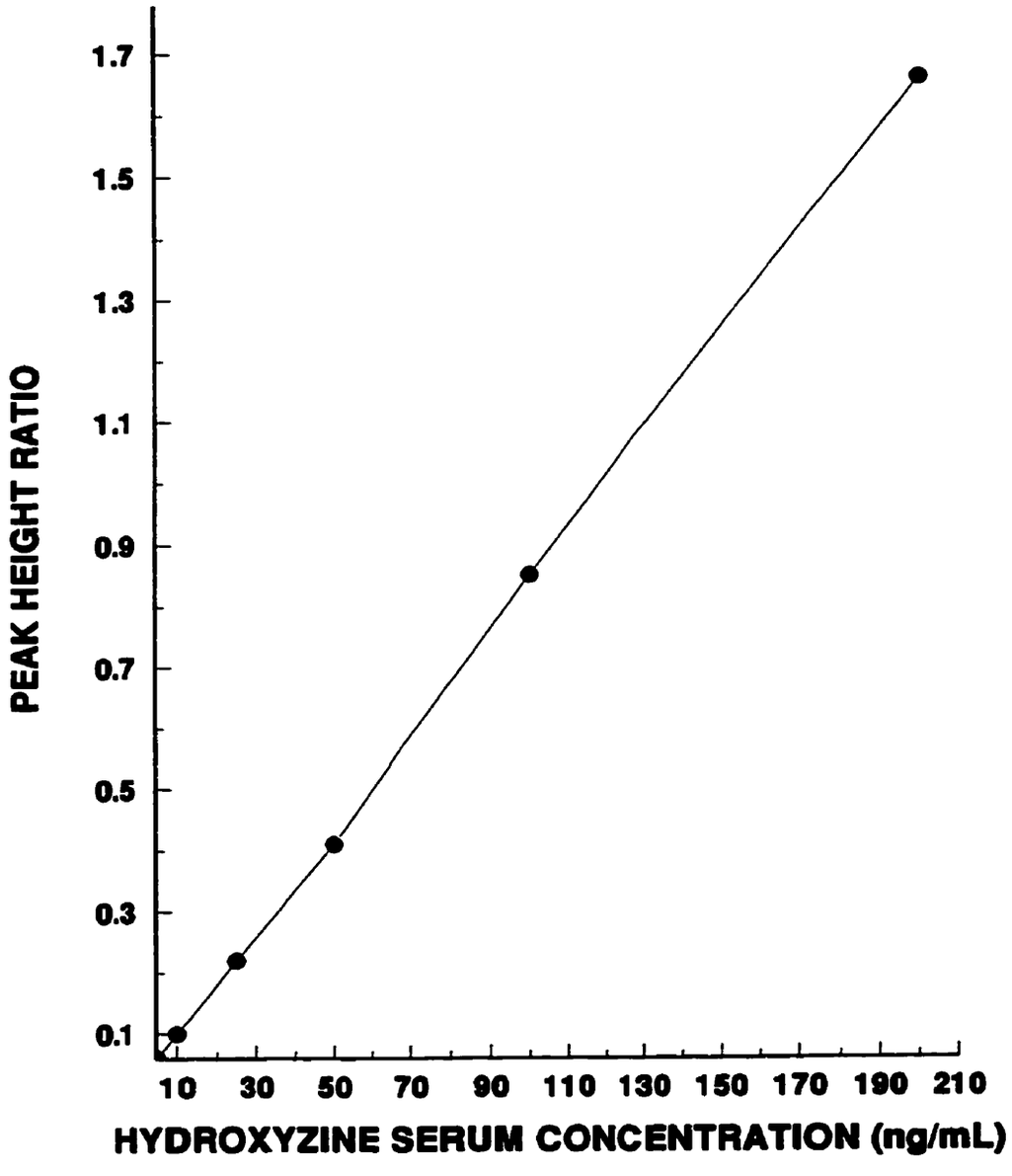


Table 2. The serum concentrations of hydroxyzine after I.V. bolus dose of 2.5 mg of hydroxyzine HCl to 5 rabbits.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	62.4	63.6	65.7	83.6	90.1	73.1	12.9
0.25	47.5	53.0	42.6	66.7	77.4	49.4	28.7
0.5	46.8	49.8	41.5	34.0	103.7	55.1	27.8
1.0	27.3	37.1	30.5	23.0	32.8	30.1	5.3
1.5	22.0	34.9	24.0	19.7	29.1	25.9	6.1
2.0	17.7	32.8	15.1	12.6	24.4	20.5	8.2
3.0	13.5	30.7	14.0	14.2	22.2	18.9	7.5
4.0	8.4	26.5	7.4	10.8	19.6	14.5	8.2
4.5	6.3	NS	4.1	7.7	14.3	8.1	4.4
5.0	5.0	14.8	0.0	6.7	12.7	7.9	5.9
5.5	0.0	9.5	0.0	6.3	11.9	5.5	5.4
6.0	0.0	6.3	0.0	6.2	7.6	4.0	3.7

* NS = No sample.

Table 3. Hydroxyzine serum concentrations after I.V. dose of 2.5 mg hydroxyzine HCl with co-administration of cimetidine 100 mg/kg for 7 days.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	137.3	182.8	154.0	152.6	154.0	155.9	16.1
0.25	77.5	167.9	133.1	123.4	133.1	127.0	32.4
0.5	66.4	140.1	112.3	106.7	122.0	109.5	27.2
1.0	56.7	112.3	108.1	99.8	99.8	95.3	22.3
1.5	47.0	105.3	105.3	91.4	92.8	88.4	24.1
2.0	41.4	98.4	97.0	87.2	88.8	82.5	23.5
3.0	30.3	91.4	85.9	69.2	85.8	72.5	25.1
4.0	15.1	77.5	80.3	63.6	80.3	63.4	27.9
4.5	12.2	66.4	66.4	52.5	74.7	54.4	25.0
5.0	9.4	49.7	58.0	45.5	69.2	46.4	22.6
5.5	8.0	44.2	51.1	39.6	53.9	39.1	18.4
6.0	6.6	37.2	30.3	30.3	49.7	30.8	15.7
8.0	3.8	29.0	27.5	26.1	35.8	24.4	12.1
10.0	3.8	21.9	21.9	16.3	21.9	17.2	7.9
12.0	3.8	10.8	13.6	8.0	16.3	10.5	4.9

Table 4. The pharmacokinetic parameters of hydroxyzine after I.V. dose of 2.5 mg hydroxyzine.

Subject	$CP_{5\ min}$ (ng/mL)	$T_{1/2}$ (h)	VD (L)	CL (mL/min)	AUC ng-h/mL
1	62.4	1.7	53.5	232.1	107.7
2	63.6	2.1	40.6	135.0	185.1
3	65.7	1.3	50.3	253.2	98.7
4	86.6	1.9	70.8	205.4	121.7
5	90.1	2.1	46.5	130.6	191.4
Mean	73.7	1.8	52.3	191.3	140.9
S.D.	13.5	0.3	11.4	56.0	44.0

Table 5. The pharmacokinetic parameters of hydroxyzine after I.V. dose of 2.5 mg hydroxyzine with co-administration of cimetidine.

Subject	$CP_{5\ min}$ (ng/mL	$T_{1/2}$ (h)	VD (L)	CL (mL/min)	AUC ng-h/mL
1	137.1	2.8	35.0	182.5	228.3
2	182.0	3.5	16.3	56.9	732.5
3	154.1	5.2	18.1	58.1	717.7
4	153.1	3.5	19.5	69.5	599.3
5	154.0	4.1	18.7	52.6	792.1
Mean	156.1	3.4	21.5	83.9	613.9
S.D.	16.0	0.5	7.6	55.5	226.6

Table 6. Cetirizine serum concentrations after I.V. bolus dose of 2.5 mg hydroxyzine HCl to 5 rabbits.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	6.6	8.9	7.2	14.9	10.9	9.7	3.3
0.25	8.9	9.8	10.6	16.6	12.3	11.6	3.0
0.5	17.4	15.7	11.5	22.5	19.9	17.4	4.2
1.0	25.5	24.2	17.4	24.2	28.4	23.9	4.0
1.5	30.8	20.8	16.6	19.1	25.9	22.6	5.7
2.0	15.7	16.6	14.0	15.7	19.9	16.4	2.2
3.0	14.9	14.0	13.2	14.9	13.2	14.0	0.8
4.0	14.0	13.2	11.5	12.3	11.5	12.5	1.1
4.5	11.5	NS	10.6	11.5	10.6	11.0	0.5
5.0	8.9	8.9	8.1	10.6	8.9	9.1	0.9
5.5	6.4	5.5	5.5	8.9	7.2	6.7	1.4
6.0	4.7	5.0	4.7	8.1	6.4	5.7	1.5

* NS = no sample.

Table 7. Cetirizine serum concentrations after I.V. bolus dose of hydroxyzine 2.5 mg with co-administration of cimetidine 100 mg/kg for 7 days.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	24.9	24.0	27.5	25.8	24.1	25.3	1.4
0.25	39.6	36.1	32.7	29.2	27.4	33.0	5.0
0.5	46.5	44.7	36.2	33.5	30.1	38.2	7.1
1.0	59.4	56.0	41.3	37.8	36.1	46.1	10.8
1.5	59.4	59.4	53.4	49.9	43.0	53.0	7.0
2.0	61.1	62.0	60.3	57.7	55.9	59.4	2.5
3.0	55.9	55.1	56.8	54.2	53.4	55.1	1.4
4.0	30.9	37.8	47.3	44.7	47.3	41.6	7.1
4.5	30.9	30.1	41.3	38.7	43.0	36.8	6.0
5.0	24.9	27.5	32.7	34.4	32.7	30.4	4.0
5.5	18.0	19.7	26.6	27.5	25.8	23.5	4.3
6.0	15.4	17.1	21.4	10.6	18.9	18.7	2.5
8.0	13.7	14.5	18.0	17.1	14.5	15.6	1.9
10.0	9.4	11.1	15.4	12.8	12.0	12.1	2.2
12.0	5.9	6.8	10.2	7.6	9.4	8.0	1.8

Table 8. The pharmacokinetic parameters of cetirizine after I.V. dose of 2.5 mg hydroxyzine.

Subject	Weight (kg)	Cp_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ng-h/mL	R
1	5.0	30.8	1.5	1.1	88.8	0.8
2	4.5	24.2	1.0	1.2	81.4	0.4
3	4.5	17.4	1.0	1.4	69.7	0.7
4	5.0	24.2	1.0	2.8	88.2	0.7
5	5.0	28.4	1.0	2.1	89.8	0.5
Mean	4.8	25.0	1.1	1.7	83.6	0.6
S.D.	0.2	5.1	0.2	0.7	8.4	0.2

Table 9. Cetirizine pharmacokinetic parameters after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of cimetidine.

Subject	Weight (kg)	CP_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ng-h/mL	R
1	5.0	61.1	2.0	3.9	320.3	1.4
2	4.5	61.9	2.0	4.5	332.1	0.5
3	4.5	55.9	2.0	4.9	326.0	0.5
4	5.0	60.3	2.0	3.7	361.6	0.6
5	5.0	57.7	2.0	4.2	339.3	0.4
Mean	4.8	59.4	2.0	3.3	335.9	0.7
S.D.	0.2	2.5	-----	0.3	16.0	0.4

Figure 10

THE MEAN SERUM CONCENTRATIONS OF HYDROXYZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF CIMETIDINE.

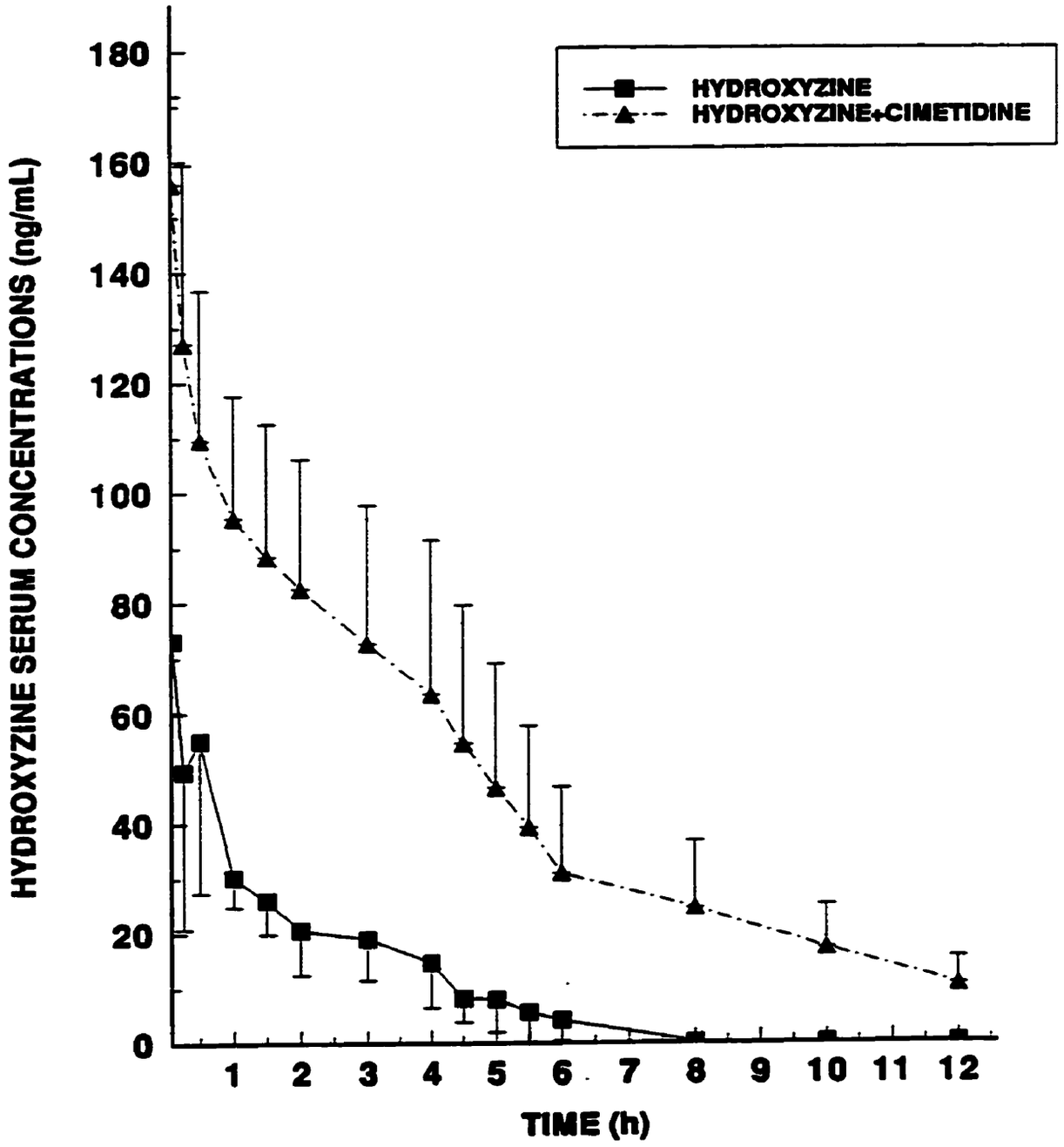
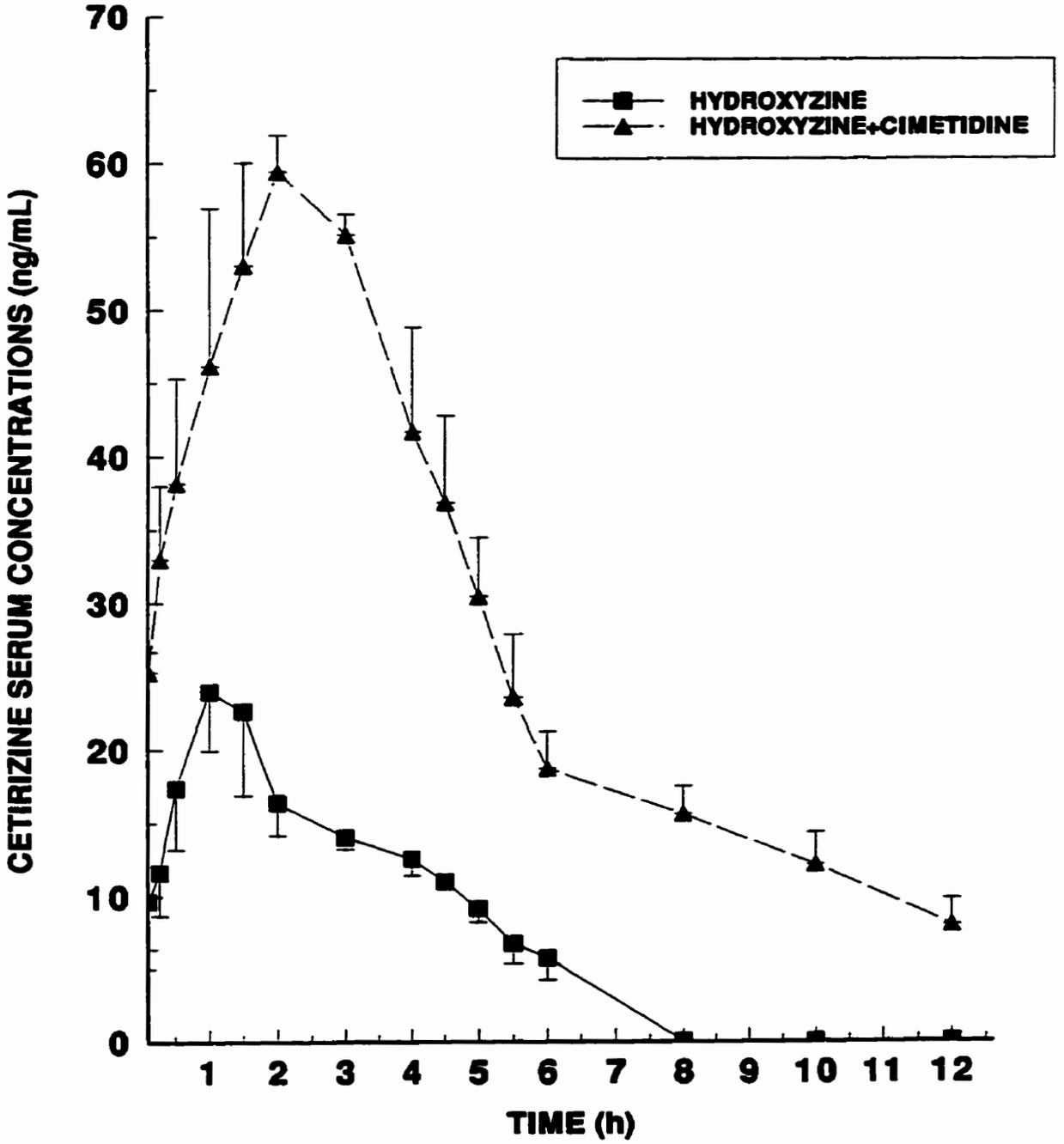


Figure 11

THE MEAN SERUM CONCENTRATIONS OF CETIRIZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF CIMETIDINE.



3.1.4 Pharmacodynamic studies of hydroxyzine.

The efficacy tests are calculated as absolute values of wheal area induced by intradermal injection of histamine phosphate after intravenous bolus injection of 2.5 mg hydroxyzine in rabbits with and without co-administration of cimetidine (100 mg/kg).

Results are listed in Tables 10, 11. Mean wheal area is plotted versus time in Figure 12.

3.2 Pharmacokinetic and pharmacodynamic studies of hydroxyzine in rabbits: the effect of famotidine.

3.2.1 Serum concentration of hydroxyzine and cetirizine.

Serum concentrations and pharmacokinetic parameters of hydroxyzine and cetirizine following the intravenous bolus dose of 2.5 mg hydroxyzine two weeks before famotidine, and with famotidine are listed in Tables 12 to 19 . The serum concentrations of hydroxyzine are listed in Tables 12 and 13, the pharmacokinetic parameters of hydroxyzine are listed in Tables 14 and 15, the serum concentration of cetirizine are listed in Tables 16 and 17, the pharmacokinetic parameters of cetirizine are listed in Tables 18 and 19. The mean serum concentrations are plotted versus time in Figures 13 and 14.

Table 10. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.00	1.31	1.70	1.23	1.01	0.99	1.25	0.29
0.08	0.78	0.70	0.85	0.78	0.70	0.76	0.06
0.25	0.69	0.74	0.69	0.68	0.70	0.72	0.04
0.5	0.74	0.57	0.78	0.51	0.54	0.63	0.12
1.0	0.64	0.68	0.69	0.62	0.70	0.67	0.03
1.5	0.74	0.67	0.51	0.68	0.53	0.63	0.09
2.0	0.51	0.58	0.56	0.49	0.55	0.54	0.03
3.0	0.49	0.52	0.50	0.51	0.52	0.51	0.01
4.0	0.51	0.44	0.47	0.42	0.59	0.49	0.06
4.5	0.41	0.49	0.42	0.41	0.46	0.44	0.03
5.0	0.29	0.44	0.42	0.28	0.46	0.38	0.08
5.5	0.46	0.40	0.49	0.46	0.49	0.46	0.04
6.0	0.56	0.51	0.55	0.50	0.56	0.54	0.03
12.0	0.79	0.66	0.86	0.92	0.82	0.81	0.09
24.0	0.96	0.80	0.96	0.89	0.94	0.91	0.07

Table 11. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of hydroxyzine with co-administration of cimetidine.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.0	0.88	0.90	1.19	0.85	0.81	0.93	0.15
0.08	0.42	0.44	0.47	0.44	0.36	0.44	0.05
0.25	0.15	0.32	0.39	0.37	0.38	0.32	0.10
0.5	0.15	0.23	0.21	0.55	0.35	0.30	0.16
1.0	0.07	0.19	0.19	0.18	0.29	0.19	0.08
1.5	0.36	0.18	0.25	0.19	0.32	0.26	0.08
2.0	0.12	0.16	0.05	0.06	0.19	0.11	0.06
3.0	0.16	0.08	0.09	0.13	0.19	0.13	0.05
4.0	0.08	0.07	0.05	0.03	0.35	0.12	0.13
4.5	0.06	0.11	0.10	0.06	0.25	0.12	0.08
5.0	0.06	0.06	0.06	0.23	0.26	0.14	0.10
5.5	0.17	0.19	0.26	0.07	0.39	0.22	0.12
6.0	0.21	0.21	0.21	0.08	0.35	0.21	0.09
12.0	0.59	0.52	0.54	0.03	0.32	0.34	0.22
24.0	0.69	0.80	0.69	0.79	0.61	0.72	0.08

Figure 12

THE MEAN SUPPRESSION OF WHEALS INDUCED BY INTRADERMALLY INJECTED HISTAMINE AFTER IV DOSE OF HYDROXYZINE 2.5MG WITH CO-ADMINISTRATION OF CIMETIDINE

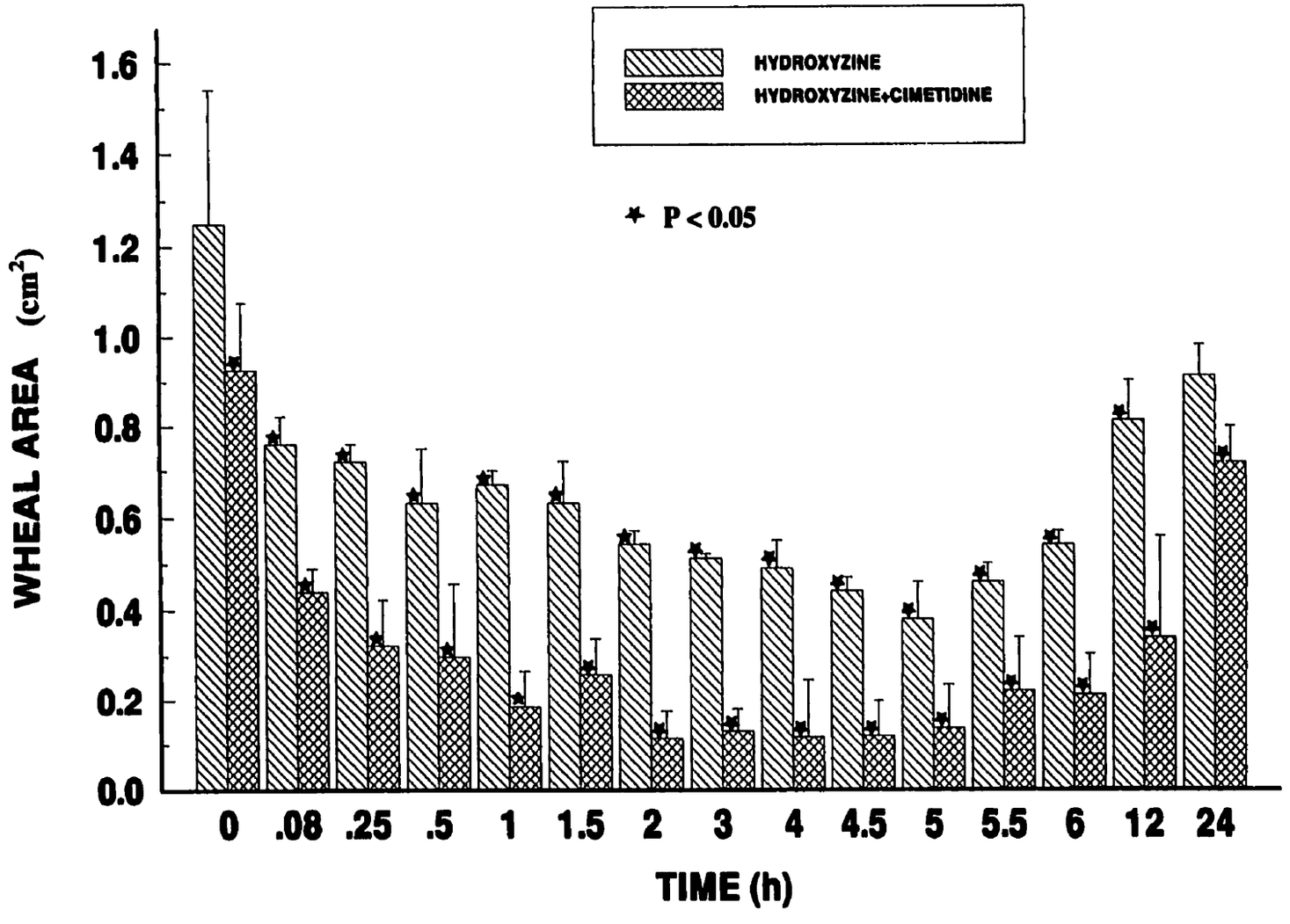


Table 12. The serum concentrations of hydroxyzine after I.V. bolus dose of 2.5 mg hydroxyzine.

Sub. / Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	176.1	194.7	165.1	145.2	110.5	158.3	32.2
0.25	126.2	145.2	125.4	120.5	61.1	115.6	31.9
0.5	72.1	95.7	95.7	58.5	36.3	71.6	25.4
1.0	57.7	70.9	93.2	43.7	21.4	57.2	27.1
1.5	37.8	36.3	67.2	19.4	7.1	33.3	22.6
2.0	21.8	26.4	44.2	6.5	2.6	20.3	16.7
3.0	6.9	21.4	39.2	4.6	1.6	14.7	15.7
4.0	5.4	11.5	27.3	3.6	NS	11.9	10.7
4.5	3.4	4.6	22.4	1.6	0.0	6.4	9.1
5.0	1.9	3.1	16.4	0.0	0.0	4.3	6.6
5.5	0.0	0.0	11.1	0.0	0.0	2.2	4.7
6.0	0.0	0.0	2.8	0.0	0.0	0.6	1.2

* NS: No Sample

Table 13. The serum concentrations of hydroxyzine after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of famotidine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	165.0	111.5	150.2	118.0	115.1	131.7	24.3
0.25	90.7	66.1	98.2	62.5	63.6	76.2	16.9
0.5	70.9	26.4	73.9	46.2	47.1	52.5	19.6
1.0	32.8	21.4	51.6	31.3	32.0	33.8	10.9
1.5	28.8	9.1	46.2	25.4	26.5	27.2	13.1
2.0	21.4	5.6	40.3	23.9	24.6	23.1	12.3
3.0	9.0	3.1	32.8	15.2	16.3	15.3	11.1
4.0	2.6	2.1	31.3	13.0	14.0	12.6	11.8
4.5	2.1	0.0	34.3	7.5	8.6	10.5	13.7
5.0	0.0	0.0	16.5	7.5	7.5	6.3	6.8
5.5	0.0	0.0	11.5	0.0	0.0	2.3	5.1
6.0	0.0	0.0	6.5	0.0	0.0	1.3	2.9

Table 14. The pharmacokinetic parameters of hydroxyzine after I.V. bolus dose of 2.5 mg hydroxyzine.

Subject	$CP_{5\ min}$ (ng/mL)	$T_{1/2}$ (h)	VD (L)	CL (mL/min)	AUC ng-h/mL
1	176.2	0.9	20.0	284.0	144.0
2	194.1	1.0	18.0	212.0	190.0
3	165.0	1.4	18.9	144.0	275.0
4	145.2	1.4	25.6	388.0	103.0
5	110.0	0.5	31.8	780.0	53.0
Mean	158.1	1.0	22.9	362.6	153.0
S.D.	31.9	0.4	5.8	250.3	85.4

Table 15. The pharmacokinetic parameters of hydroxyzine after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of famotidine.

Subject	CP_{5min} (ng/mL)	$T_{1/2}$ (h)	VD (L)	CL (mL/min)	AUC ng-h/mL
1	165.0	0.8	24.3	347.0	118.0
2	111.0	0.9	43.7	710.0	56.0
3	150.0	2.0	28.7	159.0	229.0
4	118.0	1.8	24.7	306.0	117.0
5	115.0	1.9	45.3	293.0	121.0
Mean	131.8	1.5	37.5	363.0	128.2
S.D.	24.1	0.6	10.2	206.5	52.3

Table 16. Cetirizine serum concentrations after an I.V. bolus dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	9.1	10.3	17.9	11.5	6.8	11.1	4.2
0.25	14.8	17.9	26.8	15.4	14.5	17.9	5.2
0.5	35.5	20.1	29.8	32.5	29.6	29.5	5.8
1.0	12.1	18.6	26.5	16.1	12.7	17.2	5.8
1.5	10.2	17.6	25.8	13.8	12.3	15.9	6.1
2.0	9.4	16.5	23.7	10.9	11.8	14.4	5.8
3.0	8.6	15.1	22.5	7.3	10.1	12.7	6.2
4.0	8.2	13.6	19.7	6.9	7.9	11.2	5.4
4.5	6.8	11.4	17.8	6.8	6.8	9.9	4.8
5.0	0.0	11.1	16.5	6.7	6.7	8.2	6.1
5.5	0.0	10.3	13.2	6.4	6.1	7.2	4.9
6.0	0.0	7.4	11.8	6.3	4.7	6.0	4.3

Table 17. Cetirizine serum concentration after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of famotidine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	11.8	24.0	24.0	18.4	17.9	18.9	5.4
0.25	14.1	31.2	32.2	22.1	24.0	24.7	7.4
0.5	18.2	36.5	35.5	24.0	25.8	27.9	7.9
1.0	21.8	33.4	29.3	NS	25.2	25.7	7.9
1.5	11.1	31.2	25.3	18.4	24.5	22.1	7.6
2.0	11.3	28.5	24.0	17.9	24.0	21.1	6.6
3.0	9.1	25.3	17.2	17.8	23.4	18.5	6.3
4.0	8.6	23.0	13.3	17.2	19.7	16.4	5.6
4.5	7.5	21.6	11.9	15.4	18.1	14.9	5.5
5.0	7.3	15.9	10.0	14.4	17.9	13.1	4.3
5.5	4.8	7.2	7.6	12.9	14.8	9.4	4.2
6.0	0.0	NS	6.5	0.0	11.6	4.5	5.6

NS : No sample.

Table 18. The pharmacokinetic parameters of cetirizine after I.V. dose of 2.5 mg hydroxyzine.

Subject	Weight (kg)	C_{max} (ng/mL)	T_{max} (h)	T 1/2 (h)	AUC ng-h/mL	R
1	5.0	35.5	1.0	3.8	114.5	0.8
2	3.0	20.1	0.5	2.6	85.4	0.4
3	4.0	29.5	0.5	3.0	126.9	0.5
4	5.0	32.5	0.5	2.1	57.7	0.6
5	4.0	29.6	0.3	3.6	109.0	2.0
Mean	4.2	29.5	0.6	3.0	98.7	0.9
S.D.	0.8	5.8	0.3	0.7	27.4	0.7

Table 19. The pharmacokinetic parameters of cetirizine after I.V. dose of 2.5 mg hydroxyzine with co-administration of famotidine.

Subject	Weight (kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ng-h/mL	R
1	5.0	14.8	1.0	3.6	54.8	0.5
2	3.0	36.5	0.5	2.7	132.2	2.3
3	4.0	35.5	0.5	2.1	110.9	0.7
4	5.0	24.0	0.5	3.4	98.5	0.8
5	4.0	25.8	0.5	4.1	125.6	1.0
Mean	4.2	27.3	0.6	3.2	104.4	1.1
S.D.	0.83	8.9	0.22	0.8	30.7	0.7

Figure 13

THE MEAN SERUM CONCENTRATIONS OF HYDROXYZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF FAMOTIDINE

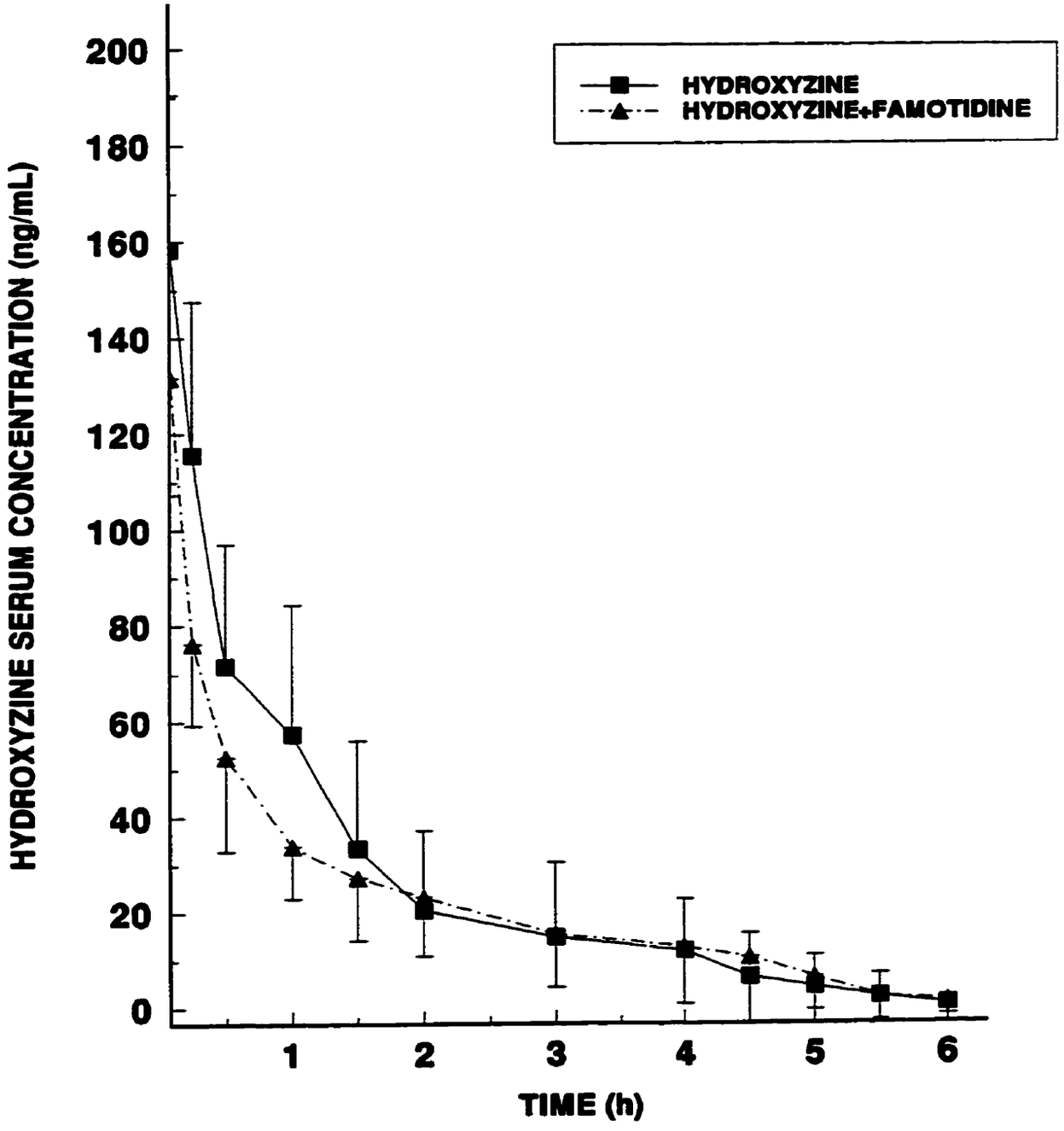
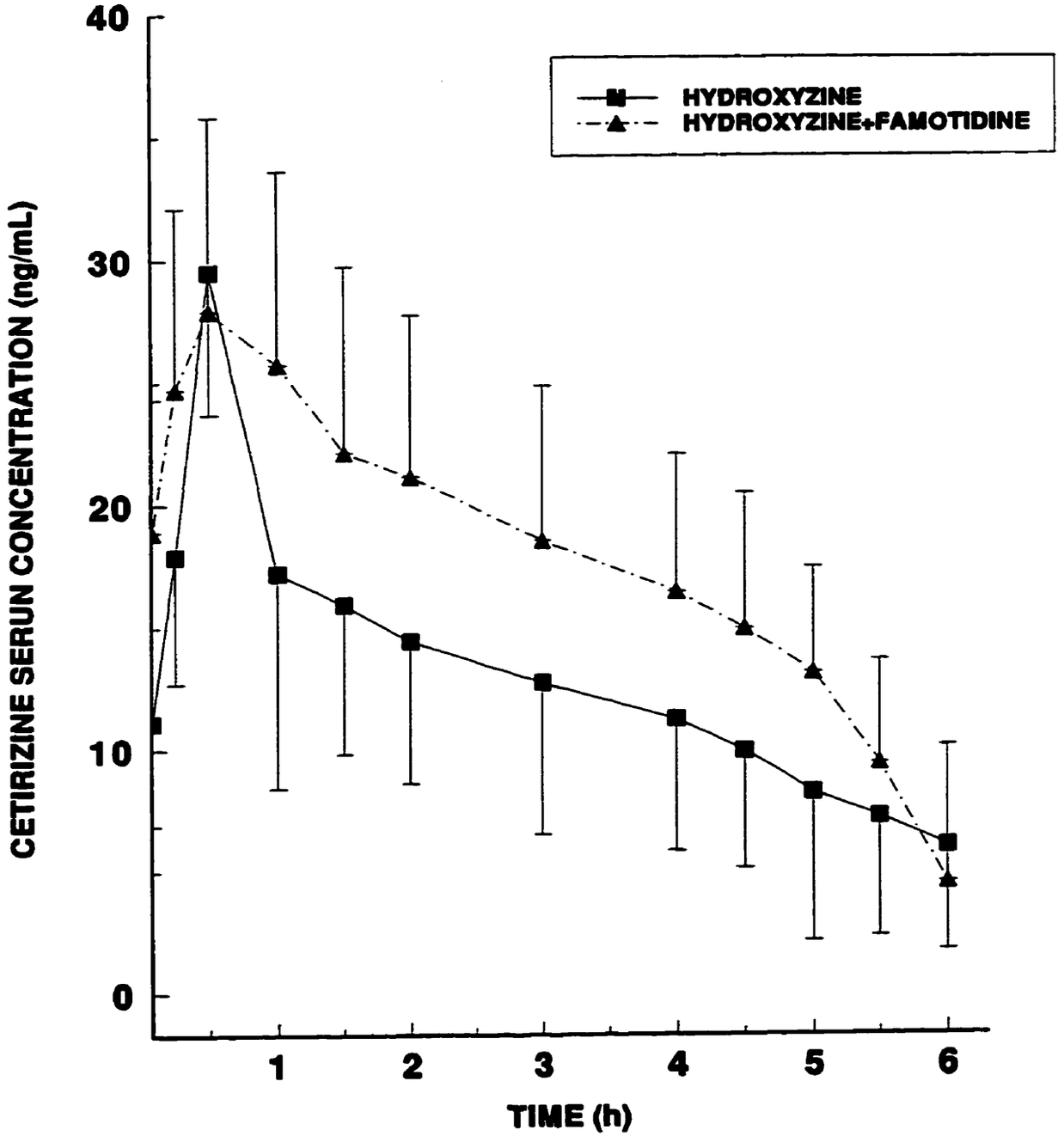


Figure 14

THE MEAN SERUM CONCENTRATIONS OF CETIRIZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF FAMOTIDINE



3.2.2 Pharmacodynamic studies of hydroxyzine.

The efficacy tests are calculated as absolute values of wheal areas induced by intradermally injected histamine, 0.05 mL of 1.0 mg/ml after intravenous bolus injection of 2.5 mg hydroxyzine in rabbits with and without co-administration of famotidine (5mg/kg). Results are listed in Tables 20 and 21. Mean wheal area is plotted versus time in Figure 15.

3.3 Pharmacokinetic and pharmacodynamic studies of hydroxyzine in rabbits: the effect of ranitidine.

3.3.1 Serum concentration of hydroxyzine and cetirizine.

Serum concentrations and pharmacokinetic parameters of hydroxyzine and cetirizine following the intravenous bolus dose of 2.5 mg hydroxyzine two weeks before ranitidine, with ranitidine are listed from Tables 22 to 29. The serum concentrations of hydroxyzine are listed in Tables 22 and 23, the pharmacokinetic parameters are listed in Tables 24 and 25, the serum concentrations of cetirizine are listed in Tables 26 and 27, the pharmacokinetic parameters are listed in Tables 28 and 29. The mean serum concentrations are plotted versus time in Figures 16 and 17.

Table 20. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.0	0.80	0.97	0.94	0.89	0.95	0.91	0.07
0.08	0.54	0.49	0.62	0.45	0.55	0.53	0.06
0.25	0.33	0.05	0.36	0.41	0.40	0.31	0.15
0.5	0.09	0.11	0.17	0.17	0.54	0.22	0.18
1.0	0.09	0.35	0.14	0.26	0.07	0.18	0.12
1.5	0.09	0.06	0.10	0.41	0.19	0.17	0.14
2.0	0.06	0.11	0.03	0.18	0.35	0.15	0.13
3.0	0.09	0.09	0.31	0.07	0.13	0.14	0.10
4.0	0.20	0.13	0.11	0.17	0.13	0.15	0.04
4.5	0.38	0.04	0.25	0.09	0.09	0.17	0.10
5.0	0.21	0.13	0.08	0.25	0.37	0.21	0.10
5.5	0.40	0.12	0.14	0.21	0.37	0.25	0.08
6.0	0.51	0.11	0.14	0.13	0.54	0.28	0.02
12.0	0.53	0.75	0.43	0.47	0.54	0.54	0.11
24.0	0.62	0.62	0.70	0.80	0.60	0.67	0.08

Table 21. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of 2.5 mg hydroxyzine with co-administration of famotidine.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.0	0.58	0.83	0.74	0.68	0.62	0.69	0.09
0.08	0.44	0.25	0.34	0.14	0.18	0.33	0.11
0.25	0.45	0.07	0.23	0.47	0.25	0.30	0.16
0.5	0.18	0.15	0.19	0.18	0.33	0.21	0.07
1.0	0.19	0.18	0.28	0.06	0.37	0.22	0.11
1.5	0.27	0.08	0.19	0.06	0.16	0.15	0.08
2.0	0.19	0.07	0.05	0.06	0.38	0.15	0.14
3.0	0.11	0.04	0.02	0.02	0.02	0.04	0.03
4.0	0.08	0.0	0.0	0.04	0.05	0.03	0.03
4.5	0.0	0.0	0.0	0.05	0.04	0.02	0.02
5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.5	0.06	0.01	0.07	0.05	0.04	0.05	0.02
6.0	0.17	0.10	0.08	0.23	0.08	0.13	0.06
12.0	0.37	0.41	0.39	0.41	0.33	0.38	0.03
24.0	0.40	0.58	0.54	0.60	0.53	0.53	0.08

Figure 15

THE MEAN SUPPRESSION OF WHEALS INDUCED BY INTRADERMALLY INJECTED HISTAMINE AFTER IV DOSE OF HYDROXYZINE 2.5 MG WITH CO-ADMINISTRATION OF FAMOTIDINE

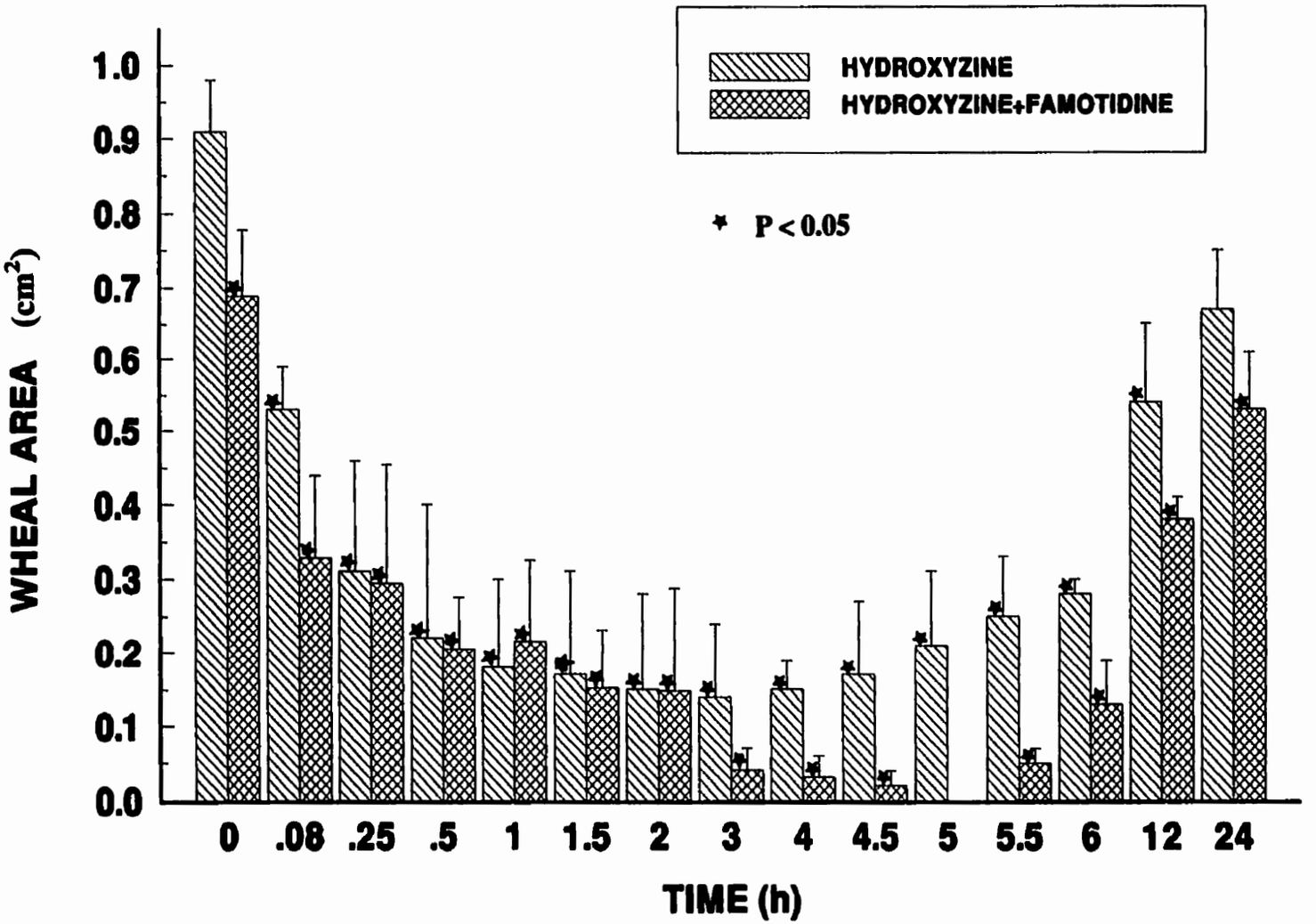


Table 22. The serum concentrations of hydroxyzine after I.V. dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	135.5	188.2	140.3	188.2	165.2	163.5	25.2
0.25	81.9	135.5	93.0	170.5	145.9	125.4	37.1
0.5	58.9	121.2	68.1	140.2	99.3	97.5	34.4
1.0	39.8	92.5	45.1	100.5	70.2	69.7	27.2
1.5	20.7	70.9	39.8	70.9	39.8	48.4	21.9
2.0	8.7	45.1	29.8	55.1	29.8	33.7	17.6
3.0	5.9	40.3	30.3	30.3	15.3	24.4	13.7
4.0	3.5	32.7	25.5	10.6	11.5	16.8	11.9
4.5	3.5	23.1	11.1	8.1	8.1	10.8	7.4
5.0	0.0	11.6	4.5	5.1	7.5	5.7	4.3
5.5	0.0	4.4	0.0	4.4	5.6	2.9	2.7
6.0	0.0	0.0	0.0	0.0	4.1	0.8	1.8

Table 23. The serum concentrations of hydroxyzine after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of ranitidine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	116.4	142.7	116.4	92.5	142.7	122.1	21.1
0.25	57.1	94.9	56.2	72.4	114.7	79.1	25.3
0.5	48.9	61.4	40.0	66.6	94.9	62.4	21.0
1.0	38.0	57.5	27.1	43.2	65.4	46.2	15.3
1.5	15.4	44.6	20.4	20.7	45.6	29.3	14.5
2.0	11.1	35.5	15.4	15.9	35.5	22.7	11.8
3.0	8.7	30.7	9.6	11.1	23.6	16.7	9.9
4.0	3.9	23.6	7.8	6.3	10.1	10.3	7.7
4.5	3.0	11.1	6.5	4.0	6.1	6.1	3.1
5.0	0.0	3.9	4.9	0.0	5.9	2.9	2.8
5.5	0.0	0.0	4.5	0.0	4.5	1.8	2.4
6.0	0.0	0.0	4.0	0.0	0.0	0.8	1.7

Table 24. The pharmacokinetic parameters of hydroxyzine after I.V. bolus dose of 2.5 mg hydroxyzine.

Subject	$CP_{5\ min}$ (ng/mL)	$T_{1/2}$ (h)	V.D. (L)	CL (mL/min)	AUC ng-h/mL
1	135.5	0.6	26.3	430.0	97.0
2	188.2	1.1	16.0	141.0	289.0
3	140.0	0.8	25.1	234.0	177.0
4	188.2	0.9	13.2	147.0	277.0
5	165.2	1.1	21.5	203.0	195.0
Mean	163.0	0.9	20.4	231.0	207.0
S.D.	25.3	0.2	5.7	117.1	78.3

Table 25. The pharmacokinetic parameters of hydroxyzine after I.V. dose of 2.5 mg with co-administration of ranitidine.

Subject	$CP_{5\ min}$ (ng/mL)	$T_{1/2}$ (h)	V.D. (L)	CL (mL/min)	AUC ng-h/mL
1	116.4	1.0	37.1	459.0	87.0
2	142.7	1.1	24.8	214.0	188.0
3	116.4	1.4	58.2	382.0	96.0
4	92.5	0.7	29.6	378.0	109.0
5	142.7	1.1	20.8	209.0	192.0
Mean	102.1	1.1	34.1	328.4	134.4
S.D.	52.0	0.3	14.8	111.2	51.6

Table 26. Cetirizine serum concentrations after an I.V. bolus dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	14.5	8.1	8.8	10.9	15.1	11.5	3.2
0.25	28.8	13.9	15.7	13.7	29.3	20.3	8.0
0.5	25.8	34.9	31.6	27.6	31.1	30.2	3.6
1.0	22.7	11.9	29.8	15.8	25.7	21.1	7.2
1.5	20.7	10.0	21.5	12.9	20.2	17.1	5.2
2.0	19.7	9.1	17.6	11.0	15.5	14.6	4.4
3.0	18.7	8.7	15.1	8.8	11.9	12.6	4.3
4.0	17.6	8.6	11.5	7.5	10.7	11.2	4.0
4.5	16.5	8.3	10.1	6.9	8.1	10.0	3.8
5.0	15.2	6.1	8.1	7.4	7.7	8.9	3.6
5.5	13.5	6.0	6.9	7.0	6.6	7.9	3.1
6.0	9.2	4.5	6.3	6.2	4.3	6.1	1.9

Table 27. Cetirizine serum concentrations after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of ranitidine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	23.1	11.5	24.0	10.7	24.0	18.7	6.9
0.25	31.3	13.6	31.2	15.2	31.2	24.5	9.3
0.5	34.4	14.3	35.5	20.5	36.5	28.3	10.1
1.0	29.3	13.5	29.3	14.2	29.3	23.1	8.4
1.5	25.3	13.3	25.3	13.0	25.3	20.4	6.7
2.0	24.5	NS	23.1	12.9	24.0	21.1	5.5
3.0	16.2	12.1	18.2	12.6	18.1	15.4	2.9
4.0	13.3	11.1	14.2	12.3	14.2	13.0	1.3
4.5	11.8	10.3	12.6	10.6	12.9	11.6	1.2
5.0	10.1	9.3	12.0	9.4	11.1	10.4	1.2
5.5	7.6	8.9	9.5	8.8	8.5	8.6	0.7
6.0	6.1	6.3	7.5	5.2	6.5	6.3	0.8

Table 28. The pharmacokinetic parameters of cetirizine after I.V. bolus dose of 2.5 mg hydroxyzine.

Subject	Weight (kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ng-h/mL	R
1	4.0	28.8	0.3	2.3	110.1	1.1
2	4.4	34.9	0.5	2.9	60.9	0.2
3	4.5	31.6	0.5	2.5	92.5	0.5
4	5.0	27.6	0.5	3.1	63.3	0.2
5	5.0	31.1	0.5	2.1	85.8	0.4
Mean	4.6	30.8	0.5	2.6	82.5	0.5
S.D.	0.4	2.8	0.1	0.4	20.7	0.4

Table 29. The pharmacokinetic parameters of cetirizine after I.V.bolus dose of 2.5 mg hydroxyzine with co-administration of ranitidine.

Subject	Weight (kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ng-h/mL	R
1	3.5	34.4	0.5	2.3	119.6	1.3
2	4.0	14.3	0.5	3.5	68.4	0.4
3	5.0	35.5	0.5	2.6	114.1	1.2
4	4.5	15.2	0.3	3.1	70.5	0.6
5	4.0	36.5	0.5	2.4	114.3	0.6
Mean	4.2	27.2	0.5	2.8	95.4	0.8
S.D.	0.6	11.3	0.1	0.5	23.8	0.4

Figure 16

THE MEAN SERUM CONCENTRATIONS OF HYDROXYZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF RANITIDINE.

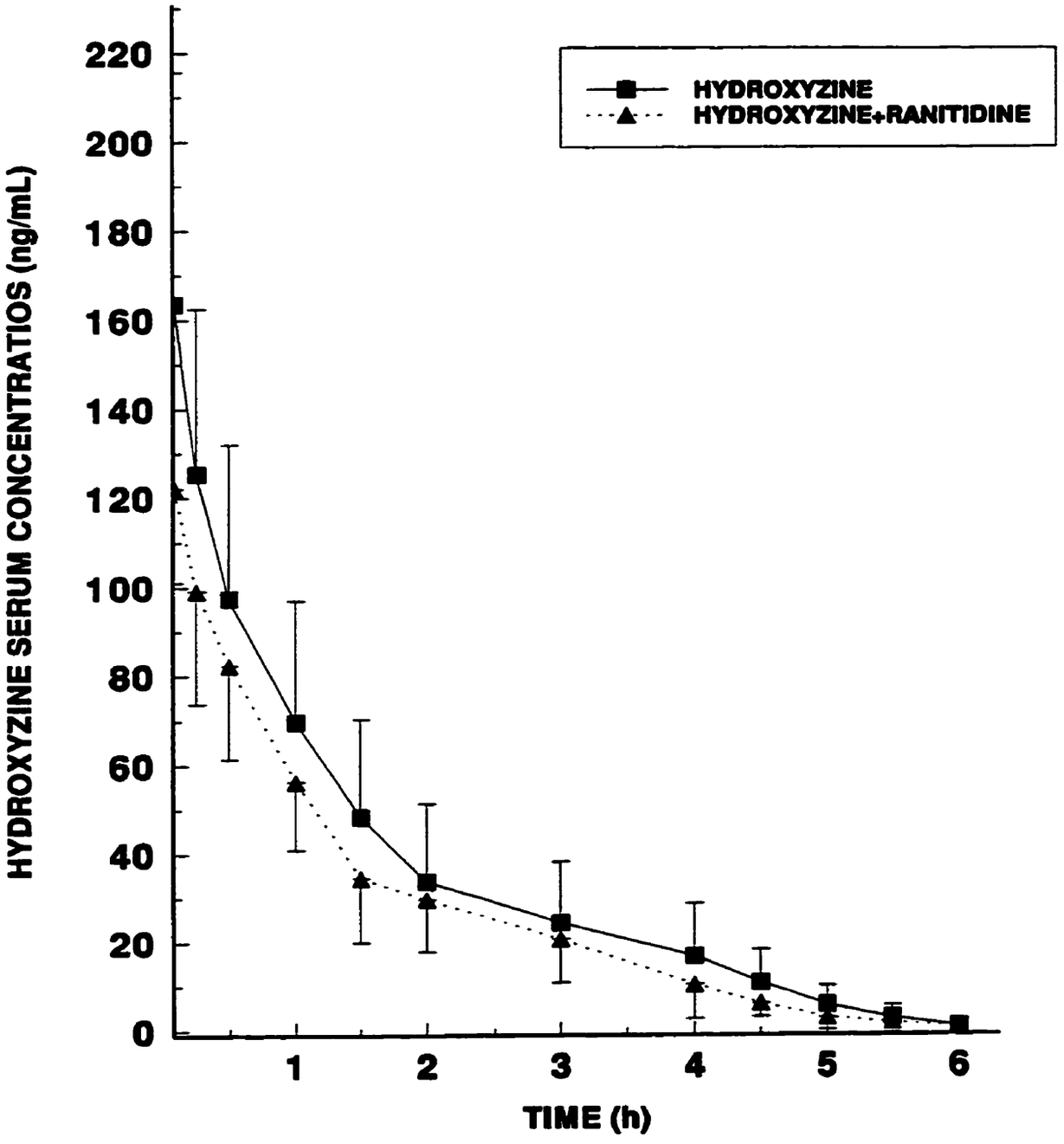
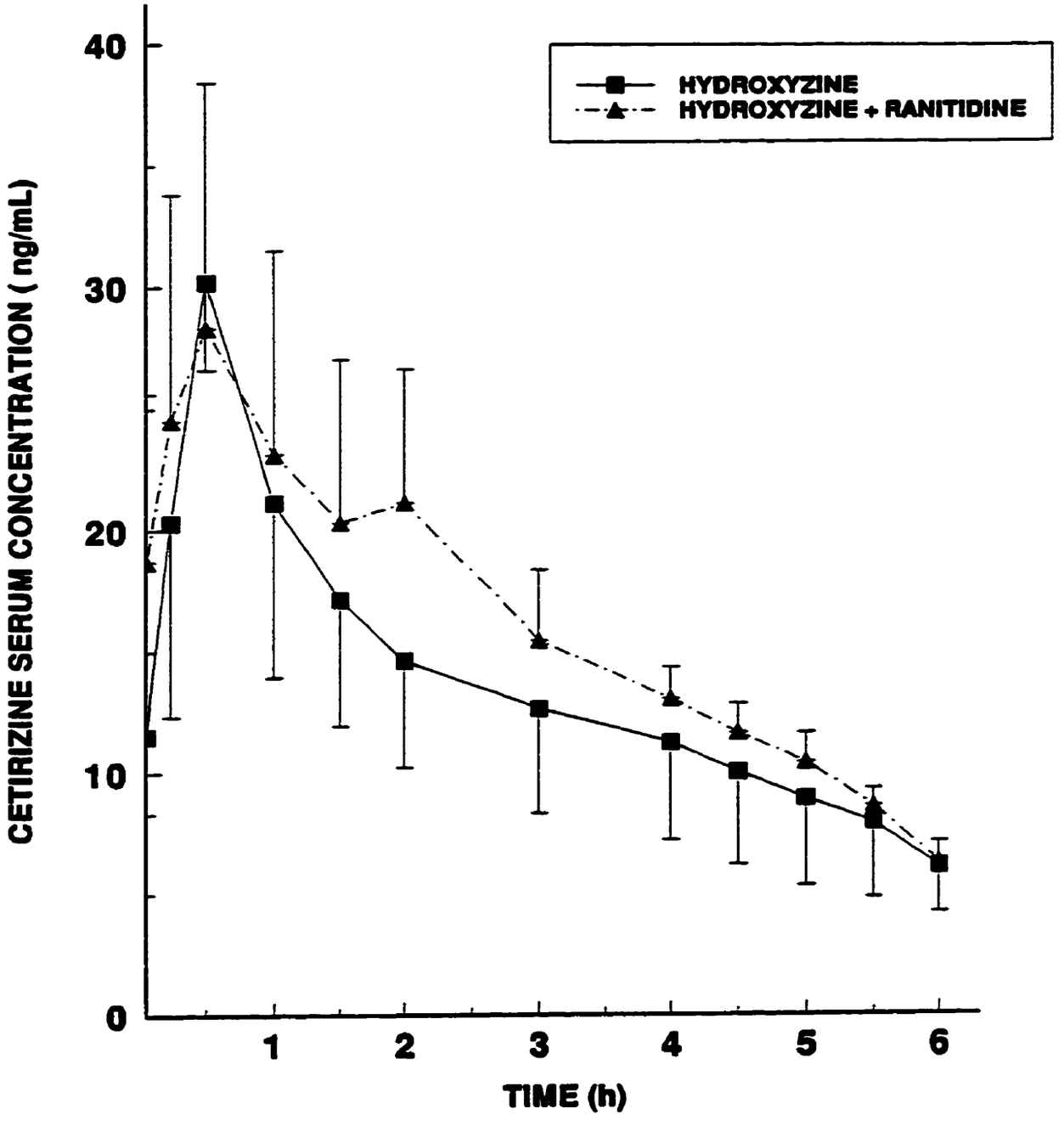


Figure 17

THE MEAN SERUM CONCENTRATIONS OF CETIRIZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF RANITIDINE



3.3.2 Pharmacodynamic studies of hydroxyzine.

Results of the efficacy tests calculated as absolute values of wheal areas induced by intradermally injected histamine after intravenous bolus dose of 2.5 mg hydroxyzine in rabbits with and without co-administration of ranitidine (1 mg/kg) are listed in Tables 30 and 31. Mean wheal area is plotted versus time in Figure 18.

3.4. Pharmacokinetic and pharmacodynamic studies of hydroxyzine in rabbits: the effect of ketoconazole.

3.4.1 Serum concentrations of hydroxyzine and cetirizine.

Serum concentration and pharmacokinetic parameters of hydroxyzine and cetirizine following intravenous bolus dose of 2.5 mg hydroxyzine two weeks before ketoconazole and with ketoconazole are listed in Tables 32 to 39. The serum concentrations of hydroxyzine are listed in Tables 32 and 33, the pharmacokinetic parameters of hydroxyzine are listed in Tables 34 and 35, the serum concentrations of cetirizine are listed in Tables 36 and 37, the pharmacokinetic parameters are listed in Tables 38 and 39. The mean serum concentrations are plotted versus time in Figures 19 and 20.

Table 30. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.0	1.28	0.73	1.19	1.06	1.05	1.06	0.21
0.08	0.62	0.25	0.44	0.47	0.66	0.49	0.16
0.25	0.38	0.36	0.38	0.38	0.33	0.37	0.02
0.5	0.28	0.22	0.37	0.28	0.30	0.29	0.05
1.0	0.27	0.19	0.29	0.12	0.17	0.21	0.07
1.5	0.19	0.15	0.25	0.14	0.29	0.20	0.06
2.0	0.16	0.17	0.12	0.07	0.12	0.13	0.04
3.0	0.09	0.16	0.29	0.26	0.28	0.22	0.09
4.0	0.25	0.20	0.23	0.29	0.08	0.21	0.08
4.5	0.08	0.23	0.24	0.33	0.18	0.21	0.09
5.0	0.15	0.25	0.17	0.55	0.13	0.19	0.05
5.5	0.34	0.42	0.28	0.57	0.37	0.40	0.11
6.0	0.58	0.42	0.51	0.47	0.44	0.49	0.06
12.0	0.74	0.57	0.51	0.78	0.54	0.63	0.12
24.0	0.70	0.50	0.52	0.81	0.80	0.67	0.15

Table 31. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of 2.5 mg hydroxyzine with co-administration of ranitidine.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.0	0.73	0.62	0.67	0.50	0.61	0.63	0.08
0.08	0.35	0.23	0.26	0.24	0.27	0.27	0.05
0.25	0.27	0.17	0.10	0.19	0.26	0.20	0.07
0.5	0.20	0.14	0.15	0.12	0.16	0.15	0.03
1.0	0.27	0.19	0.14	0.06	0.05	0.14	0.09
1.5	0.23	0.16	0.07	0.04	0.02	0.11	0.09
2.0	0.18	0.12	0.06	0.01	0.01	0.08	0.07
3.0	0.05	0.07	0.04	0.0	0.0	0.06	0.01
4.0	0.08	0.06	0.01	0.0	0.0	0.05	0.03
4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.0	0.12	0.16	0.09	0.07	0.03	0.09	0.05
5.5	0.34	0.23	0.16	0.17	0.07	0.19	0.10
6.0	0.50	0.27	0.27	0.32	0.22	0.32	0.11
12.0	0.55	0.38	0.35	0.37	0.33	0.39	0.09
24.0	0.71	0.54	0.36	0.43	0.47	0.50	0.13

Figure 18

THE MEAN SUPPRESSION OF WHEALS INDUCED BY INTRADERMALLY INJECTED HISTAMINE AFTER IV DOSE OF HYDROXYZINE AND WITH CO-ADMINISTRATION OF RANITIDINE

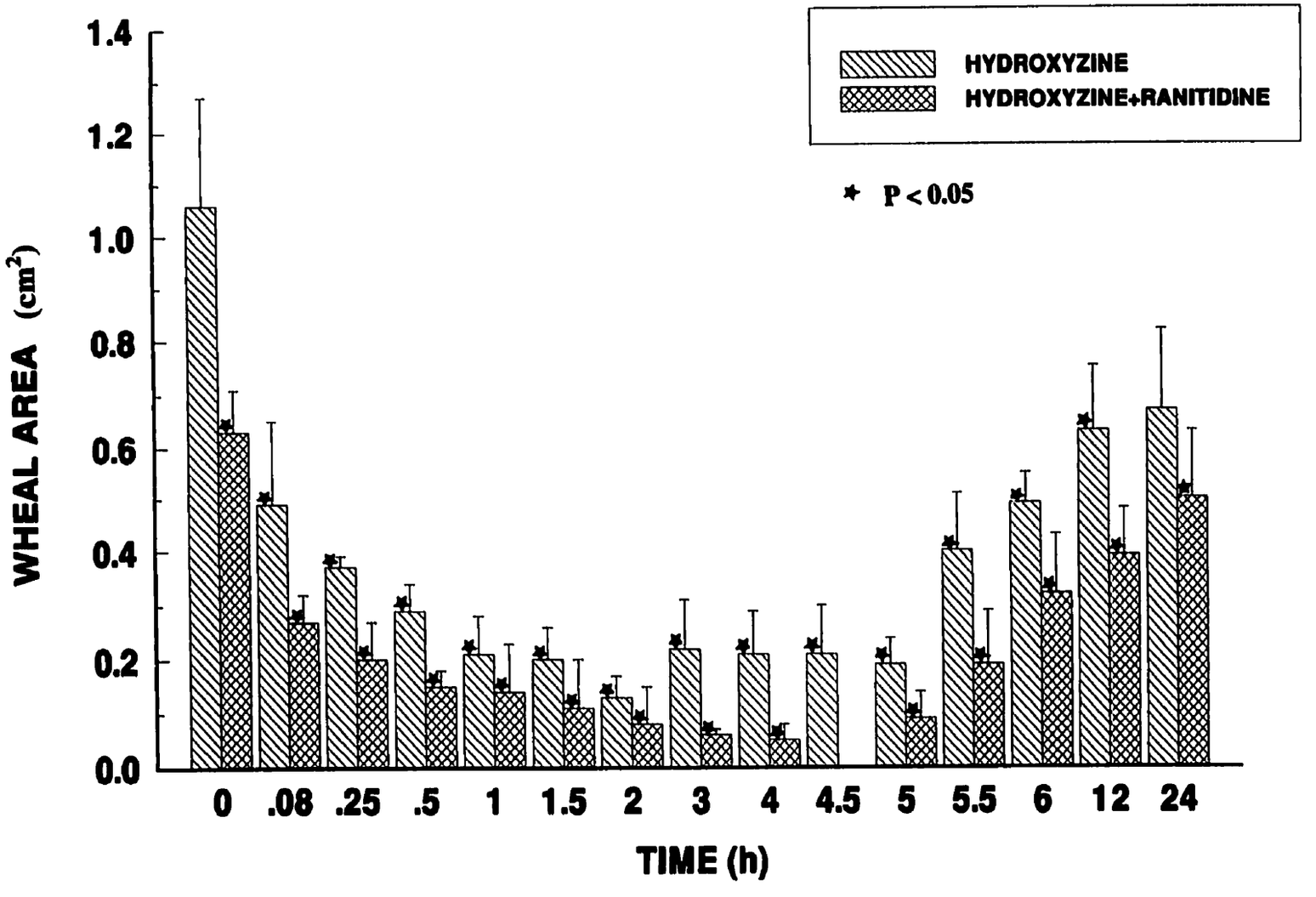


Table 32. The serum concentrations of hydroxyzine after I.V. bolus dose of 2.5 mg of hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	62.4	63.6	65.7	83.6	90.1	73.1	12.9
0.25	47.5	53.0	42.6	66.7	77.4	49.4	28.7
0.5	46.8	49.8	41.5	34.0	103.7	55.1	27.8
1.0	27.3	37.1	30.5	23.0	32.8	30.1	5.3
1.5	22.0	34.9	24.0	19.7	29.1	25.9	6.1
2.0	17.7	32.8	15.1	12.6	24.4	20.5	8.2
3.0	13.5	30.7	14.0	14.2	22.2	18.9	7.5
4.0	8.4	26.5	7.4	10.8	19.6	14.5	8.2
4.5	6.3	NS	4.1	7.7	14.3	8.1	4.4
5.0	5.3	14.8	0.0	6.7	12.7	7.9	5.9
5.5	0.0	9.5	0.0	6.3	11.9	5.5	5.4
6.0	0.0	6.3	0.0	6.2	7.6	4.0	3.7

* NS = No sample.

Table 33. The serum concentrations of hydroxyzine after I.V. dose of 2.5 mg hydroxyzine with co-administration of ketoconazole.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	162.3	155.1	159.1	NS	185.0	165.4	13.4
0.25	100.7	111.2	101.1	120.1	152.7	117.2	21.4
0.5	88.0	78.2	95.7	109.2	129.4	100.1	19.9
1.0	62.6	65.1	67.6	100.1	97.4	78.6	18.6
1.5	61.5	54.0	75.7	78.6	75.7	65.5	11.0
2.0	57.2	46.3	43.0	73.1	82.7	60.5	17.1
3.0	35.0	43.0	40.3	54.8	46.2	43.9	7.4
4.0	26.5	36.4	38.5	35.0	NS	34.1	5.3
4.5	20.1	30.9	31.4	29.1	34.2	29.2	5.4
5.0	14.8	23.5	25.9	23.2	29.7	23.4	5.5
5.5	13.7	25.2	23.0	13.7	25.0	20.1	5.9
6.0	12.6	18.9	18.3	NS	20.8	17.7	3.5
8.0	11.6	16.7	13.7	10.6	15.0	13.6	2.5
10.0	9.5	14.0	12.0	9.6	NS	11.3	2.2
12.0	6.4	8.4	6.4	NS	8.4	7.4	1.2

* NS = No sample

Table 34. The pharmacokinetic parameters of hydroxyzine after I.V. dose of 2.5 mg hydroxyzine.

Subject	$CP_{5\ min}$ (ng/mL)	$T_{1/2}$ (h)	VD (L)	CL (mL/min)	AUC ng-h/mL
1	62.4	1.7	53.5	386.8	107.7
2	63.6	2.1	40.6	225.1	185.1
3	65.7	1.3	50.3	422.0	98.7
4	86.6	1.9	70.8	342.4	121.7
5	90.1	2.1	46.5	217.7	191.4
Mean	73.7	1.8	52.3	318.8	140.9
S.D.	13.5	0.3	11.4	93.3	44.0

Table 35. The pharmacokinetic parameters of hydroxyzine after I.V. bolus dose of 2.5 mg of hydroxyzine with co-administration of ketoconazole.

Subject	$CP_{5\,min}$ (ng/mL)	$T_{1/2}$ (h)	VD (L)	CL (mL/min)	AUC ng-h/mL
1	162.3	5.6	39.0	112.1	371.7
2	155.1	3.9	32.3	99.8	417.6
3	159.1	3.5	30.5	107.0	389.3
4	120.1	3.6	23.0	104.0	400.6
5	185.0	3.1	20.7	86.6	481.2
Mean	156.3	3.9	29.1	101.8	412.1
S.D.	23.4	0.9	7.4	9.7	42.1

Table 36. Cetirizine serum concentrations after I.V. bolus dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	6.6	8.9	7.2	14.9	10.9	9.7	3.3
0.25	8.9	9.8	10.6	16.6	12.3	11.6	3.0
0.5	17.4	15.7	11.5	22.5	19.9	17.4	4.2
1.0	25.5	24.2	17.4	24.2	28.4	23.9	4.0
1.5	30.8	20.8	16.6	19.1	25.9	22.6	5.7
2.0	15.7	16.6	14.0	15.7	19.9	16.4	2.2
3.0	14.9	14.0	13.2	14.9	13.2	14.0	0.8
4.0	14.0	13.2	11.5	12.3	11.5	12.5	1.1
4.5	11.5	NS	10.6	11.5	10.6	11.0	0.5
5.0	8.9	8.9	8.1	10.6	8.9	9.1	0.9
5.5	6.4	5.5	5.5	8.9	7.2	6.7	1.4
6.0	4.7	5.0	4.7	8.1	6.4	5.7	1.5

* NS = no sample.

Table 37. Cetirizine serum concentrations after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of ketoconazole.

Sub./ Time	1	2	3	4	5	Mean ng/mL	S.D.
0.08	43.9	NS	33.5	36.1	38.7	38.1	4.4
0.25	76.6	14.5	67.2	58.5	61.1	55.6	23.9
0.5	115.5	48.2	96.5	83.5	79.2	84.6	24.7
1.0	123.3	72.3	110.3	99.1	105.1	102.0	18.9
1.5	130.1	83.5	127.5	114.6	118.9	114.9	18.7
2.0	155.2	130.1	144.8	131.9	132.7	138.9	10.8
3.0	125.6	128.4	107.7	111.2	122.4	119.0	9.1
4.0	106.8	94.8	94.8	105.1	110.3	102.3	7.2
4.5	99.5	59.4	84.4	87.9	89.6	84.1	14.9
5.0	94.8	55.9	68.0	68.0	77.5	72.8	14.4
5.5	90.4	50.8	58.5	58.5	62.0	64.1	15.3
6.0	82.7	41.3	41.3	53.4	43.9	52.5	17.6
8.0	59.4	39.3	33.5	34.4	34.4	40.2	10.9
10.0	34.4	19.7	27.5	22.3	20.6	24.8	6.1
12.0	18.9	15.4	15.4	18.0	14.5	16.4	1.9

* NS = No sample.

Table 38. The pharmacokinetic parameters of cetirizine after I.V. dose of 2.5 mg hydroxyzine.

Subject	Weight (kg)	Cp_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ng-h/mL	R
1	5.0	30.8	1.5	1.1	88.8	0.8
2	4.5	24.2	1.0	1.2	81.4	0.4
3	4.5	17.4	1.0	1.4	69.7	0.7
4	5.0	24.2	1.0	2.8	88.2	0.7
5	5.0	28.4	1.0	2.1	89.8	0.5
Mean	4.8	25.0	1.1	1.7	83.6	0.6
S.D.	0.2	5.1	0.2	0.7	8.4	0.2

Table 39. Cetirizine pharamacokinetic parameters after I.V. bolus dose of 2.5 mg hydroxyzine with co-administration of ketoconazole.

Subject	Weight (kg)	CP_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ng-h/mL	R
1	5.0	155.2	2.0	3.2	964.1	2.6
2	4.5	130.1	2.0	3.1	662.8	1.6
3	4.5	144.8	2.0	3.2	753.9	1.9
4	5.0	131.9	2.0	3.2	747.7	1.9
5	5.0	132.7	2.0	3.3	756.3	1.6
Mean	4.8	138.9	2.0	3.2	773.4	1.9
S.D.	0.2	10.8	----	0.1	112.9	0.4

Figure 19

THE MEAN SERUM CONCENTRATIONS OF HYDROXYZINE AFTER IV DOSE OF HYDROXYZINE AND WITH CO-ADMINISTRATION OF KETOCONAZOLE.

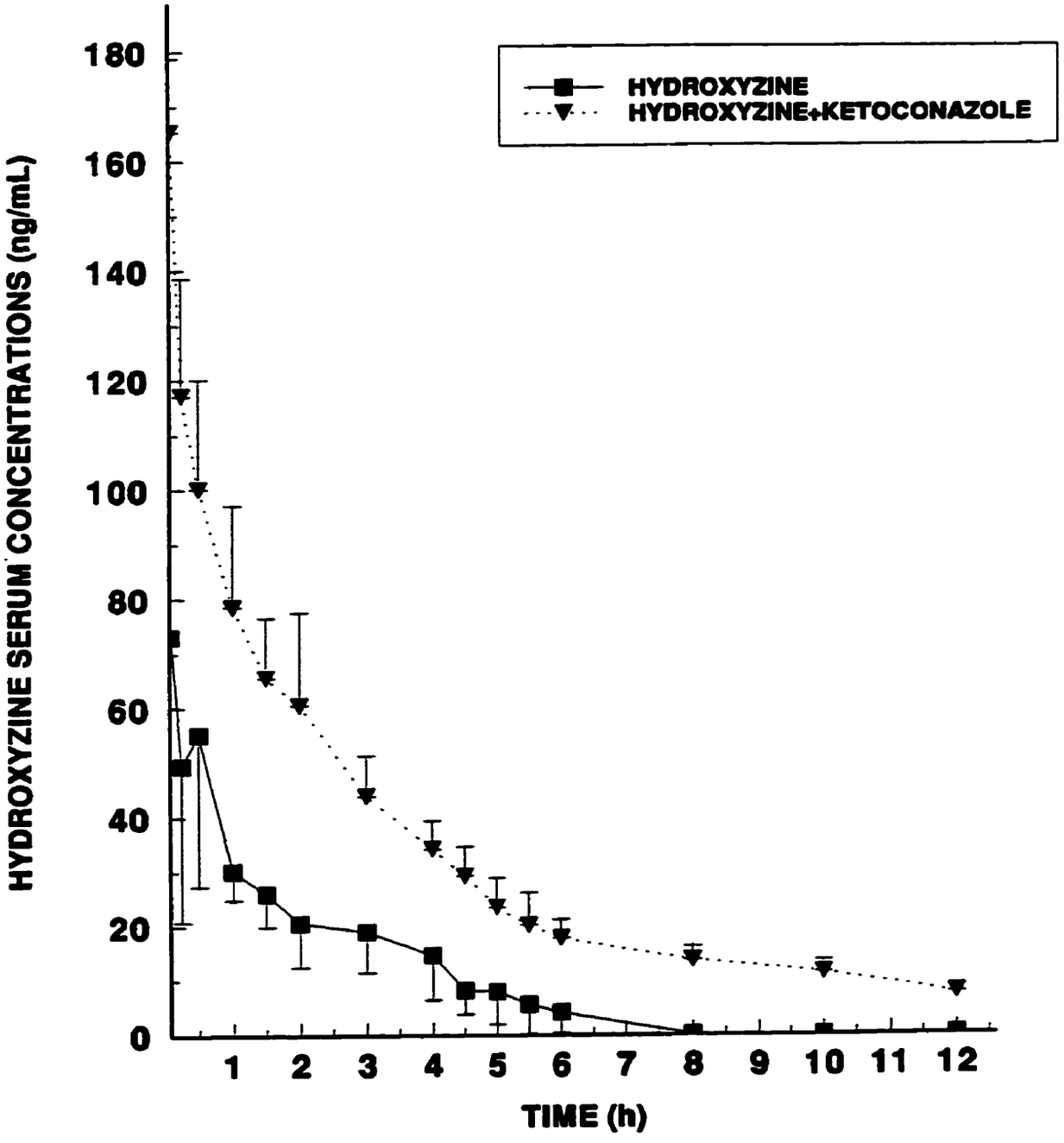
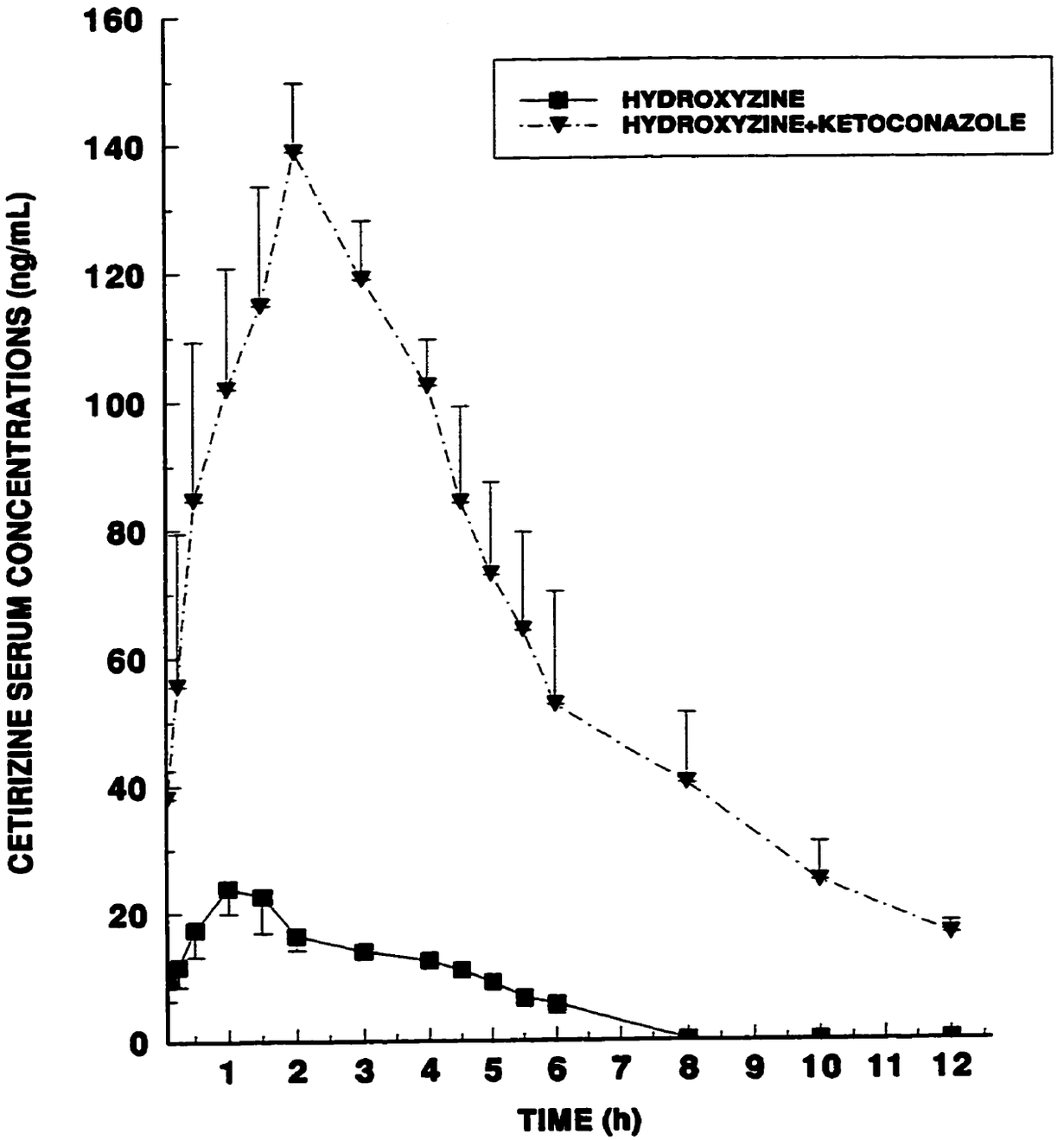


Figure 20

THE MEAN SERUM CONCENTRATIONS OF CETIRIZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF KETOCONAZOLE.



3.4.2 Pharmacodynamic studies of hydroxyzine.

The efficacy tests are calculated as absolute values of wheal area induced by intradermal injection of histamine phosphate after intravenous bolus injection of 2.5 mg hydroxyzine in rabbits with and without co-administration of ketoconazole (200 mg tablet).

Results are listed in Tables 40 and 41. Mean wheal area is plotted versus time in Figure 21.

3.5 Pharmacokinetic and pharmacodynamic studies of cetirizine in patients with primary biliary cirrhosis.

3.5.1 HPLC assay for cetirizine.

Representative chromatograms for cetirizine and its internal standard hydroxyzine in serum and urine are shown in Figures 25 and 26.

The retention times of cetirizine and hydroxyzine as internal standard are 6.9 and 10.16 minutes respectively. No interfering peaks were observed.

Table 40. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of 2.5 mg hydroxyzine.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.00	1.31	1.70	1.23	1.01	0.99	1.25	0.29
0.08	0.78	0.70	0.85	0.78	0.70	0.76	0.06
0.25	0.69	0.73	0.69	0.68	0.70	0.72	0.04
0.5	0.74	0.57	0.78	0.51	0.54	0.63	0.12
1.0	0.64	0.68	0.69	0.62	0.70	0.67	0.03
1.5	0.74	0.66	0.51	0.68	0.54	0.63	0.09
2.0	0.51	0.58	0.56	0.49	0.55	0.54	0.03
3.0	0.49	0.52	0.50	0.51	0.52	0.51	0.01
4.0	0.51	0.44	0.47	0.42	0.59	0.49	0.06
4.5	0.41	0.49	0.42	0.41	0.46	0.44	0.03
5.0	0.29	0.44	0.42	0.28	0.46	0.38	0.08
5.5	0.46	0.40	0.49	0.46	0.49	0.46	0.04
6.0	0.56	0.51	0.55	0.50	0.56	0.54	0.03
12.0	0.79	0.66	0.86	0.92	0.82	0.81	0.09
24.0	0.97	0.80	0.96	0.89	0.94	0.91	0.07

Table 41. The wheal area (cm^2) induced by intradermally injected histamine after I.V. dose of 2.5 mg hydroxyzine with co-administration of ketoconazole.

Sub./ Time	1	2	3	4	5	Mean	S.D.
0.00	1.28	1.09	1.39	1.21	1.54	1.30	0.17
0.08	0.67	0.62	0.81	0.41	0.42	0.59	0.17
0.25	0.71	0.50	0.75	0.41	0.48	0.57	0.15
0.5	0.57	0.24	0.78	0.26	0.54	0.48	0.22
1.0	0.52	0.33	0.56	0.41	0.51	0.46	0.09
1.5	0.27	0.29	0.52	0.28	0.49	0.37	0.12
2.0	0.44	0.15	0.42	0.45	0.46	0.38	0.12
3.0	0.29	0.34	0.37	0.28	0.42	0.34	0.06
4.0	0.14	0.23	0.43	0.22	0.44	0.29	0.13
4.5	0.11	0.26	0.34	0.20	0.43	0.27	0.12
5.0	0.18	0.21	0.36	0.16	0.47	0.27	0.13
5.5	0.15	0.25	0.36	0.23	0.33	0.26	0.08
6.0	0.25	0.27	0.35	0.35	0.44	0.33	0.12
12.0	0.47	0.45	0.72	0.54	0.41	0.52	0.12
24.0	1.00	0.89	1.12	0.97	0.80	0.95	0.12

Figure 21

THE MEAN SUPPRESSION OF WHEALS INDUCED BY INTRADERMALLY INJECTED HISTAMINE AFTER IV DOSE OF HYDROXYZINE WITH CO-ADMINISTRATION OF KETOCONAZOLE.

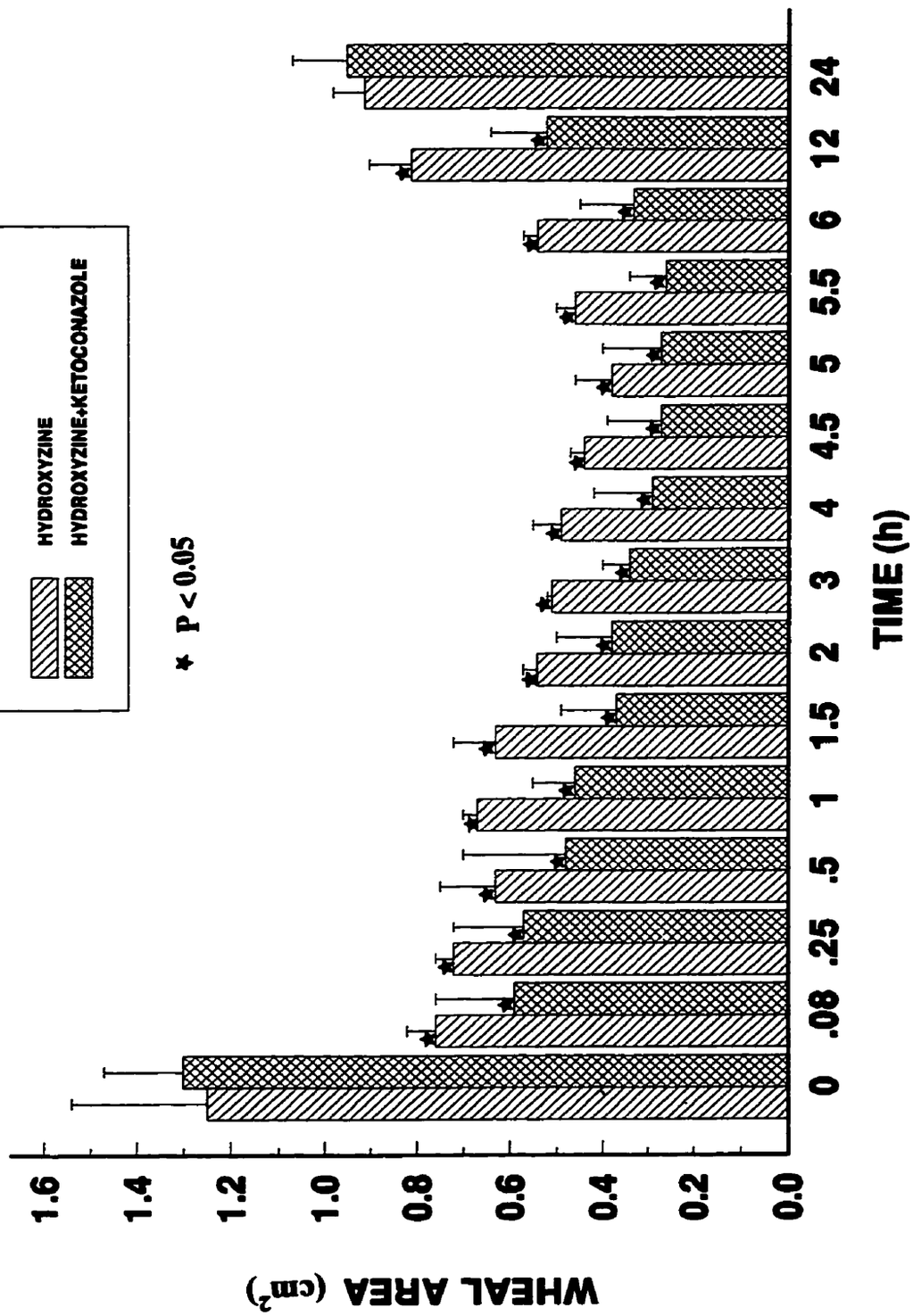


Figure 22

THE MEAN SERUM CONCENTRATIONS OF HYDROXYZINE AFTER IV DOSE OF HYDROXYZINE AND WITH CO-ADMINISTRATION OF KETOCONAZOLE AND CIMETIDINE.

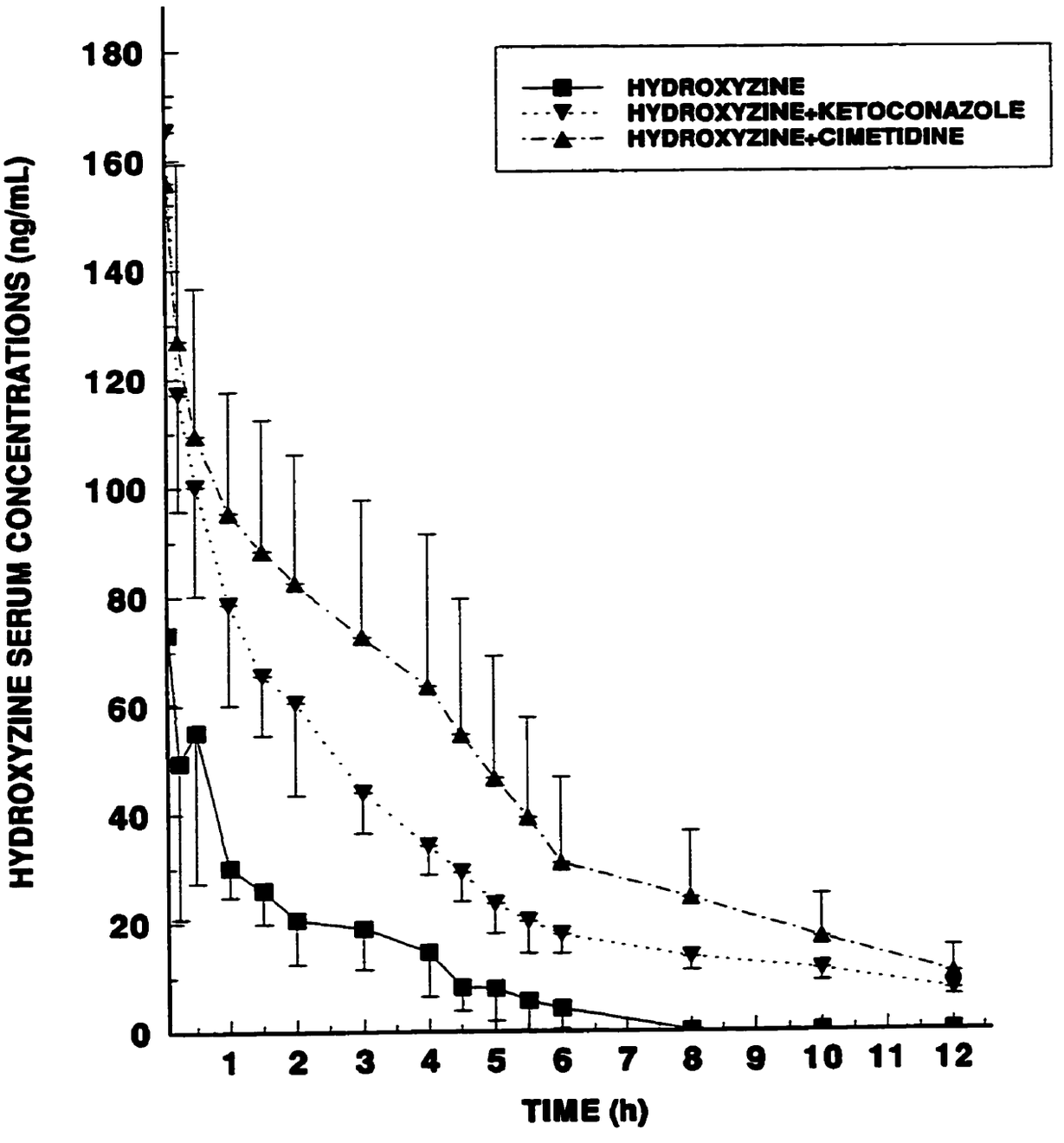


Figure 23

THE MEAN SERUM CONCENTRATIONS OF CETIRIZINE AFTER IV DOSE OF 2.5 MG HYDROXYZINE AND WITH CO-ADMINISTRATION OF KETOCONAZOLE & CIMETIDINE

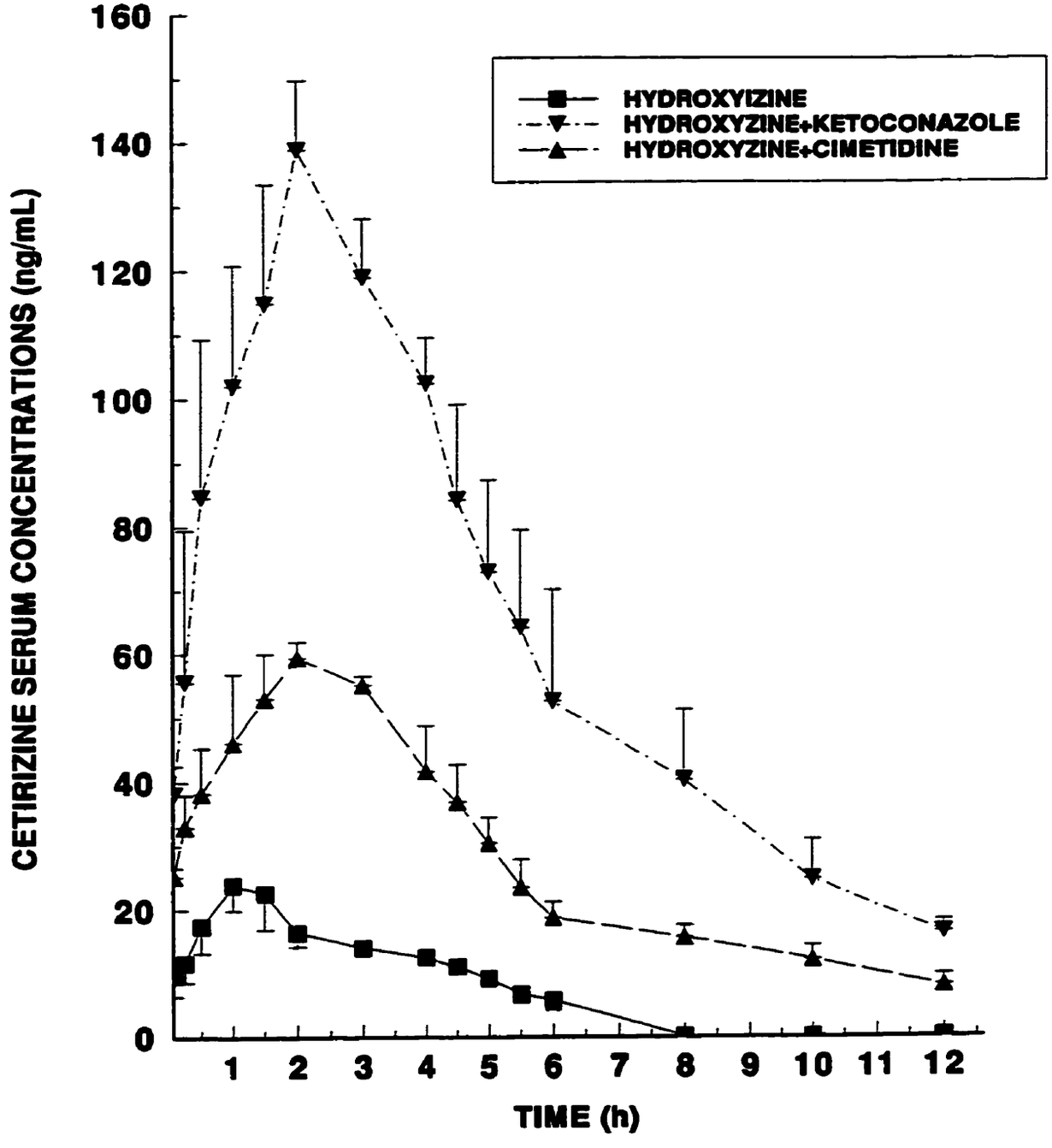
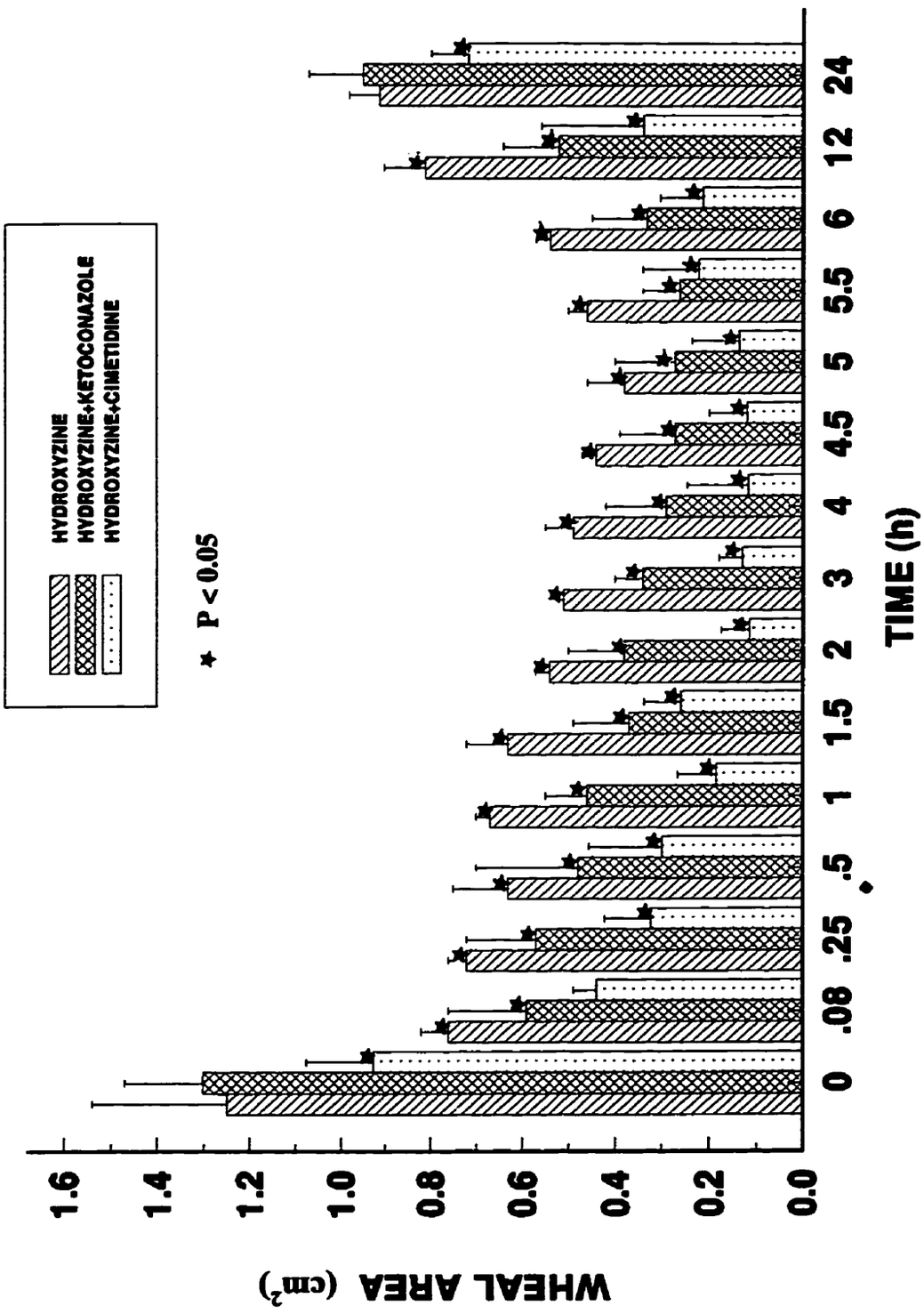


Figure 24

THE MEAN SUPPRESSION OF WHEALS INDUCED BY INTRADERMALLY INJECTED HISTAMINE AFTER IV DOSE OF HYDROXYZINE WITH KETOCONAZOLE AND CIMETIDINE.



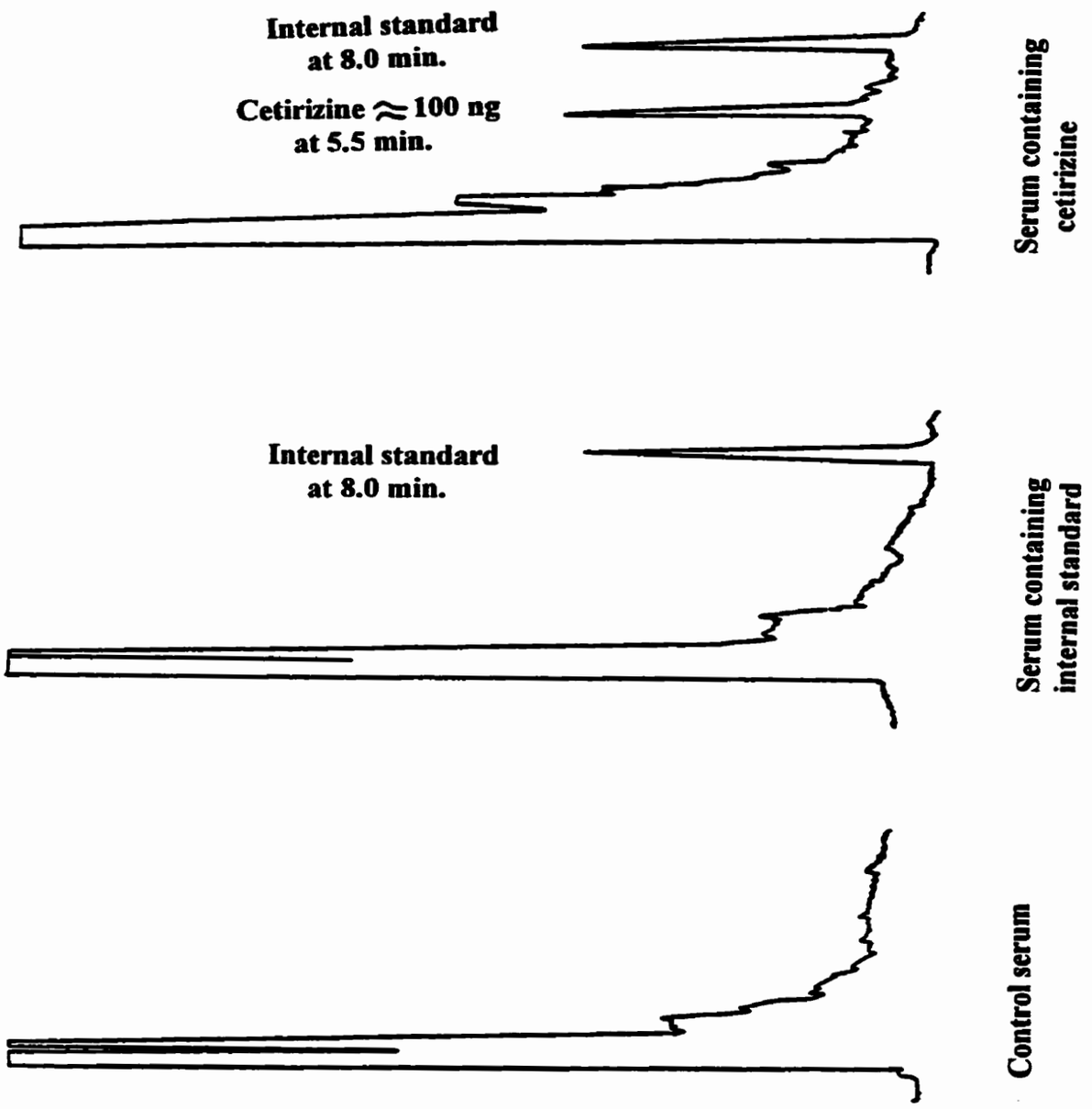


Figure 25. The HPLC chromatograms of serum cetirizine.

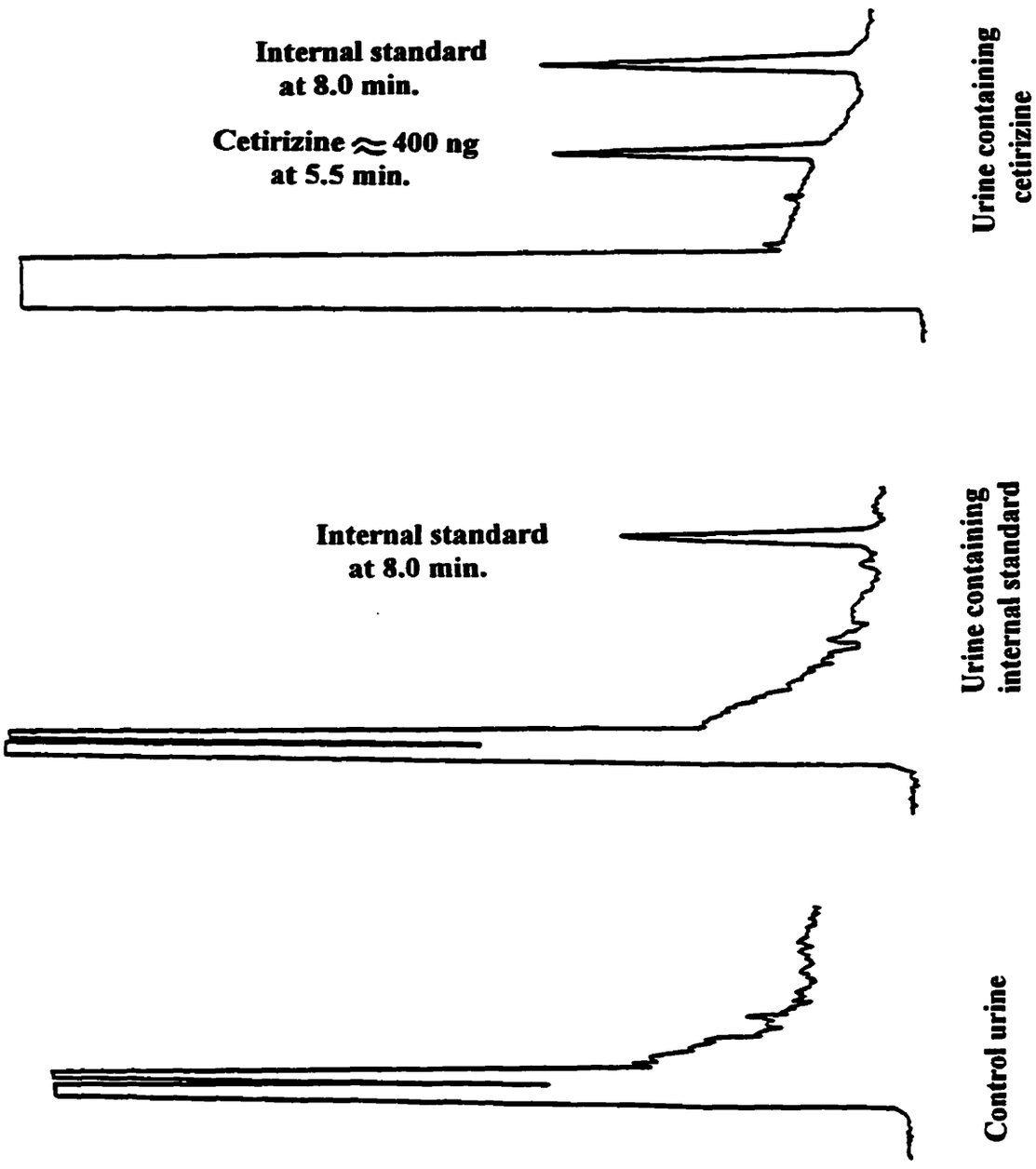


Figure 26. The HPLC chromatograms of urine cetirizine.

3.5.2 Calibration curve for cetirizine

The calibration curves for cetirizine were constructed by plotting cetirizine to hydroxyzine peak height ratios versus concentrations of cetirizine. The calibration curve was analyzed periodically during the study period using concentrations of cetirizine from 5 ng/mL to 200 ng/mL and from 100 ng/mL to 4000 ng/mL when measuring urine cetirizine. The calibration curves are linear over these ranges of concentrations. The variabilities in the serum cetirizine calibration curves over a period of 6 months were calculated from 10 calibration curves, each having 4 samples at every concentration. The variability is expressed as the coefficient of variation as shown in Table 42. The variabilities in the urine cetirizine calibration curves over a period of 2 months were calculated from 6 calibration curves, each having 3 samples at every concentration. The variability is expressed as the coefficient of variation as shown in Table 43. The calibration curves for cetirizine in serum and urine are shown in Figures 27 and 28 respectively.

Table 42. Variability in HPLC calibration curves for serum cetirizine.

Concentration ng/mL	Peak height ratio ± SD	C.V. %
5	0.05 ± 0.01	6.1
10	0.14 ± 0.01	7.1
25	0.30 ± 0.02	5.1
50	0.55 ± 0.02	3.6
100	1.10 ± 0.14	12.7
200	2.17 ± 0.04	1.8

Table 43. Variability in HPLC calibration curves for urine cetirizine.

Concentration ng/mL	Peak height ratio ± SD	C.V. %
100	0.04 ± 0.01	12.5
200	0.10 ± 0.01	3.4
500	0.24 ± 0.01	1.3
1000	0.51 ± 0.04	7.3
1500	0.75 ± 0.05	6.1
2000	1.02 ± 0.06	5.4
4000	1.98 ± 0.08	4.1

Figure 27

CALIBRATION CURVE FOR SERUM CETIRIZINE

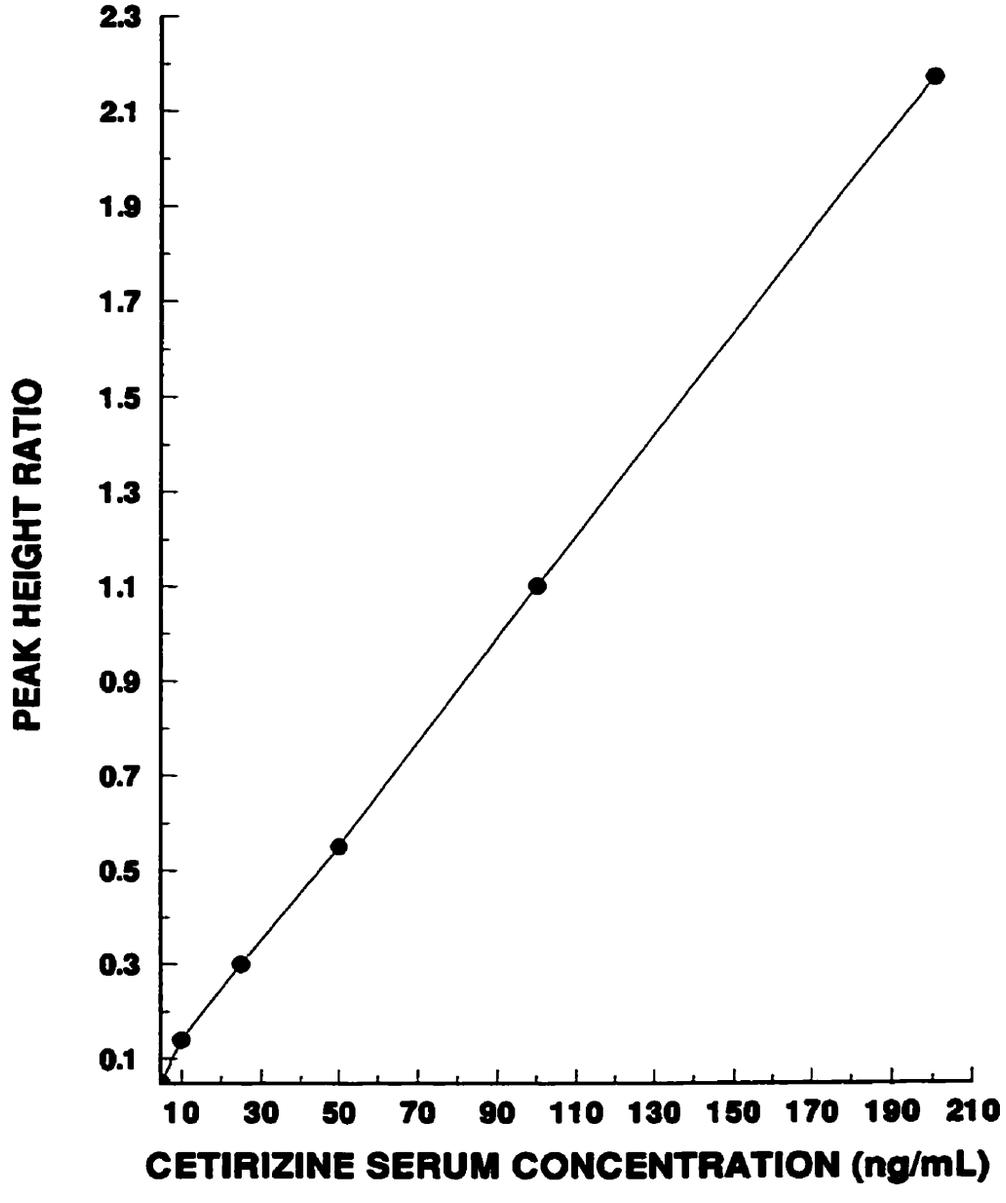
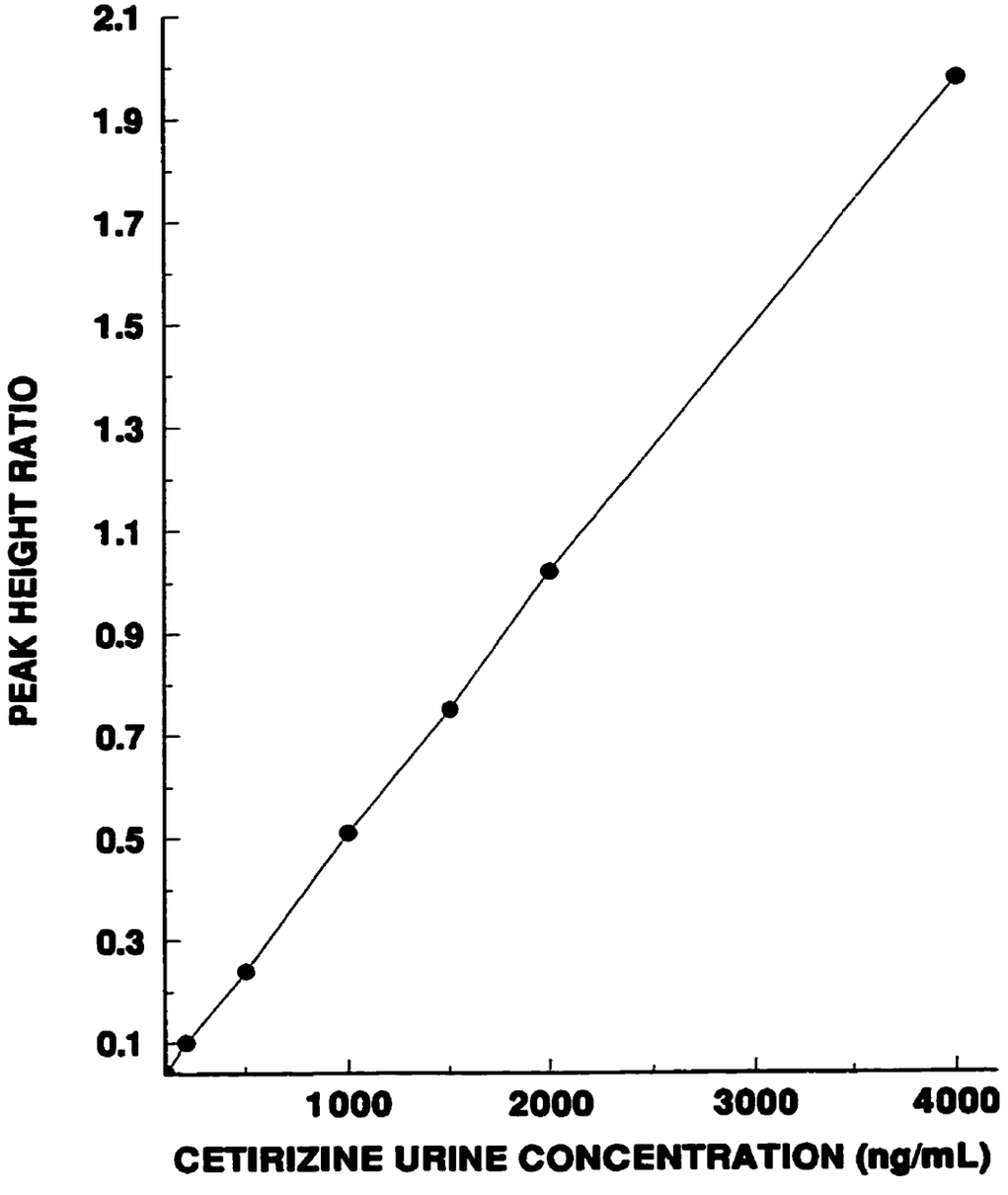


Figure 28

CALIBRATION CURVE FOR URINE CETIRIZINE



3.5.3 Serum concentrations of cetirizine in patients with primary biliary cirrhosis.

Six outpatients, five women and one man, mean age 64±8 years, completed the study. All had biopsies compatible with primary biliary cirrhosis, positive tests for antimitochondrial antibodies, abnormal liver biochemistry tests, and normal renal function as evidenced by normal blood urea nitrogen and serum creatinine measurement, and normal creatinine clearance (Table 44).

Serum cetirizine concentrations are shown in Table 45. The peak cetirizine level was 498 ± 118 ng/mL, occuring at 1.0 ± 0.4 h. In some patients, cetirizine was still measurable in serum 96 h post-dose. Pharmacokinetic values are shown in Table 46 . The mean serum elimination half-life value of cetirizine was 13.8 ± 1.8 h as shown in Figure 29 . The area under the serum concentration versus time curve was 6438 ± 1621 ng/mL/kg. the fraction of the dose excreted as unchanged drug in the urine was 0.32±0.14 as shown in Figure 30.

One patient with severe pruritus took cetirizine, 10 mg q.24.h. for several weeks after the single dose study. At steady state, recovery of

Table 44. Dermographic, hepatic function, and renal function data.

	$\bar{X} \pm SD$	Range: PBC patients	Range: Normal*
Age (yr)	64 ± 8	_____	_____
Hepatic function			
Bilirubin (mol/L)	21 ± 19	2-56	3-18
Alanine amino transferase (μ/L)	86 ± 52	32-167	0-18
Alkaline phosphatase (μ/L)	678 ± 188	474-845	30-120
Aspartase aminotransferase (μ/L)	85 ± 53	34 -167	10-32
γ-glutamyl transferase	571± 429	40-1038	5-38
Renal function			
Blood urea nitrogen	6.3 ± 0.6	5.7 -7.2	2.8-7.1
Serum creatinine (mol/L)	92.8 ± 7.1	85-101	70-110
Creatinine clearance (mL/min/1.73 m^2	74.7 ± 8.5	62-83	65-110

* Health Sciences Centre clinical chemistry laboratory normal values.

Table 45. Cetirizine serum concentration after oral dose of 10 mg cetirizine to patients with primary biliary cirrhosis.

Sub./ Time (h)	1	2	3	4	5	6	Mean ng/mL	S.D.
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
1	439.2	445.1	319.5	562.3	564.9	656.8	498.0	119.9
2	412.3	388.9	324.3	350.5	435.5	587.4	416.5	92.9
3	349.6	327.1	315.7	428.1	440.7	414.9	379.3	54.9
4	246.5	301.8	269.5	379.5	364.6	400.9	327.1	63.3
5	264.4	276.5	207.0	292.7	376.0	283.5	283.4	54.6
6	259.9	265.2	NA	262.6	315.7	319.8	284.7	30.3
8	170.3	217.5	164.7	177.0	289.5	229.2	288.0	47.8
10	152.3	211.8	145.4	177.0	237.0	239.6	193.9	41.6
12	147.8	192.2	140.6	142.3	219.5	227.1	178.3	39.8
24	44.7	60.1	60.8	34.7	114.6	135.6	75.1	40.5
48	15.6	34.8	14.6	17.3	28.9	42.1	25.6	11.5
72	NA	15.1	4.0	12.7	13.2	NA	11.3	4.9
96	NA	3.9	0.0	2.3	0.0	NA	2.1	1.9

* NA = Not available

Table 46. Comparison of pharmacokinetic parameters of cetirizine 10 mg oral dose in different populations.

Parameter	Children	Adults	Elderly	Renal dysfunction	Hepatic dysfunction
Age (yr)	8 ± 0.6	53 ± 9	77 ± 5	67 ± 4	64 ± 8
c_{Pmax} (ng/mL)	978 ± 341	384 ±103	460± 59	357 ± 172	498 ± 118
T_{max} (h)	0.8 ± 0.4	1.0 ± 0.5	0.9 ± 0.3	2.2 ± 1.1	1.0 ± 0.4
$T_{1/2}$ (h)	6.9 ± 1.6	10.6 ± 0.5 7.4 ± 1.8	11.8 ± 5.4	20.9 ± 4.4	13.8 ± 1.8
AUC (ng/mL/kg)	6375 ±1874	3300 ± 900	5600 ±1800	10700 ± 2400	6438±1621
cl mL/min/kg	1.10 ± 0.13	0.6 ± 0.04	0.5 ± 0.03	0.25 ± 0.06	0.44 ± 0.1
vd (L/kg)	0.7 ± 0.1	0.50 ± 0.07	0.38 ± 0.06	0.54 ± 0.21	0.44 ± 0.04
Ae^{∞} (%)	39 ± 14	65 ± 2	51 ± 6	------	32 ± 14
cl_R mL/min/kg	0.38 ± 0.08	0.39	0.26	2.8±1.5 mL/min	0.15 ± 0.08
Reference	93	91,92,39	39,41	41	------

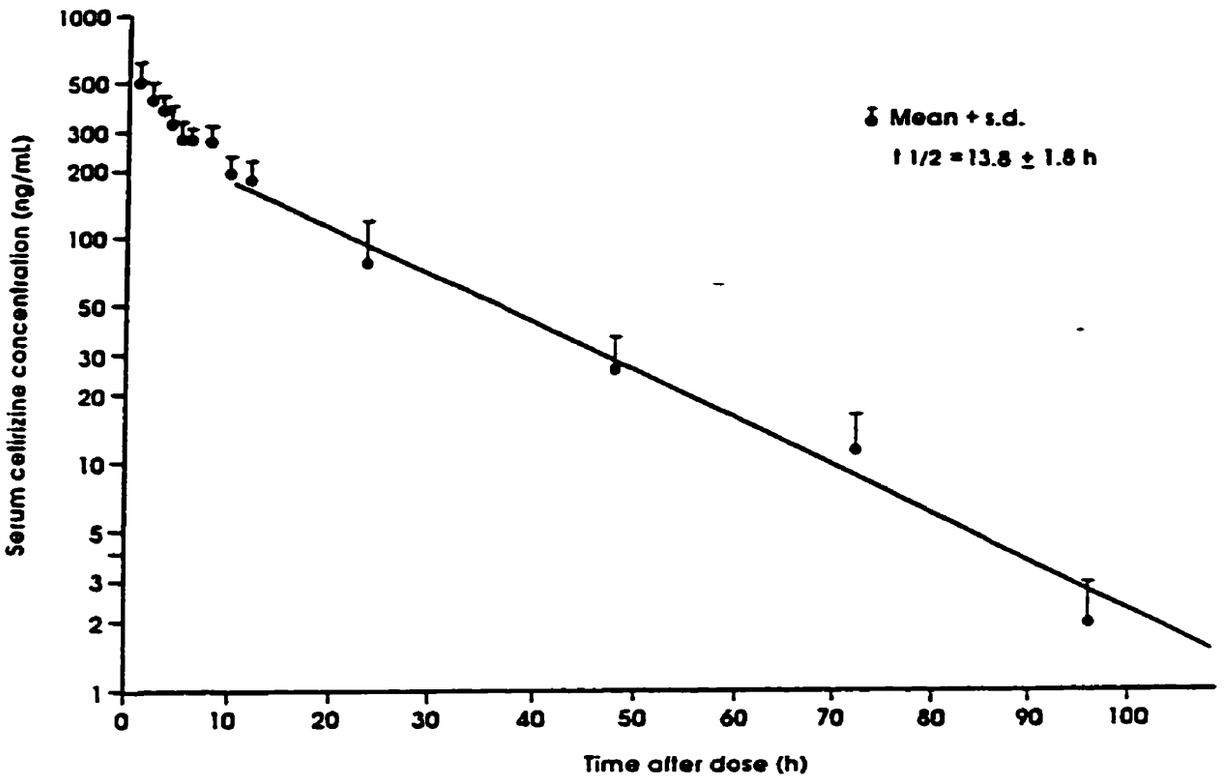


Figure 29. The mean serum cetirizine concentrations after an oral dose of 10 mg in patients with primary biliary cirrhosis.

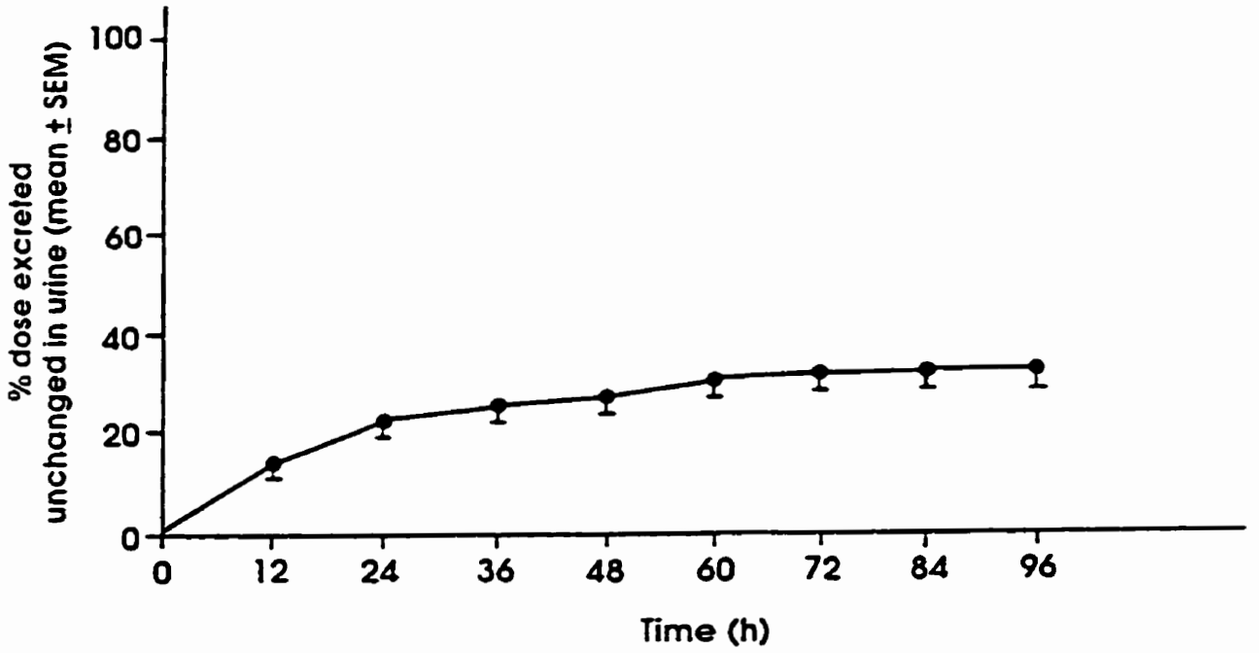


Figure 30. Mean urinary excretion of unchanged cetirizine over 96 hours after administration of cetirizine, 10 mg, as a single dose in patients with primary biliary cirrhosis.

unchanged cetirizine in her urine over a 24 hour period was 11.2% at the end of week 1, and 11.6% at the end of week 2. Her dose was then increased to 20 mg q.24.h. Recovery of unchanged cetirizine in the urine at the end of week 3 was 13.3%.

3.5.4 Pharmacodynamic studies of cetirizine in patients with primary biliary cirrhosis.

After a single dose of cetirizine, the mean wheal areas were significantly suppressed from 1-48 h, compared to the mean pre-dose wheal areas, and the mean flare areas were significantly suppressed from 1-72 h, compared to the mean pre-dose flare area ($p<0.01$) (Figure 31).

Cetirizine subjectively relieved itching at the wheal and flare site in 5 of the 6 patients, from 2-48 h. The sixth patient had no generalized itching on the day of the study and did not perceive any itch at the wheal and flare site in the day of the study.

All patients reported transient sedation during the first six hours of the study. Four patients actually fell asleep for a mean of 2.25 h.

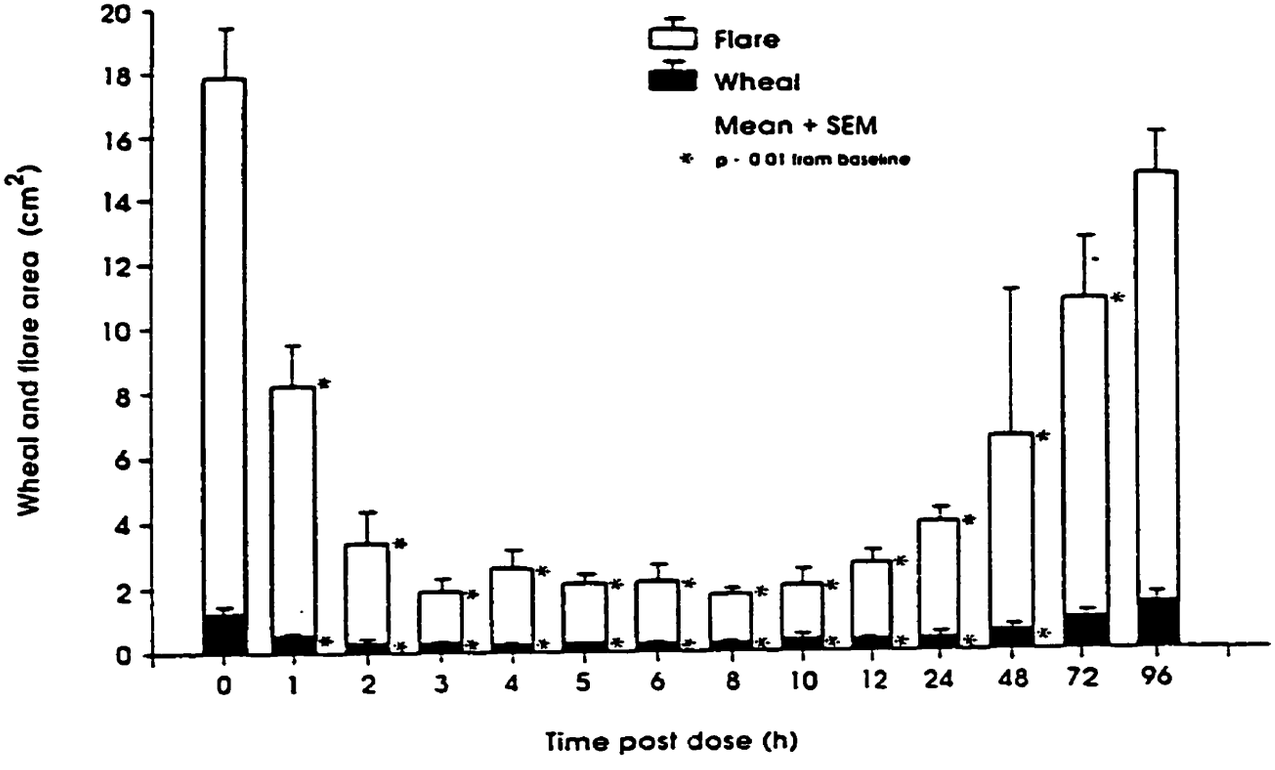


Figure 31. The mean suppression of wheals and flare induced by intradermally injected histamine after a single oral dose of 10 mg cetirizine in patients with primary biliary cirrhosis.

CHAPTER IV

DISCUSSION

4.1 The effect of cimetidine on the pharmacokinetics and pharmacodynamics of hydroxyzine in rabbits.

4.1.1 HPLC assays.

Simons *et al.* (1984) (24) described an HPLC method for measuring serum hydroxyzine concentrations with a sensitivity of 3 ng/mL. In the present study, mobile phases of different composition were evaluated to optimize the degree of resolution of hydroxyzine peaks from extraneous peaks. The mobile phase used in the present study was acetonitrile: phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.5), 35:65 v/v. The flow rate in the hydroxyzine analysis was set at 0.8 mL/min. The retention times of hydroxyzine and the internal standard were 11.0 and 5.0 minutes respectively.

Serum and urine cetirizine concentration were analyzed also using HPLC methods developed previously in our laboratory (24). The lower limit of sensitivity for cetirizine was 2.0 ng/mL.

Within the concentration range of 5 ng/mL to 200 ng/mL for both hydroxyzine and cetirizine, the calibration curves were linear (Fig. 9,27,28), with a coefficient of variation at all concentrations of less than 15 % over a 6 month period (Tables 1, 42, 43).

4.1.2 Pharmacokinetics of hydroxyzine.

The co-administration of cimetidine with hydroxyzine to increase the anti-histaminic effect has been studied extensively in humans (55), but no serum hydroxyzine concentrations were measured in these investigations. In 1986, Salo and his colleagues showed the combination of hydroxyzine and cimetidine to be more effective than hydroxyzine alone in the treatment of chronic urticaria. They reported plasma hydroxyzine concentrations were higher during the combination treatment than during hydroxyzine treatment alone (76).

In order to determine if this effect was a pharmacokinetic or pharmacodynamic interaction, or both, Chen *et al.* (1990) studied the effect of the co-administration of the H_2-receptor antagonist cimetidine with the H_1-receptor antagonist hydroxyzine in rabbits. The hydroxyzine dose used in these studies was 10 mg given intravenously. Cimetidine significantly

inhibited the elimination rate of hydroxyzine, so that serum concentrations of hydroxyzine were increased.

Suppression of histamine-induced wheals by hydroxyzine administration was used to evaluate the peripheral H_1-effect of hydroxyzine. The suppression of histamine-induced wheals by hydroxyzine administered alone ranged from 95-98 %. The co-administration of cimetidine had an effect on the pharmacodynamics of hydroxyzine. Suppression of the histamine-induced wheals increased from 98 to 100 %, but the inter-animal variability made it difficult to differentiate between the pharmacodynamic effects of cimetidine on hydroxyzine when compared to hydroxyzine administered alone.

In this present study the dose of hydroxyzine was reduced to 2.5 mg intravenously. Preliminary studies with both 2.5 and 5 mg hydroxyzine intravenous doses showed that 2.5 mg is a suitable dose to assess the effect of cimetidine on hydroxyzine pharmacokinetics and pharmacodynamics. It produced suppression of the histamine-induced wheal area which was significantly different from baseline, but this suppression did not exceed 75 %.

After the 2.5 mg dose, serum hydroxyzine concentrations were quantifiable with sufficient specificity and sensitivity for pharmacokinetic parameters of hydroxyzine to be calculated. Blood samples to measure serum hydroxyzine concentrations were withdrawn only until 6 hours after dose administration because after this time the amount of drug in serum is very small and cannot be quantified with sufficient accuracy.

When cimetidine was co-administered, the increase in the suppression of histamine-induced wheals can be clearly seen with detectable hydroxyzine serum concentration that can be measured up to 6 hours (Table 2, Fig. 10).

The dose independent parameters such as the elimination half life, clearance and volume of distribution from 2.5 mg intravenous dose of hydroxyzine HCl were equivalent to values obtained by Chen *et al.* (77) after the 10 mg intravenous dose ($p>0.05$). The area under the seum concentration versus time curve was about 1/4 the value obtained by Chen *et al.* (77) using 10 mg intravenous hydroxyzine. These values support the concept that hydroxyzine shows no dose dependent pharmacokinetics at these doses.

When 2.5 mg hydroxyzine was administered intravenously to the rabbits, it produced serum concentrations as shown in Table 2. When cimetidine was co-administered, the hydroxyzine serum concentrations

increased as shown in Table 3. The concentrations of hydroxyzine when cimetidine was co-administered were significantly higher than concentrations from hydroxyzine alone. The serum concentrations versus time plots (Fig. 10) clearly show the two curves with different areas under serum concentration versus time plots ($p<0.05$).

The pharmacokinetic parameters after administration of 2.5 mg hydroxyzine alone are shown in Table 4. When cimetidine was co-administered with hydroxyzine, these parameters were significantly different ($p<0.05$) (Table 5). The mean serum concentration at 5 min. was 73.7 ± 13.5 ng/mL. When cimetidine was co-administered, it increased to 156.1 ± 16.0 ng/mL. The mean elimination half life of 1.8 ± 0.3 h, lengthened significantly ($p<0.05$) to 3.4 ± 0.5 h when cimetidine was given with hydroxyzine.

The area under serum concentration versus time curve is the most important parameter used to relate the amount of the drug in the body to the dose administered. The mean area under serum concentration versus time curve was 140.9 ± 44.0 ng-h/mL with 2.5 mg hydroxyzine. When cimetidine was given as well, this parameter significantly increased to 613.9 ± 226.6 ng-h/mL ($p<0.05$).

When administered alone, the mean clearance of hydroxyzine (2.5 mg intravenously), 191.3 ± 56.0 mL/min decreased significantly ($p<0.05$) to 83.9 ± 55.5 mL/min. with cimetidine co-administration.

The volume of distribution of hydroxyzine after administration of 2.5 mg hydroxyzine was 52.3 ± 11.4 L. When cimetidine was co-administered, it decreased significantly to 21.5 ± 7.6 L ($p<0.05$). It is possible that the volume of distribution is reduced as a result of the method used to calculate this value from the AUC. The AUC value is a function of both dose and clearance. The apparent volume of distribution is calculated from the clearance value by taking into account the contribution by the elimination rate constant. The elimination rate constant value is also reduced as a result of the co-administration of cimetidine leading to the increase in half life. The dose remains constant, but the reduction in both the clearance value and the elimination rate constant with the increase in AUC must lead to a decrease in the apparent volume of distribution value that may have no physiological explanation.

The decrease in the volume of distribution could be the result of reduced diffusion of hydroxyzine into tissues due to the compitition with cimetidine for distribution. Another possibility is that there may have been a

loss of weight due to the treatment with cimetidine. Cimetidine is an H_2-receptor antagonist which reduces the acidity in the stomach. This might result into decreased food ingestion by the animal or reduced absorption of food ingested. Any weight loss might have been the result of reduced body fat which could affect the volume of distribution of hydroxyzine, a lipophilic molecule. Animal weight was not evaluated during the study, so there are no data to support this speculation.

During the experiment, it was observed that the handling of the animal for dosing, blood sampling and histamine testing, appears to reduce fluid intake, although water was provided *ad libitum*. Since minimal urine was produced during the study, the reduced fluid intake might also have had an effect on the reduced voume of distribution of hydroxyzine in the animal's body.

Any of the above hypotheses might contribute to the reduced volume of distribution calculated in this study following the co-administration of cimetidine with hydroxyzine.

4.1.3 Pharmacokinetics of cetirizine.

Cimetidine is an H_2-receptor antagonist which interacts with cytochrome P-450 and reduces the enzyme's capacity to metabolize other drugs (71). The imidazole nucleus of cimetidine and the cyano portion of the side chain bind to the haem protein of cytochrome P-450 and prevent it from binding with other drugs which are metabolized by cytochrome P-450 (71).

After administration of hydroxyzine, about 60 % of the dose is metabolized to cetirizine (8). Cetirizine serum concentrations after hydroxyzine 2.5 mg given intravenously are shown in Table 6. When cimetidine was co-administered with hydroxyzine, the serum concentrations of cetirizine were significantly higher ($p<0.05$) (Table 7, Fig. 11).

The pharmacokinetic parameters of cetirizine after the administration of a 2.5 mg hydroxyzine intravenously were significantly ($p<0.05$) higher than those values calculated when cimetidine was co-administered (Tables 8,9). After the administration of hydroxyzine alone, the mean maximum cetirizine serum concentration was 25.0 ± 5.1 ng/mL. When cimetidine was co-administered, the mean maximum serum concentration significantly increased to 59.4 ± 2.5 ng/mL ($p<0.05$).

The time taken to achieve the maximum serum concentration was 1.1 ± 0.2 h after hydroxyzine alone, and it significantly increased ($p<0.05$) to 2.0 ± 0.0 h when cimetidine was co-administered.

The mean elimination half life of cetirizine after the administration of 2.5 mg hydroxyzine intravenously was 1.7 ± 0.7 h which increased significantly ($p<0.05$) to 3.3 ± 0.3 h after the co-administration of cimetidine. The elimination half life values of cetirizine after the administration of hydroxyzine 2.5 mg intravenously were equivalent to the values obtained after administration of hydroxyzine 10 mg intravenously by Chen *et al.* (77).

The mean area under serum cetirizine concentration versus time curve was 83.6 ± 8.4 ng-h/mL when hydroxyzine was given alone. After the co-administration of cimetidine, this area increased significantly ($p<0.05$) to 335.9 ± 16 ng-h/mL.

When hydroxyzine is administered 60-70 % of the dose is metabolized to cetirizine. Cetirizine is also an H_1-receptor antagonist itself. When cetirizine is administered *de novo*, about 80 % of the dose is excreted unchanged in the urine (8). Some preliminary studies in the past showed no

hydroxyzine in urine in humans given hydroxyzine orally (personal communication).

In this study, the renal clearances of hydroxyzine or cetirizine were not measured. It was not possible to collect urine in a metabolic cage. Although rabbits were allowed food and water *ad libitum,* handling of the rabbits for dosing appears to reduce fluid intake. Also the lack of urine produced may be due to the anticholinergic effects of hydroxyzine which may inhibit the urinary excretion.

Renal excretion of most drugs is achieved by glomerular filtration and the active tubular secretion. Renal tubular secretion has been shown to require two active transport mechanism, one for weak acids such as has been shown for penicillin and probenecid (87), and one for weak bases. Cimetidine has been shown to reduce the clearance of weak basic drugs by competition for the active tubular secretion pathway for weak bases as shown for procainamide and it's metabolite N-acetyl procainamide (68).

Cetirizine is predominately excreted as unchanged drug. Renal clearance in humans is approximately 50 mL/min. This is lower than the glomerular filtration rate of 120 mL/min. and this is indicative of passive tubular reabsorption, although active secretion may occur as well (93).

The R value is the ratio of cetirizine $AUC_{0-\infty}$ to hydroxyzine $AUC_{0-\infty}$. With cimetidine it was expected that the R value will be reduced as the metabolism of hydroxyzine to cetirizine is reduced, yielding an increase in hydroxyzine AUC and a decrease in cetirizine AUC. However, in this study when cimetidine was given, the AUC of both hydroxyzine and cetirizine were significantly increased, so that the ratio did not change significantly (Tables 8,9). This is a further support for the decrease in the elimination of cetirizine.

It was also found by Chen *et al.* 1990 (77) that serum levels of cetirizine were significantly higher when cimetidine was co-administered with hydroxyzine compared to the control group. We speculate that cimetidine competes with cetirizine for the active tubular secretion mechanisms for weak organic bases in rabbits. Cimetidine has no effect on cetirizine given orally to humans (101), so the effect in rabbits may be due to the relatively large dose of cimetidine in thses animals.

4.1.4 Pharmacodynamics of hydroxyzine.

The peripheral H_1 effects of the H_1-receptor antagonists can be monitored by evaluating the suppression of histamine-induced wheal and

flare. This technique has been used for many years and has become a standard, well-accepted technique (55).

The wheals were generated by 0.05 mL intradermal injections of histamine phosphate (1 mg/mL). Histamine increases the permeability of post capillary venules leading to the passage of plasma proteins and fluids to extracellular spaces, resulting in edema and the formation of a wheal (8). Due to the pink skin colors of rabbits, only wheals can be identified. Evans Blue dye was injected into the animal to facilitate the identification of the wheal perimeters as the edematous fluid will show a dark blue colour to distinguish the area of the wheals (96). The wheal shows up as a dark blue spot against the pale blue skin.

Chen *et al.* (1990) found out that the co-administration of cimetidine with hydroxyzine caused an increase in the suppression of wheal area compared with the suppression caused by hydroxyzine alone. The 10 mg dose Chen used (77) induced maximum wheal suppression of 95-98 % . The suppression of wheals with the co-administration of cimetidine was generally greater than 98-100 %. This suppression was greater than observed when hydroxyzine was given alone, but the differences were not statistically significant. Hydroxyzine alone is a very potent H_1-receptor antagonist and

provided a considerable protection against the production of histamine-induced wheal. In this study the hydroxyzine dose was reduced to 2.5 mg which reduced the maximum suppression to 70 %. The pharmacodynamic effect was evaluated by calculating the absolute wheal area suppression compared to baseline wheal area in order to compare to Chen's results (77). Each wheal area was measured three times, and then the mean of the closest two values was taken to insure accuracy and reproducibility. The suppression in the wheal area induced by intradermally injected histamine after intravenous dose of 2.5 mg hydroxyzine was significantly less ($p<0.05$) than when cimetidine was co-administered (Fig 12). This indicated that the co-administration of cimetidine potentiates the histamine-induced wheal suppression caused by hydroxyzine.

Human skin blood vessels possess both H_1 and H_2 receptors (8). When an H_1-receptor antagonist such as hydroxyzine is used, only H_1-receptors will be blocked leaving H_2-receptors available to react with histamine. Allergic dermatitis conditions such as eczema, pruritis and urticaria are treated with oral H_1-receptor antagonists such as hydroxyzine. In many cases the H_1-receptor antagonists do not provide complete relief from the allergic disorder. An increase in the dose of H_1-receptor antagonist may improve the

treatment, but will usually increase the incidence of adverse effects such as sedation. There are H_2-receptors on the skin blood vessels which will not be blocked by H_1-antagonists and still available to react with histamine action to yield histaminergic activity.

In this investigation the effects of an H_2-antagonist alone can only be evaluated at the pre-H_1-antagonist administration baseline values. There is a slight antihistaminic effect of cimetidine at baseline. The mean wheal area at pre-H_1 dose of 1.25 ± 0.29 cm^2 (Table 10), compared to the pre-dose mean wheal area of 0.93 ± 0.15 cm^2 (Table 11), after cimetidine 100 mg/kg intravenously q 12 h for one week were significantly different ($p<0.05$). These effects could be due to the H_2-effect of cimetidine on H_2-receptors on the skin blood vessels.

Chen *et al.* (1990) did not find any effect of cimetidine at baseline (77). This may be due to the fact that the effects are very weak and may not be always detected with cimetidine.

In order to confirm and clarify these results, H_2-receptor antagonists that are reported to have no pharmacokinetic interactions with other drugs (78) and which may also not affect the pharmacokinetics of hydroxyzine such as famotidine and/or ranitidine will be evaluated

4.2 The effects of famotidine and ranitidine on the pharmacokinetics and pharmacodynamics of hydroxyzine in rabbits.

4.2.1 Pharmacokinetics of hydroxyzine.

In the studies represented in the previous section 4.1.2, the effects of cimetidine on the pharmacokinetics and pharmacodynamics of hydroxyzine described by Chen (77) were partially resolved by reducing the dose of hydroxyzine from 10 mg to 2.5 mg. To support the conclusions from results in the previous section, the effects of H_2-receptor antagonists which do not interact with cytochrome P-450 such as famotidine and ranitidine (78) on the pharmacokinetics and pharmacodynamics of hydroxyzine were evaluated and compared to the results when famotidine and ranitidine were co-administered. The control results for the pharmacokinetic studies of hydroxyzine alone for famotidine were similar to those for ranitidine (Tables 14,24).

4.2.1.1 The effect of famotidine.

The hydroxyzine serum concentration versus time curve after an intravenous administration of 2.5 mg hydroxyzine was not significantly

different ($p>0.05$) when given alone compared to results when famotidine was co-administered, (Tables 12, 13, Fig. 13).

The pharmacokinetic parameters of hydroxyzine 2.5 mg (Table 14) were not significantly different ($p>0.05$) from those obtained when famotidine was co-administered (Table 15).

The hydroxyzine serum concentration at 5 min.in the untreated group, 158.1 ± 31.9 ng/mL, was not significantly different ($p>0.05$) from the serum concentration at 5 min. when famotidine was co-administered, 131.8 ± 24.1 ng/mL. The mean elimination half life of 2.5 mg hydroxyzine of 1.0 ± 0.4 h was not statistically different ($p>0.05$) from that obtained after the co-administration of famotidine, 1.5 ± 0.6 h.

The apparent volumes of distribution were 22.9 ± 5.8 L and 37.5 ± 10.2 L before and after the co-administration of famotidine respectively, and not significantly different ($p>0.05$).

The area under serum concentration versus time curve was 153.0 ± 85.4 ng-h/mL from 2.5 mg hydroxyzine alone. With the co-administration of famotidine, the mean area, 128.2 ± 52.3 ng-h/mL, was not statistically different ($p>0.05$). The area under serum concentration versus time curve resulting from co-administration of famotidine (Fig. 14), was lower than that

of the control values, but the results were not statistically significant ($p>0.05$).

The clearance of hydroxyzine after the 2.5 mg dose was 362.6 ± 250.3 mL/min. When famotidine was co-administered, the clearance was 363.0 ± 206.5 mL/min, not significantly different ($p>0.05$).

4.2.1.2 The effect of ranitidine.

The serum concentrations after the administration of 2.5 mg hydroxyzine intravenously are shown in Table 22. When ranitidine was co-administered there was no significant difference ($p>0.05$) between the two groups (Table 23).

The pharmacokinetic parameters (Table 24) resulting from the administration of 2.5 mg hydroxyzine were not statistically different ($p>0.05$) from those obtained after the co-administration of ranitidine (Table 25).

When hydroxyzine 2.5 mg was administered alone, the mean serum concentration at 5 min. was 163.0 ± 25.3 ng/mL. After the co-administration of ranitidine, the mean serum concentration at 5 min. was 102.1 ± 52.0 ng/mL. There is a trend towards a decrease in the serum

concentration at 5 min., but the results were not statistically significant ($p>0.05$).

The mean elimination half life after administration of 2.5 mg hydroxyzine was 0.9 ± 0.2 h. With ranitidine co-administration, the mean elimination half life was 1.1 ± 0.3 h, which is not statistically significant ($p>0.05$).

The mean apparent volume of distribution was 20.4 ± 5.7 L. With ranitidine co-administration, the mean apparent volume of distribution was 34.1 ± 14.8 L, which was not statistically different ($p>0.05$).

The mean area under serum concentration versus time curve of 207.0 ± 78.3 ng-h/mL after 2.5 mg hydroxyzine and when ranitidine was co-administered, this area decreased to 134.4 ± 51.6 ng-h/mL, but the two values were not statistically ($p>0.05$) different (Fig.16). The area under the serum concentration versus time curve resulting from co-administration of ranitidine was lower than that of the control values, but the results were not statistically significant ($p>0.05$).

The mean clearance of hydroxyzine 2.5 mg was 231.0 ± 117.1 mL/min. When ranitidine was co-administered, the mean clearance was

328.4 ± 111.2 mL/min. The two mean values of the clearance rates are not statistically different ($p>0.05$).

The pharmacokinetic parameters of a drug are the major factors used to evaluate the pharmacokinetic interaction of two co-administered drugs. According to the results of this study, the pharmacokinetics of hydroxyzine did not change when famotidine or ranitidine was co-administered. This is probably due to the lack of interaction between famotidine or ranitidine and cytochrome P-450, so the metabolic capacity of the enzyme did not change (83). The metabolism of hydroxyzine by cytochrome P-450 was not inhibited by the co-administration of famotidine nor ranitidine. Famotidine and ranitidine are H_2-receptor antagonists which do not interact with cytochrome P-450 (83).

Cimetidine, an older H_2-receptor antagonist, does interact with cytochrome P-450 (88), probably due to difference in structure from famotidine and ranitidine (Fig.7). Cimetidine possesses 2 important structures for the interaction with cytochrome P-450. These are the imidazole nucleous and the cyano portion of the side chain (76). Famotidine and ranitidine do not possess either of these 2 structures (Fig. 7).

However, both famotidine and ranitidine seem to decrease $C_{5\ min}$ and AUC, but this effect was not statistically significant. We speculate that this effect could be explained by the fact that both medications famotidine and ranitidine induce the metabolism of small fraction of hydroxyzine co-administered dose. This has not been reported in other potential drug interactions studies with famotidine and ranitidine, and may need further investigation.

4.2.2 Pharmacokinetics of cetirizine.

4.2.2.1 The effect of famotidine.

The serum concentrations of cetirizine as a metabolite after the administration of 2.5 mg hydroxyzine (Table 16) were not statistically ($p>0.05$) different from those obtained following the co-administration of famotidine (Table 17).

The pharmacokinetic parameters of cetirizine before and after co-administration of famotidine (Tables 18&19) were not statistically different ($p>0.05$). The mean maximum serum concentration of cetirizine was 29.5 ± 5.8 ng/mL. When famotidine was co-administered, the maximum serum concentration was 27.3 ± 8.9 ng/mL. There was no statistical difference

between the two values ($p>0.05$). The mean time for the maximum cetirizine serum concentration to occur after the administration of 2.5 mg hydroxyzine was 0.6 ± 0.3 h compared to 0.6 ± 0.2 h when famotidine was co-administered. These results were not statistical different ($p>0.05$). The mean elimination half life of cetirizine after administration of 2.5 mg hydroxyzine was 3.0 ± 0.7 h. When famotidine was co-administered, the mean elimination half life was 3.2 ± 0.8 h. The two half life values are not statistically ($p>0.05$) different. The area under the serum concentration versus time curve of cetirizine after the administration of 2.5 mg hydroxyzine was 89.7 ± 27.4 ng-h/mL. When famotidine was co-administered, it becomes 104.4 ± 30.7 ng-h/mL. There was a slight increase in the area under serum concentration versus time curve (Fig. 14) compared to the control group, but they were not statistically different ($p>0.05$). This is consistent with the decrease in AUC of hydroxyzine when famotidine was co-administered. Famotidine may be inducing metabolism of hydroxyzine to cetirizine. This will need further study to confirm or explain these results.

4.2.2.2 The effect of ranitidine.

The serum concentrations of cetirizine after the administration of 2.5 mg hydroxyzine (Table 26) were not statisticallydifferent ($p>0.05$) when ranitidine was co-administered (Table 27). The pharmacokinetic parameters of cetirizine after administration of 2.5 mg hydroxyzine (Table 28) were not statistically different ($p>0.05$) from those parameters obtained after the co-administration of ranitidine (Table 29).

The mean maximum serum concentrations of cetirizine after the administration of 2.5 mg hydroxyzine was 30.8 ± 2.8 ng/mL. When ranitidine was co-administered, the mean maximum serum concentration of cetirizine was 27.2 ± 11.3 ng/mL. These two values of mean concentrations are not statistically different ($p>0.05$).

The mean time of maximum serum cetirizine concentration appearance was 0.5 ± 0.1 h, not statistically different ($p>0.05$) from 0.5 ± 0.1 h obtained when ranitidine was co-administered.

The mean elimination half life of cetirizine after the administration of 2.5 mg hydroxyzine was 2.6 ± 0.4 h. When ranitidine was co-administered,

the value was 2.8 ± 0.5 h. These two elimination half life values are not statistically different ($p>0.05$).

The mean area under the serum concentration versus time curve was 82.5 ± 20.7 ng-h/mL. When ranitidine was co-administered it was 95.4 ± 23.8 ng-h/mL. The area under serum concentration versus time curve of both groups were not statistically different ($p>0.05$) (Fig. 17). Again, a slight, although not statistically significant increase is observed in cetirizine AUC after ranitidine co-administration with hydroxyzine. This is also consistent with the desrease in AUC of hydroxyzine when ranitidine was co-administered. These effects were observed for famotidine as well, and may require further investigation.

4.2.2.3 Effect of cimetidine, famotidine and ranitidine on cetirizine renal clearance.

Cetirizine is the major metabolite of hydroxyzine which is also an active H_1-receptor antagonist and can be administered *de novo*. Unlike most other H_1-receptor antagonists which are extensively metabolized, it is unique in that more than 80 % of it is excreted unchanged in the urine (8). Since the pharmacokinetics of the parent drug hydroxyzine were not affected by co-

administration of famotidine or ranitidine, it would be expected that there would also be no effect on the metabolite.

The renal clearance of cetirizine was not determined in these studies due to difficulties in collecting urine. Urinary retention is one of the pharmacological adverse effects of hydroxyzine (8). When hydroxyzine is administered, about 60 % of a dose is metabolized to cetirizine.

In the cimetidine interaction study, a dose of 100 mg/kg cimetidine was used. For a rabbit of approximately 5 kg weight, a dose of 500 mg would be administered. Chen (77) studied the effect of cimetidine 100 mg/kg administered intravenously on the pharmacokinetics and the pharmacodynamics of hydroxyzine. The pharmacokinetics of the active metabolite cetirizine which is primarily excreted as unchanged drug were not expected to change.

The serum concentrations of cetirizine from hydroxyzine after the co-administration of cimetidine were higher than the control study. Also, when cetirizine was administered *de novo,* co-administration of cimetidine also increased the serum cetirizine concentrations and decreased the rate of elimination (77). This effect was not observed in humans (101). It was

concluded that cimetidine might compete with cetirizine for active tubular secretion in the kidney.

About 80 % of a dose of cetirizine is excreted as unchanged drug in urine, so 20% of a dose is eliminated by other pathways, possibly metabolism. The cetirizine metabolite (P026) which has been identified, accounted for only 1 % of the dose excreted in the feces, and was not detected in urine (91). Other metabolites were detected in urine, but accounted for less than 3 % of the dose and have not been identified to date (93). Any metabolism of cetirizine which may occur. no matter how small a fraction of the dose involved, may be inhibited by the co-administration of cimetidine, yielding increased serum cetirizine concentrations.

In this study, the serum concentrations of cetirizine as a metabolite of hydroxyzine were also higher when cimetidine 100 mg/kg was co-administered intravenously with 2.5 mg intravenous hydroxyzine. In contrast the dose of ranitidine is 1 mg/kg or approximately 5 mg/rabbit. The dose of ranitidine, 5 mg, is similar to the dose of cetirizine. The serum concentrations of cetirizine before and after co-administration of ranitidine were not significantly different ($p>0.05$). This suggests that ranitidine did not compete with cetirizine for active tubular secretion or have any effect on the

metabolism of cetirizine. There appears to be no pharmacokinetic interaction between cetirizine and ranitidine. However, these results need to be evaluated by the administration of cetirizine *de novo* with ranitidine.

The dose of famotidine was approximately 5 mg/kg. The rabbit weights were about 5 kg, so a dose of approximately 25 mg was administered to each rabbit. The serum concentrations of cetirizine before and after co-administration of famotidine were not significantly different ($p>0.05$). This seems to indicate that famotidine did not compete with cetirizine for active tubular secretion or affect cetirizine metabolism. There appears to be no pharmacokinetic interaction between cetirizine and famotidine. However, these results need to be evaluated by the administration of cetirizine *de novo* with famotidine.

The dose of cimetidine is 20 times the dose of famotidine and 100 times greater than the ranitidine dose. The chance of competition for active renal tubular secretion pathways between cetirizine and cimetidine might be expected to be greater than might occur for cetirizine with famotidine or ranitidine. When Chen (77) studied cetirizine administered *de novo* with cimetidine, it was concluded that the prolonged cetirizine elimination

observed in his studies could be caused by the fact that cimetidine competes with cetirizine for the active weak base renal tubular secretion pathways.

4.2.3 Pharmacodynamics of hydroxyzine, and it's active metabolite cetirizine.

Famotidine and ranitidine are H_2-receptor antagonists which do not interact with cytochrome P-450 (54), and have been shown to have no significant effect ($p>0.05$) on the pharmacokinetics of hydroxyzine or cetirizine.

Neither the hydroxyzine or cetirizine pharmacokinetic parameters changed after the co-administration of either famotidine or ranitidine. These results indicated that the H_2-receptor antagonists famotidine and ranitidine did not interact with hydroxyzine or cetirizine. If there is an increase in the peripheral antihistaminic effect as measured by the suppression of the histamine-induced wheals by hydroxyzine and cetirizine when famotidine or ranitidine are co-administered, it will be evidence of the involvement of the pharmacodynamic antihistaminic effect of ranitidine and famotidine on the H_2-receptors in the skin.

4.2.3.1 The effect of famotidine.

There is a statistically significant difference in the wheal area suppression when hydroxyzine alone is compared to hydroxyzine with famotidine co-administration (Fig. 15). The effect of famotidine with hydroxyzine on the suppression of wheal area was greater than the effect of hydroxyzine alone.

The mean pre-dose wheal area at zero time in the control group was $0.91 \pm 0.7 cm^2$ (Table 20). When famotidine 1 mg/kg was administered for one week, the mean pre-dose wheal area at zero time was $0.69 \pm 0.09\ cm^2$ (Table 21). These results were significantly different ($p<0.05$). The baseline data of famotidine treated group shows suppression of wheal area even before hydroxyzine was given. This indicates the involvement of the H_2-receptor antagonist on the H_2- receptors on the blood vessels in the skin. The effect of famotidine and hydroxyzine together on wheal area suppression was significantly greater at all times up to 24 hours after hydroxyzine injection. The maximum effect was at 5 hour where the suppression was 100 %, and wheal area was zero, compared to the suppression caused by hydroxyzine alone which was 75% (Tables 20&21).

4.2.3.2 The effect of ranitidine.

Ranitidine is an H_2-receptor antagonist. In single dose studies, Millen *et al.* 1989 (94) found that ranitidine (150 mg) yielded suppression of the immediate skin reaction to histamine base (5 mg/mL), producing 22 % suppression of wheal and flare response to histamine ($p<0.05$).

The administration of 2.5 mg hydroxyzine caused significant suppression of the wheal area compared to pre-dose wheal areas from 0.25 to 24 h ($p<0.05$) (Table 30). The maximum suppression was 75 % compared to baseline values. After the co-administration of ranitidine, the suppression obtained from hydroxyzine was statistically significantly greater ($p<0.05$) (Table 31) than when hydroxyzine was administered alone. The comparison between the results in Table 30 and Table 31 serves to demonstrate that there are dramatic increases in the suppression of the wheal areas induced by intradermal injection of histamine when ranitidine was co-administered with hydroxyzine compared to the effect of hydroxyzine alone.

In the control study, before the administration of hydroxyzine, the pre-dose , mean wheal area was 1.06 ± 0.21 cm^2. In the treated group when ranitidine was co-administered for seven days, the pre-dose wheal area on

the seventh day was 0.63 ± 0.08 cm^2. There is a statistically significant difference between the two baseline values ($p<0.05$).

The maximum effect for ranitidine was similar to the effect of famotidine on the wheal area which was totally suppressed at 4.5 hours (Table 31). The increase in the suppression was caused by co-administration of ranitidine and reached the maximum effect at 4.5 hours (Fig. 18). These results show that there is a statistical increase ($p<0.05$) in the degree of suppression when ranitidine was co-administered with hydroxyzine. However, the pharmacokinetic results indicated that ranitidine did not interact with the metabolism of hydroxyzine, so the measured effects when ranitidine was co-administered are due to the effect of this H_2-receptor antagonist on the H_2-receptors on the blood vessels in the skin.

4.3 Pharmacokinetic and pharmacodynamic studies of hydroxyzine in rabbits: the effect of ketoconazole.

4.3.1 Pharmacokinetics of hydroxyzine.

Ketoconazole is an antifungal agent which interacts with cytochrome P-450 and interferes with the metabolism of many drugs including H_1-receptor antagonists such as terfenadine and astemizole (88). It inhibits the

isozyme CYP3A4 activity and reduces the rate of terfenadine biotransformation to terfenadine acid metabolite I by this enzyme. Ketoconazole possesses the capacity to modulate mixed function oxidase activity in man and experimental animals (95). The prolongation of prothrombin time in rat plasma has been attributed to inhibition of metabolism of co-administered anticoagulant. Ketoconazole has also been shown to reduce the clearance of cyclosporin (95).

Ketoconazole was used in the present studies to provide information that could be used to differentiate between the pharmacokinetic and pharmacodynamic effect of cimetidine when co-administered with hydroxyzine. Chen *et al.* (1990) proved that cimetidine interacts with hydroxyzine (77). They found that the metabolism of hydroxyzine was delayed by co-administration of cimetidine. The dose of hydroxyzine used in those studies was 10 mg. Chen was not able to differentiate as to whether the interaction between the two drugs was a pharmacokinetic or pharmacodynamic effect.

In the present study, the dose of hydroxyzine was reduced to 2.5 mg to yield a statistically significant suppression of histamine-induced wheals, and at the same time a reduced percentage of maximum suppression.

Ketoconazole is an anti-fungal agent which possesses no anti-histaminic activity, but it does inhibit some of the P-450 isozymes. Any enhanced anti-histaminic effect of hydroxyzine with ketoconazole co-administration, would be due to ketoconazole causing an increase in serum hydroxyzine concentrations, thus yielding increased anti-histaminic effects.

The H_1-receptor antagonist, hydroxyzine is metabolized by the cytochrome P-450 enzyme system. Serum concentrations (Table 32) were statistically significantly higher ($p<0.05$) when ketoconazole was co-administered (Table 33). The change in the serum concentrations of hydroxyzine when it was co-administered with ketoconazole were dramatic and resulted in changes in the pharmacokinetic parameters of hydroxyzine when compared to values obtained when hydroxyzine was administered alone (Tables 34, 35).

The mean serum concentration at 5 min. after administration of 2.5 mg hydroxyzine was 73.7 ± 13.5 ng/mL. When ketoconazole was co-administered, the serum concentration at 5 min. increased significantly ($p<0.05$) to 156.3 ± 23.4 ng/mL.

The mean serum elimination half life after administration of 2.5 mg hydroxyzine alone was 1.8 ± 0.3 h. When ketoconazole was co-

administered, the mean elimination half life increased to 3.9 ± 0.9 h which is significantly different ($p<0.05$) from when hydroxyzine was administered alone.

The mean apparent volume of distribution after administration of 2.5 mg hydroxyzine was 52.34 ± 11.40 L. This value is statistically significantly different ($p<0.05$) from that of 29.10 ± 73.50 L after co-administration of ketoconazole.

The mean area under serum concentration versus time curve of hydroxyzine 2.5 mg was 140.9 ± 44.0 ng-h/mL. When ketoconazole was co-administered, the area under serum concentration versus time curve increased significantly ($p<0.05$) to 412.1 ± 42.1 ng-h/mL (Fig. 19).

The mean clearance of hydroxyzine in the control group was 318.8 ± 93.3 mL/min. When ketoconazole was co-administered, the clearance was significantly reduced to 101.8 ± 9.7 mL/min ($p<0.05$).

From the above results it can be determined that there is an interaction between ketoconazole and hydroxyzine in rabbits. Ketoconazole is an enzyme inhibitor that inhibits the isozyme CYP3A4 activity (88).Since the metabolism of hydroxyzine is inhibited by ketoconazole, this suggests that the isozyme CYP3A4 plays an important rule in the metabolism of

hydroxyzine. The isozyme CYP3A4 could be one of several isozymes that contribute to the metabolism of hydroxyzine.

4.3.2 The pharmacokinetics of cetirizine.

The serum concentrations of cetirizine, the active metabolite of hydroxyzine were also statistically higher ($p<0.05$) when ketoconazole was co-administered (Tables 36,37).

The pharmacokinetic parameters of cetirizine as a metabolite after administration of hydroxyzine are shown in Table 38. When ketoconazole was co-administered (Table 39), these parameters were significantly different ($p<0.05$) from the control group when hydroxyzine was administered alone.

The mean maximum serum concentrations of cetirizine after administration of 2.5 mg hydroxyzine were 25.0 ± 5.1 ng/mL. When ketoconazole was co-administered, the mean maximum serum concentration of cetirizine increased to 138.9 ± 10.8 ng/mL. The two values were statistically significantly different ($p<0.05$).

The mean time at which maximum serum concentrations of cetirizine were achieved after the administration of 2.5 mg hydroxyzine was 1.1 ± 0.2

h. When ketoconazole was co-administered, the maximum time increased significantly ($p<0.05$) to 2.0 ± 0.0 h.

The mean elimination half life of cetirizine from hydroxyzine alone was 1.7 ± 0.7 h. This was significantly shorter ($p<0.05$) than the value of 3.2 ± 0.1 h after the co-administration of ketoconazole.

The mean area under serum concentration versus time curve of cetirizine after administration of 2.5 mg hydroxyzine was 83.6 ± 8.4 ng-h/mL. After co-administration of ketoconazole, this area increased significantly ($p<0.05$) to 773.4 ± 112.9 ng-h/mL (Fig.20).

In this study, when hydroxyzine was administered alone, the R value (the ratio of cetirizine $AUC_{0-\infty}$ to hydroxyzine $AUC_{0-\infty}$) was 0.6 ± 0.2. When ketoconazole was co-administered, the R value was dramatically increased to 1.9 ± 0.4. This indicated that the elimination of cetirizine was inhibited by the co-administration of ketoconazole, yielding a significant increase in cetirizine AUC.

These results indicate that ketoconazole induced significant changes in the pharmacokinetics of cetirizine after hydroxyzine administration. Ketoconazole interacts with cytochrome P-450, and is a metabolic inhibitor in human and animals (95). D'mello *et al.* 1989 (99) found that ketoconazole

did not significantly alter the excretion of cyclosporin and various cyclosporin metabolites in bile/urine mixture. Inhibition of hepatic drug metabolizing enzymes appears to be the primary reason for the ketoconazole induced elevation in cyclosporin concentrations. No intact ketoconazole has been shown to be excreted in the urine and only very small amounts in bile (100). There appears to be no chance for renal competition between ketoconazole and other drugs and the same is probably the case for cetirizine.

Ketoconazole probably does not have any effect on the active tubular secretion of cetirizine unlike the explanation proposed for the effect of cimetidine on cetirizine elimination. Cetirizine is mainly excreted unchanged in urine (80 %), but the rest is probably metabolized. The metabolite (P026) has been identified, but accounts for only 1 % of the dose excreted in the feces probably via biliary secretion, and is not seen in urine (91). Ketoconazole may have an effect on the fraction of a cetirizine dose which does undergo metabolism. Other cetirizine metabolites have been observed in urine, but account for less than 3 % of a dose of cetirizine and thus have not been identified or quantified (93).

4.3.3 Pharmacodynamics of hydroxyzine.

It was hypothesized that the suppression of the histamine-induced wheal caused by the administration of 2.5 mg hydroxyzine will be increased by co-administration of ketoconazole. The suppression by hydroxyzine of the wheal area induced by intradermally injected histamine in the control group is shown in Table 40. When ketoconazole was co-administered, the suppresion (Table 41) was significantly increased ($p<0.05$) (Fig.21).

At pre-dose, zero-time when hydroxyzine was administered alone, the mean wheal area was 1.25 ± 0.29 cm^2. After the co-administration of ketoconazole for seven days, the predose mean wheal area was 1.30 ± 0.17 cm^2 on the seventh day. There was no significant difference ($p>0.05$) in these pre-dose values. These results confirmed that ketoconazole has no effect on the suppression of the histaminic-induced wheals.

After hydroxyzine administration, the wheal suppression observed was due to the presence of hydroxyzine. The percent suppression of hydroxyzine plus ketoconazole,was statistically greater ($p<0.05$) than when hydroxyzine was given alone. These increased effects were due to the increased serum hydroxyzine concentrations because of the inhibition of

hydroxyzine metabolism by ketoconazole. The maximum suppression resulting from hydroxyzine in the presence of ketoconazole was at 5.5 hr. The wheal area at this time was 0.26 ± 0.08 cm^2 significantly smaller ($p<0.05$) than the study of hydroxyzine alone which was 0.46 ± 0.04 cm^2 at 5.5 h. The effect of ketoconazole on the suppression caused by hydroxyzine was statistically significant ($p<0.05$) at each time up to 12 h.

Serum cetirizine concentrations were also increased following the co-administration of ketoconazole with hydroxyzine. These increased serum cetirizine concentrations probably contributed to the enhanced suppression of the histamine-induced wheals when ketoconazole was co-administered with hydroxyzine.

4.3.4 The comparison between the effects of cimetidine and ketoconazole on hydroxyzine.

4.3.4.1 Pharmacokinetics of hydroxyzine.

The three serum hydroxyzine concentration versus time curves show different areas under serum concentration versus time curves (Fig 22). When the upper 2 curves, hydroxyzine plus cimetidine and hydroxyzine plus ketoconazole are compared with the lower curve, hydroxyzine alone, the

serum hydroxyzine concentrations of the upper 2 curves were statistically significant different ($p<0.05$) from the data in the lower curve. When the upper 2 plots were compared to each other, they were found to be not statistically different from each other ($p>0.05$). These results indicate that the extent to which both drugs, cimetidine and ketoconazole, impair the metabolism of hydroxyzine is similar at the dose given.

4.3.4.2 Pharmacokinetics of cetirizine.

The serum cetirizine concentration versus time curves for the two studies, hydroxyzine with ketoconazole and hydroxyzine with cimetidine, (Tables 37,7) were statistically different from each other and when compared to serum cetirizine concentration versus time curve resulting from administration of hydroxyzine alone (Tables 6,36) (Fig. 23).

The pharmacokinetic parameters of cetirizine as a metabolite in the three different groups are statistically different ($p< 0.05$) as shown in Tables 9,38,39.

Both cimetidine and ketoconazole appear to inhibit the metabolism of hydroxyzine to a similar extent since both drugs inhibit the P-450 enzyme system.

4.3.4.3 Pharmacodynamics of hydroxyzine.

When the suppression of histamine-induced wheals in Fig.22 are compared with the suppression of wheals area in Fig. 24, it can be seen that the wheal area reduced by co-administration of cimetidine was more than the reduction caused by co-administration of ketoconazole, in comparison to the reduction caused by administration of hydroxyzine alone. The wheal area was the lowest when cimetidine was co-administered compared to when ketoconazole was co-administered. These results suggest that because cimetidine is an H_2-receptor antagonist, the wheal area reduction is the sum of two effects; the metabolic inhibition of the H_1-receptor antagonist hydroxyzine which result in the increased hydroxyzine serum concentrations, yielding an increased peripheral H_1-effect, and the effect of cimetidine on the H_2-receptors on the vasculature. Ketoconazole is a metabolic inhibitor with no H_2-receptor effect (Tables 11,40,41). So the increased suppression of the histamine-induced wheal formation is due to the increased H_1-receptor effect due to the increased serum hydroxyzine and cetirizine concentrarions.

4.4 Pharmacokinetic and pharmacodynamic studies of cetirizine in patients with primary biliary cirrhosis.

4.4.1 Pharmacokinetics of cetirizine.

Cetirizine, the active metabolite of hydroxyine, is eliminated from the body primarily by excretion of unchanged drug in the urine. More than 60% of a dose is excreted unchanged in the urine within 24 hours, and only small amounts of cetirizine metabilites are recovered from blood, feces, and urine in humans (91). Mean serum elimination half-life values have been reported as 6.6-10.6 hours in healthy adults(39,91,92), 7 hours in children (93), 11.8 hours in elderly adults with a mean age of 76 years, and a mean creatinine clearance of 57.4 ± 26.3 mL/min (39), and 20.9 hours in adults with a mean age of 40 years, and moderately reduced renal function with a mean creatinine clearance of 19 mL/min (41).

In patients with hepatic dysfunction secondary to primary biliary cirrhosis, pharmacokinetic values for cetirizine were different from those reported by other investigators in young healthy subjects (Tables 45,46). The mean serum elimination half life value was prolonged, although not as greatly prolonged as in patients with renal dysfunction; clearance rates were

slower, maximum serum concentrations were higher, and the area under the serum concentration versus time curve was increased (Fig. 29). The time at which the maximum serum concentration was achieved did not differ from that found in other populations.

The fraction of the dose excreted as unchanged cetirizine in the urine was low, compared to the fraction reported in studies in young and elderly adults with normal hepatic and renal function. Age, as well as primary biliary cirrhosis may have contributed to reduced hepatic dysfunction, yielding decreased metabolism of cetirizine, and prolonged cetirizine serum elimination half-life values. Age-related decrease in hepatic function, including decreased liver blood flow, smaller liver size, diminshed number and function of hepatocytes, and reduced metabolizing capacity for H_1-receptor antagonists and for other medications have been well described (26). Any reduction in renal function was consistent with increased subject age.

One patient with severe pruritis took cetirizine, 10 mg q.24.h. for several weeks after the single dose study. At steady state, recovery of unchanged cetirizine in her urine over a 24 hour period was 11.2% at the end of week 1, and 11.6% at the end of week 2. Her dose was then increased to

20 mg q.24.h. Recovery of unchanged cetirizine in the urine at the end of week 3 was 13.3%. These multiple dose results are consistent with the results from the single dose study and confirm the reduced renal clearance in patients with primary biliary cirrhosis.

The patients of this study were considered to have normal renal function related to their age, but is reduced when compared to healthy young adults as can be noted from the reduced renal clearance (Table 46). It was expected that the amounts of excreted cetirizine unchanged in the urine would be 60-80 %. From the results shown in Table 46, this amounts was reduced to 32 ± 14% (Fig. 30). This may be due to reduced renal function of these patients comparing to healthy young adults. The disposition of the remaining 20 % of the cetirizine dose in these subjects with primary biliary cirrhosis has not been identified

4.4.2 Pharmacodynamics of cetirizine.

In healthy adults, a single 10 mg dose of cetirizine suppresses the histamine-induced wheal and flare within one hour and the suppression persists for 24 hours (92). In patients with primary biliary cirrhosis the duration of action of the medication was prolonged, as objectively measured

by suppression of the histamine-induced wheal and flare. The mean wheal areas were significantly suppressed from 1-48 h, compared to the mean pre-dose wheal area, and the mean flare areas were significantly suppressed from 1-72 h, compared to the mean pre-dose flare area ($p<0.05$) as shown in Fig. 31.

Transient sedation, uncommon in young adults after 10 mg dose, was noted by 100 % of patients with primary biliary cirrhosis. Although cetirizine is eliminated primarily by renal excretion, some further metabolism of this medication seems to occur.

4.5 Pharmacokinetics of cetirizine under perturbed conditions.

Cimetidine significantly inhibited the elimination rate of hydroxyzine in these animal studies and has been shown to have some effect in humans (101). When hydroxyzine is administered about 60-70 % of the dose is metabolized to cetirizine, which is an H_1-receptor antagonist. When cetirizine is administered *de novo,* about 80 % of the dose is excreted as unchanged drug in urine in healthy adult volunteers(8).

Renal excretion of most drugs is achieved by glomerular filtration and active tubular secretion while passive tubular reabsorption also occurs.

Renal tubular secretion has been shown to require two active transport mechanism, one for weak acids such as penicillin and probenecid, and one for weak bases. Probenecid has been shown to compete with penicillin for the active secretion process for weak acids and decrease the renal excretion rate of penicillin (87). Cimetidine has been shown to reduce the clearance of weak basic drugs by competition for the active tubular secretion pathway for weak bases as shown for procainamide and it's metabolite N-acetyl procainamide (68).

Cetirizine is predominately excreted as unchanged drug with a renal clearance rate of about 50 mL/min. Glomerular filtration, active tubular secretion and passive tubular reabsorption probably all contribute to cetirizine excretion (93). It was also found by Chen *et al.* 1990 (77) that serum cetirizine levels were significantly higher when cimetidine was co-administered with hydroxyzine compared to the results when hydroxyzine was administered alone. We speculate that cimetidine competes with cetirizine for the active tubular secretion mechanisms for weak organic bases in rabbits. This has not been shown in humans (101).

Cimetidine may also inhibit cetirizine hepatic metabolism, although this pathway accounts for a very small fraction of the elimination process.

The cetirizine metabolite (P026) accounts for only 1 % of the dose excreted in feces, and it is not detected in urine (91). However, the disposition of about 2 % of a dose of cetirizine is still unknown, so other metabolic pathways may be involved especially in this animal model. Cimetidine may affects these pathways and further studies of this effect may be required.

When famotidine or ranitidine were co-administered with hydroxyzine 2.5 mg intravenously, the serum concentrations of cetirizine before and after co-administration of ranitidine or famotidine were not significantly different ($p>0.05$). This seems to indicate that neither ranitidine nor famotidine compete with cetirizine for active tubular secretion. However, these results need to be evaluated by the administration of cetirizine *de novo* with either famotidine or ranitidine.

Ketoconazole was co-administered with hydroxyzine to get information that could be useful to differentiate between the effect of cimetidine on the pharmacokinetics and on the pharmacodynamics of hydroxyzine. The results showed that ketoconazole also induced significant changes in the pharmacokinetics of cetirizine after co-administered with hydroxyzine. The effect of ketoconazole is mainly through interaction with cytochrome P-450 in human and animals (95). No intact ketoconazole was

excreted in urine and only very small amounts in bile (100). This indicated that ketoconazole might have an effect on the 20 % of cetirizine that is possibly further metabolized. These results need to be evaluated by administration of cetirizine (*de novo)* with ketoconazole.

In patients with PBC cetirizine was administered *de novo.* In these patients the pharmacokinetic values for cetirizine were different from those reported by other investigators in young healthy subjects (Tables 45,46). The mean serum elimination half life value was prolonged, although not as greatly prolonged as in patients with renal dysfunction; clearance rates were slower, maximum serum concentrations were higher, and the area under serum concentration versus time curve was increased (Fig. 29). The time at which the maximum serum concentration was achieved did not differ from that found in other populations.

The fraction of the dose excreted as unchanged cetirizine in the urine was very low, compared to the fraction reported in studies in young and elderly adults with normal hepatic and renal function. Age, as well as primary biliary cirrhosis may have contributed to reduced hepatic dysfunction, yielding decreased metabolism of cetirizine, and prolonged cetirizine serum elimination half-life values. This condition should

theoretically have resulted in increase in renal clearance of cetirizine. This was not observed, suggesting that when liver function is decreased, any metabolism of cetirizine is reduced yielding an increase of cetirizine serum concentrations. The renal clearance of unchanged drug did not seem to be increased to compensate for this effect. There was no attempt to identify other possible metabolites. Similar effects were found in rabbits when cimetidine inhibits liver enzymes involved in the metabolism of hydroxyzine to cetirizine. However, serum cetirizine concentrations also increased suggesting that reduced liver function can reduce cetirizine elimination. Renal clearance was not evaluated in the rabbit studies.

Further studies will be required to determine why reduced liver function by disease or drugs such as cimetidine, which inhibit the metabolism of other drugs, can effect the clearance of drugs such as cetirizine which are eliminated primarily by excretion as unchanged drug.

CHAPTER V
CONCLUSION

In rabbits, the co-administration of cimetidine resulted in a significant increase in the elimination half-life, and the AUC of hydroxyzine, and a significant reduction in the systemic clearance of hydroxyzine. These is also a significant increase in the cetirizine elimination half life and AUC.

Pharmacodynamic studies showed that cimetidine contributed to the suppression of the intradermally injected histamine-induced wheals.

These results suggested that the increased therapeutic efficacy of hydroxyzine and cimetidine could be due to both the pharmacokinetic and pharmacodynamic interactions. That is, increased serum H_1-receptor antagonist concentrations and the inhibitory effect of cimetidine on the H_2-receptors on the skin vasculature.

The co-administration of famotidine or ranitidine with hydroxyzine did not affect the pharmacokinetics of hydroxyzine, but they enhanced the suppression of the histamine-induced wheal formation. It was concluded that neither of these medicatios had an effect on the pharmacokinetics of hydroxyzine. The co-administration of famotidine or ranitidine also did not

affect the pharmacokinetic parameters of cetirizine. Both famotidine and ranitidine were significantly involved in suppressing intradermally injected histamine-induced wheals at pre H_1-receptor antagonist doses. These results suggested that the enhanced therapeutic efficacy of hydroxyzine and famotidine or hydroxyzine and ranitidine was probably due to the inhibition of the H_2-receptors on the vasculature in the histamine-induced cutaneous response. This effect is not due to impairment of hydroxyzine metabolism by famotidine or ranitidine, yielding increased serum H_1-antagonist concentration.

Ketoconazole, an antifungal agent that has been shown to inhibit the metabolism of many co-administered drugs, was studied as an agent that might inhibit the metabolic disposition of hydroxyzine, and possibly also cetirizine, without itself having any inherent H_1- or H_2-receptor activity. The co-administration of ketoconazole with hydroxyzine resulted in a significant increase in elimination half-life, AUC of hydroxyzine and a significant reduction in systemic clearance of hydroxyzine.

Ketoconazole has no H_1 or H_2-receptor activity. The increase in the suppression of histamine-induced wheals when ketoconazole was co-administered with hydroxyzine was probably due to the increased serum H_1-

receptor antagonist concentration due to the pharmacokinetic interaction and the delayed hydroxyzine metabolism and clearance. The pharmacokinetic interaction caused by co-administration of ketoconazole with hydroxyzine was not significantly different from that caused by co-administration of cimetidine with hydroxyzine. However, there is a significant difference between the suppression of histamine-induced wheals caused by the co-administration of cimetidine and that caused by co-administration of ketoconazole.

It was concluded that cimetidine inhibited the metabolism of hydroxyzine, yielding increased serum hydroxyzine and cetirizine concentrations. This effect leads to increased the H_1-receptor antagonism effect, and also enhanced the suppression of histamine-induced wheals by inhibition of the H_2-receptors on the skin vasculature. These changes resulted in the enhanced suppression of the histamine-induced wheals compared to administration of hydroxyzine alone or even hydroxyzine with ketoconazole co-administration.

In humans, the pharmacokinetics and the pharmacodynamics of cetirizine in patients with primary biliary cirrhosis were studied after the oral administration of cetirizine 10 mg. It was found that the mean serum

elimination half-life value of cetirizine, 13.8 ± 1.8 h, was longer than found in healthy volunteers. The mean cetirizine clearance rate, 0.44 ± 0.1 mL/min/kg, was slower, the mean maximum serum cetirizine concentration, 498 ± 118 ng/mL, was higher, and the mean area under the curve, 6438 ± 1621 ng/mL/kg, was increased compared to the healthy young adults. However, the mean fraction of the dose excreted as unchanged cetirizine in the urine, 0.32 ± 0.14, was lower than reported for the healthy young adults. The mean wheal areas were significantly suppressed from 1-48 hours, and the mean flare areas were also significantly suppressed from 1-72 hours.

In patients with primary biliary cirrhosis, pruritus may be intractable. There are a limited number of additional therapeutic options available for the relief of intractable pruritus in these patients. Cetirizine, a potent antipruritic, should be administered with caution in patients with primary biliary cirrhosis. Although ceririzine is eliminated primarily by renal excretion, some further metabolism of this medication seems to occur in these patients and requires further investigation.

In this study we tried to evaluate the effect of the co-administration of the H_2-receptor antagonists cimetidine, famotidine and ranitidine, and the antifungal agent ketoconazole on the pharmacokinetics and the

pharmacodynamics of H_1-receptor antagonist hydroxyzine and its active metabolite cetirizine in rabbits. Also, the pharmacokinetics and pharmacodynamics of cetirizine in patients with primary biliary cirrhosis.

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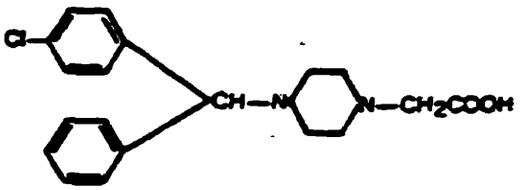
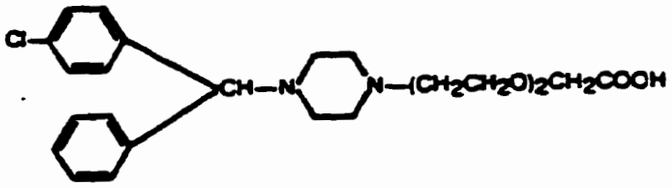
Appendix I

Chemical structure of cetirizine metabolite P026



Appendix II

Chemical structure of P_{265}



Appendix III

Chemical structure of antazoline