PHARMACOKINETIC AND PHARMACODYNAMIC EVALUATION OF THE RELATIONSHIP BETWEEN PERIPHERAL H_1-RECEPTOR BLOCKADE WITH SERUM AND SKIN CONCENTRATIONS OF H_1-RECEPTOR ANTAGONISTS HYDROXYZINE AND CETIRIZINE AFTER ORAL ADMINISTRATION IN HUMANS

AND

EVALUATION OF THE DISTRIBUTION AND EFFICACY OF HYDROXYZINE AND CETIRIZINE IN SOLUTION AND LIPOSOME FORMULATIONS APPLIED TOPICALLY TO RABBITS

BY

YI ZHENG

A thesis submitted to
the Faculty of Graduate Studies
in partial fulfilment of the requirements
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Abstract

Hydroxyzine and Cetirizine produce prompt, long-lasting peripheral H_1-blockade in skin after a single dose and are widely used to treat urticaria and other allergic skin disorders. We hypothesized that after oral dosing with these medications, skin concentrations would be higher than serum concentrations, and that liposome formulations of these agents would provide a higher efficiency and longer effective time with lower serum concentration after topical administration than aqueous solutions and these concentrations could be correlated with the extent of peripheral H_1-blockade.

In a randomized, double-blinded, parallel-group study in 13 healthy subjects, skin biopsies, venipunctures and epicutaneous tests with histamine were performed before ingestion of cetirizine 10 mg or hydroxyzine 50 mg and at 1, 3, 6, 9 and 24 hours after dosing. Subjects then took the same doses for six consecutive days. All tests were repeated 12 hours after the final dose at 168 hours. The liposome formulation tests were carried out in a four-way cross over design in six rabbits with topical application of 10 mg aqueous solution or liposome formulation of these medications on the depilated back of rabbits. Blood samples were collected following an epicutaneous tests with histamine, at pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post-dose. Extra skin tests were performed after 24 hours of dosing.

In human study, skin cetirizine concentrations were found to be lower than serum concentrations from 1-9 hours, but were higher at 24 hours after a single dose and at steady-state after multiple dose. Skin hydroxyzine concentrations were higher than serum concentrations at all times. After hydroxyzine dosing, cetirizine, the active metabolite of hydroxyzine arising *in vivo*, was found in skin and serum. Single doses of cetirizine or hydroxyzine produced significant suppression of wheals and flares from 3-24 hours, inclusively, and this was maintained at steady-state. Neither cetirizine nor hydroxyzine caused any adverse effects. Following topical liposome formulations to rabbits, lower serum hydroxyzine but higher serum cetirizine concentrations were observed and higher persisted histamine wheal suppression was observed for the liposome formulations, especially at 24 hours after topical administration.

It is concluded that cetirizine and hydroxyzine enter the skin easily and their sustained, high concentrations in skin after oral single or multiple dosing may contribute to their efficacy in symptomatic control of urticaria and other skin disorders in which histamine plays a role. Liposome formulations of these medications had a better suppression of wheal than their solutions, particularly 24 hours after their topical administration. Extremely low serum concentrations resulting from the liposome administration could prevent also systemic adverse effects.

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I would like to extend my sincere gratitude to Dr. K. J. Simons for his supervision, encouragement, patience and financial support throughout this study. I would also like to thank the other members of my advisory committee: Dr. S. Venkataram of the Faculty of Pharmacy and Dr. F. Burczynski of Faculty of Medicine for their valuable criticism in the preparation of this thesis. I am also grateful to Dr. F. E. R. Simons of Department of Pediatrics, Faculty of Medicine for her many constructive suggestions in the course of this project. Finally, I would like to thank my parents and friends for their continued concern, support, encouragement and understanding throughout my many school years.

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CHAPTER I. INTRODUCTION

1.1. Histamine

1.1.1. Chemistry

Histamine, synthesized in 1907 and later isolated from mammalian tissue, is found in most tissues of the body. Histamine, 2-(4-imidazole)ethylamine (Figure 1), is comprised of an amino group connected by a two-carbon atom chain to an imidazole ring. Both the amino and the imidazole ring nitrogen atoms of the histamine molecule are protonated in acidic solutions. Titration of histamine in aqueous solution gives three pK_a values. The first one (pK_a=5.8) corresponds to the dissociation of the ring -NH-, and the second (pK_a=9.4) corresponds to the dissociation of the side chain $-NH^+_3-$ group. In strong alkali, the ring ionizes at -N- (pK_a=14) to give an amino group (1).

Histamine is a very hydrophilic molecule. It has a strong capacity for hydrogen bonding, in which both the ammonium and imidazolium cations act as hydrogen donors and the unchanged ring acts both as hydrogen donor and receptor (2).

1.1.2. Distribution of histamine

Histamine is present in high concentrations in mast cells, in the lung and the skin. The large number of mast cells in the gastrointestinal tract also accounts for high

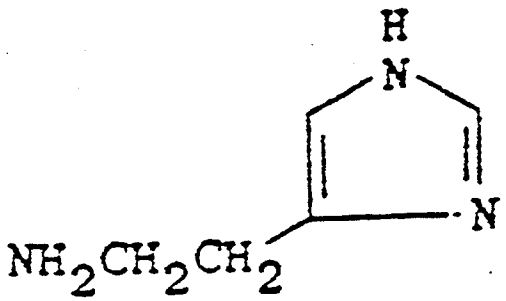

Figure 1. The chemical structure of histamine.

concentrations of histamine. Most tissue histamine exists in bound form, in granules of mast cells or basophils (3).

Histamine is released from its storage sites as the free active form by a secretory process during inflammatory and allergic reactions.

1.1.3. Histamine receptors and antagonists

Receptors may be identified by exposure to specific agonists and antagonists in the living animal. Histamine, when released from its storage sites, exerts its physiological effects through three well-defined subclasses of histamine receptors: H_1-receptors, H_2-receptors,and H_3-receptors. Additional types or subclasses of histamine receptors may exist (4) (Table 1).

1.1.3.1. H_1-receptor and antagonists

Effects mediated via H_1-receptors include increased vascular permeability, pruritus, contraction of smooth muscle in the respiratory and gastrointestinal tracts, release of mediators of inflammation, and recruitment of inflammatory cells. Histamine induces vasodilation and increases vascular permeability via both H_1- and H_2- receptors. These functions are important in the local cutaneous wheal and flare response, in urticaria, and in production of symptoms of anaphylaxis, such as hypotension, flushing, headache and tachycardia. The

Table 1. Histamine-receptor

	H_1	H_2	H_3
Location	bronchial & Gastrointestinal smooth muscle, brain	gastric mucosa, uterus, brain	brain, bronchial smooth muscle
Function	↑vascular permability ↑pruritus constrict smooth muscle ↑cyclic GMP ↑prostaglandin generation ↓atrioventricular node conduction time activation of airway vagal afferent nerves ↑hypotension ↑flushing ↑headache ↑tachycardia ↑release of mediators of inflammation* ↑recuitment of inflammatory cells*	↑gastric acid secretion bronchial smooth muscle relaxation ↑cyclic AMP ↑mucous secretion esophageal relaxation stimulation of T-suppressor cell ↓basophi histamine release ↓neutrophil and basophil chemotaxis and enzyme release ↑hypotension ↑flushing ↑headache ↑tachycardia	vasodiation of cerebral vessels prevents excessive bronchoconstriction
Agonists	2-methylhistamine betahistamine 2-pyridylethylamine 2-thiazolylethylamine	4(5)-methylhistamine betazole dimaprit impromidine	α-methylhistamine
Antagonists	chlorpheniramine diphenhydramine hydroxyzine terfenadine astemizole loratadine cetirizine	cimetidine ranitidine famotidine nizatidine etintidine	thioperamide impromidine

*not antagonized by all H_1-receptor antagonists

affinity of histamine for H_1-receptors in the vasculature is approximately 10 times the affinity for H_2-receptors in the vasculature (5).

Numerous H_1-receptor antagonists have emerged since 1930's. These drugs have considerable differences in their basic pharmacology, clinical pharmacology, pharmacokinetics, pharmacodynamics and benefit/risk ratio. Many old first-generation H_1-receptors antagonists are no longer used in the clinical treatment of allergic disorder, but some of them, such as chlorpheniramine, diphenhydramine and hydroxyzine are still used due to their effectiveness, availability of parenteral formulations and low cost. The new non-sedating second-generation H_1-receptor antagonists, such as astemizole, terfenadine, loratadine, and cetirizine, are now widely used for prevention and relief of sneezing, itching and rhinorrhoea in patients with allergic rhinoconjunctivitis.

1.1.3.2. H_2-receptor and antagonists

The best known effect of histamine via H_2-receptor activation is the increase in gastric acid secretion, while other actions of histamine mediated by H_2 receptors may be of considerable importance in allergic disorders. Activation of H_2-receptors may produce bronchodilatation and increase mucous secretion.

Specific H_2-receptor antagonists such as cimetidine,

ranitidine, and famotidine have revolutionized the treatment of peptic ulcer disease and related acid-peptic disorders. The role of H_2-receptor antagonists in the treatment of immediate hypersensitivity disorder and of primary and secondary immune deficiency diseases is being elucidated (6).

1.1.3.3. H_3-receptors and antagonists

In the 1980's it was observed that histamine inhibits its own synthesis and release in brain slices by a negative feedback process operating at the level of histaminic nerve-endings (7). It was suggested that these actions of histamine are mediated by a novel subclass of receptors, the H_3 receptor, and specific agonists and antagonists were later identified. It is clear now that H_3-receptors are involved in the negative feedback control of histamine synthesis and release, in inhibition of sympathetic neurotransmission in perivascular nerves, and in vasodilation of cerebral vessels. Recently, H_3-receptors activated by low concentrations of histamine have been found to inhibit acetylcholine release from vagal nerves in human airways and also inhibit neurogenic microvascular leakage in the airways. The physiologic significance of these H_3-receptors is still uncertain, but they may play some defensive role against excess bronchoconstriction (7).

1.2. Allergic Reaction

1.2.1. Histamine Release

The release of histamine is induced when an antigen, such as animal dander, pollen, food, stinging insect venom, or certain drugs, such as penicillin, and reaginic antibodies (IgE) attached to the mast cell and basophils surface. The ensuing process involves a series of reactions that require calcium and metabolic energy and terminates with the extrusion of the contents of secretory granules by the process of exocytosis. Histamine can also be released directly from its storage sites as the active form by some drugs such as opiates, peptides, and other chemical agents such as compound 48/80 and other surfactants.

In other clinical conditions, such as cold urticaria, vibratory urticaria and solar urticaria, histamine can be released in susceptible patients. Histamine release also occurs whenever there is nonspecific cell damage from any cause. Histamine also plays a primary role in allergic rhinitis, anaphylaxis, and to a lesser degree, asthma.

1.2.2. Immediate Skin Reaction

The immediate reaction in skin depends on mast cells that are rapidly degranulated after allergen challenge (8). Histamine release begins about five minutes after injection of allergen and reaches the peak effect within 30 minutes (9).

The injection of histamine into skin by the epicutaneous prick test or intradermal technique mimics the allergen-induced wheal-and-flare reaction well (10).

The relationship between neurogenic reflex and cellular inflammation in the generation of the cutaneous immediate hypersensitivity reaction is beginning to be understood. The skin contains a rich supply of nerve fibres. The terminal arborization of C-fibres contains substance P, neurokinin A and calcitonin gene-related peptide (CGRP) (11). Some vascular effects of inflammation in the skin are neurogenic. Substance P produces dose-related wheal-and-flare reactions in human skin. Histamine, more than any other mediators, can trigger the release of substance P by axon reflex, and this neuromediator enhances the immediate reaction by causing the release of histamine by the skin mast cell (12).

Clinical expression of allergic diseases actually depends on the actions of multiple mediators, many of which are derived from mast cells. Histamine is prominent among them. Histamine, through its H_1-receptors, can mediate all three pathologic components of urticaria: pruritus, vasodilation, and increased vascular permeability involving the superficial dermis. Classical histamine H_1-receptor antagonists therefore represent powerful drugs in the treatment of acute urticaria reactions.

Some experiments have shown that a histamine H_2-receptor

exists on human skin vessels and that by application of an additional H_2-receptor antagonist flush and itch reaction can be treated more effectively than when H_1-receptor antagonists are used alone (13).

1.3. H_1-receptor antagonists

1.3.1. Antiallergic effects

H_1-receptor antagonists (H_1-RA) have been widely used in the treatment of allergic disorders such as rhino-conjunctivitis and urticaria for more than five decades. In recent years, relatively non-sedating H_1-receptor antagonists have become available and have caused renewed interest in this class of medication.

In vitro, many H_1-receptor antagonists have been shown to prevent the release of mediators of inflammation from human basophils and mast cells. These effects vary with the stimulus for mediator release, the mediator being measured, and the H_1-antagonist concentrations (14).

In vivo, pretreatment with some second-generation H_1-receptor antagonists decreases mediator release after antigen challenge to the skin of patients naturally sensitized to the antigen. For example, pretreatment of the skin with cetirizine decreases the amounts of histamine and platelet-activating factor release, and inhibits the migration of eosinophils, neutrophils, and basophils induced by antigen or by platelet-

activating factor (15).

First generation histamine H_1-receptor antagonists are no longer widely used in the treatment of allergic rhinoconjunctivitis or chronic urticaria. They are still used in patients with atopic dermatitis, in whom the antipruritic effect of the newer medications had not been satisfactorily demonstrated (16), and as adjunctive medication in patients with anaphylaxis who require parenteral formulations of H_1-receptor antagonists (17).

Some of the first-generation H_1-RA are disappearing from clinical use because of their short serum elimination half-life values and their relative lack of efficacy combined with a high incidence of sedation and other adverse effects. Other first-generation H_1-RA, such as chlorpheniramine and hydroxyzine, continue to be used because of their availability in formulations for parenteral use, relatively high benefit-risk ratio, long half-life and suitability for once-daily administration (18,19).

The second-generation histamine H_1-receptor antagonists are now widely used for prevention and relief of sneezing, itching and rhinorrhoea in patients with allergic rhinoconjunctivitis and for relief of pruritus in patients with urticaria. In asthma patients, they have a mild dose-related bronchodilator effect, and they protect against bronchoconstriction produced by stimuli such as exercise,

inhalation of cold, dry air, and histamine, but they have been shown to be less effective in the treatment of atopic dermatitis (20-22).

1.3.2. Measurements of H_1-receptor antagonists

Most H_1-receptor antagonists could be measured by high performance liquid chromatography (HPLC), radioimmunoassays (RIAs) and gas liquid chromatography (GLC) (Tables 2 and 3) (4). Major second generation H_1-receptor antagonists present low concentrations in serum and are quantitated using RIAs in which there may be cross-reactivity of the active metabolite with the parent compound, such as terfenadine acid metabolite with terfenadine. HPLC methods are used to measure those serum concentrations which are high after intentional overdose or in situations where the metabolites may be present in much higher concentrations than the respective parent compounds. The only second-generation H_1-receptor antagonist currently readily measurable by HPLC or GLC after a single dose is cetirizine, which is, itself, the active carboxylic acid metabolite of the first-generation H_1-antagonist hydroxyzine (4).

1.3.3. Pharmacokinetics of H_1-receptor antagonists

The pharmacokinetics of H_1-receptor antagonists have been thoroughly studied in the past ten years (Tables 2 and 3) (4) and the knowledge obtained has influenced the dosage regimens (23-25).

Table 2. Pharmacokinetics and pharmacodynamics of first-generation H_1-receptor antagonists (4)

H_1-receptor antagonist (metabolite)	C_{max}(ng/ml)	$t_{1/2}$(h)	Cl(/ml/min/kg)	V_d(L/kg)	Protein binding(%)	Assay
Chlorpheniramine	na	22.3	6	3.4	na	HPLC
	2.8(0.8)	27.9(8.7)	na	na	na	HPLC
	na	24.4(6.6)	7.8(1.8)	5.9(0.9)	na	HPLC
		21.0(4.9)	4.4(1.4)	7.7(2.1)	na	HPLC
Hydroxyzine	2.1(0.4)	20.0(4.0)	9.8(3.3)	16.0(3.0)	na	HPLC
	na	14	69.3(25)(L/h)	na	na	GLC
Diphenhydramine	1.7(1.0)	9.2(2.5)	23.3(9.4)	14.6(4.0)	na	HPLC
	na	8.5(1.0)	6.2(0.5)	na	na	GLC
	2.3(0.2)	9.2(1.2)	na	na	na	GLC

Table 3. Pharmacokinetics and pharmacodynamics of second-generation H_1-receptor antagonists (4)

H_1-receptor antagonist (metabolite)	t_{max}(ng/ml)	$t_{1/2}$(h)	cl(/mL/min/kg)	Vd(L/kg)	Protein binding(%)	Assay
Terfenadine	078-1.1	16-23	na	na	97	RIA
(terfenadine carboxylic acid	3	17(metabolite)	35.9-41.2	na	70	HPLC
Astemizole (desmethylastemizole)	0.5(0.2)	24	1.5 L/h	na	96.7	RIA
	0.7(0.3)	9.5days(metabolite)	na	na	na	
Loratadine (descarboethoxyloratadine)	1.0(0.3)	11.0(9.4)	202	na	97-99	RIA
	1.2(0.3)	7.8(4.2)	142			
	1.5(0.7)	17.3(6.9)(metabolite)	na	na	23-70	HPLC
	2.0(1.3)					RIA
	1.6	14.4	na	na	na	HPLC
	3	18.7 (metabolite)			na	HPLC
Cetirizine	1.0(0.5)	7.4(1.6)	na	0.4(0.1)	93	HPLC
	1.2(0.3)	10.9(2.2)	1.0(0.2)	na		GLC
	1	6.7, 7.7	0.93(0.99)	na		GLC
	1.1(0.2)	10.6(0.5)	0.64(0.04)			GLC
Azelastine (demethylazelastine)	4 to 5	25	na	na	78 to 88	HPRIAC
	4.2(2.3)	42(metabolite)	na	na		RIA
	na	22(5)	8.9(3.5)	14.5(3.9)	na	HPLC
	5.3(1.6)	22(4)	8.5(3.2)	14.5(4.9)		HPLC
	34.5	54(18)(metabolite)				HPLC
	20.5	54(15)(metabolite)				HPLC
Ebastine (carebastine)	3.6(1.1)	10.3(2.6)(metabolite)	na	na	na	HPLC
	3.7(0.8)	12.5(1.9)(metabolite)		na	na	HPLC

H_1-receptor antagonists are well absorbed when administered orally with peak serum concentrations being reached approximately 2 hours after dosing in fasting patients. The low serum concentrations measured after a single oral dose may suggest considerable first-pass hepatic extraction and extensive distribution into body tissues. The volume of distribution reported for most second-generation H_1-receptor antagonists, except for azelastine (26-28), are uncorrected for bioavailability, because intravenous formulations are not available for comparison with oral formulations.

Serum elimination half-life values of the second-generation H_1-receptor antagonists are extremely variable in young adults, ranging from less than 7 hours for cetirizine to 1.1 days for astemizole. The elimination half-life of active metabolites may also differ from those of the parent compound, such as 1.1 days for astemizole and 9.5 days for its active metabolite, desmethylastemizole. Children generally have shorter H_1-receptor antagonist half-life values than that of adults, while the longer serum half-life values and correspondingly slower clearance rates in elderly patients are attributed to age-related decreases in hepatic function including decreased liver blood flow, reduction in liver size, and diminished number and metabolizing capacity of hepatocytes (29,30).

1.3.4. Pharmacodynamics of H_1-receptor antagonists

In most studies of the efficacy of H_1-receptor antagonists, effectiveness has been assessed using subjective scoring systems for symptoms such as nasal itching, relief of pruritus in patients with urticaria, and have shown weak correlations with serum concentrations. The efficacy of H_1-receptor antagonists has been assessed by using suppression of the histamine- or antigen-induced wheal-and-flare response in order to get possible dose-effect relationship. This well-defined standardized biologic assay is valid in patients of all ages, and could be used to monitor the dose-peripheral antihistaminic effect relationship of H_1-receptor antagonists. It has been widely used when corrected with serum H_1-antagonist concentration measurement (31,32).

1.4. Hydroxyzine

1.4.1. Chemistry and pharmacology of hydroxyzine

Hydroxyzine hydrochloride, 2-[2-[4-[(4-chlorophenyl)-phenylmethyl]-1-piperazinyl]ethanol dihydrochloride(Figure2), a member of the piperazine class of H_1-receptor antagonists is a strong antipruritic and antiwhealing agent and is often recommended as the antihistamine of choice in treatment of allergic skin disorders (19). It is also one of a few first-generation H_1-receptor antagonists which is still used clinically due to its relative long elimination half-life and

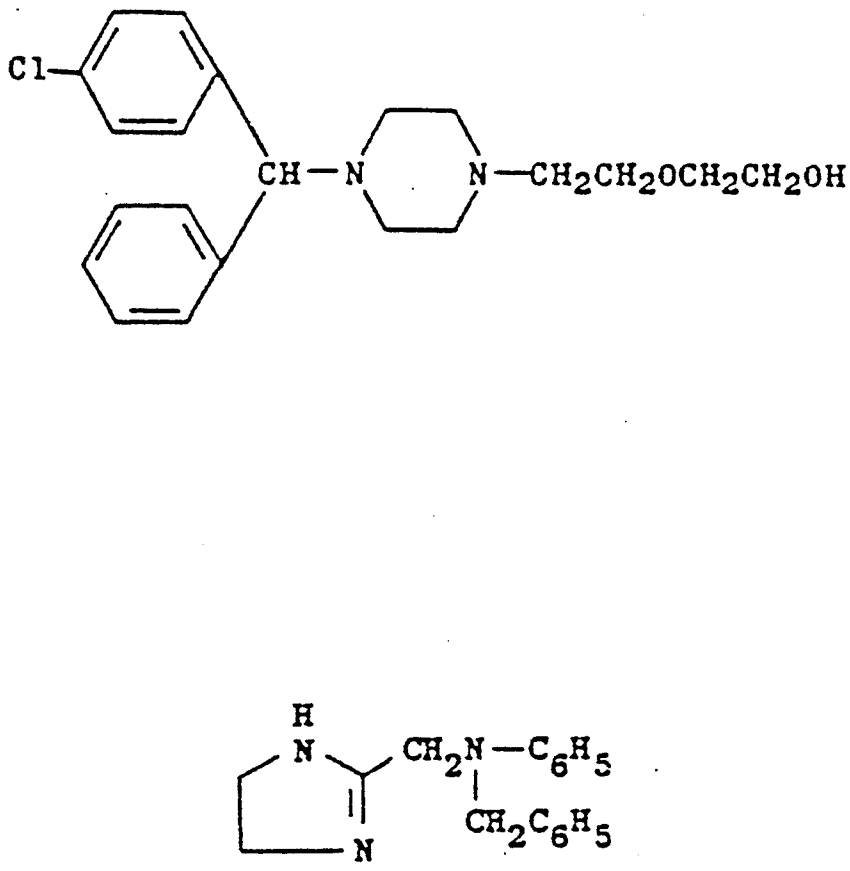

Figure 2a. The chemical structures of hydroxyzine (upper) and its internal standard antazoline (below);

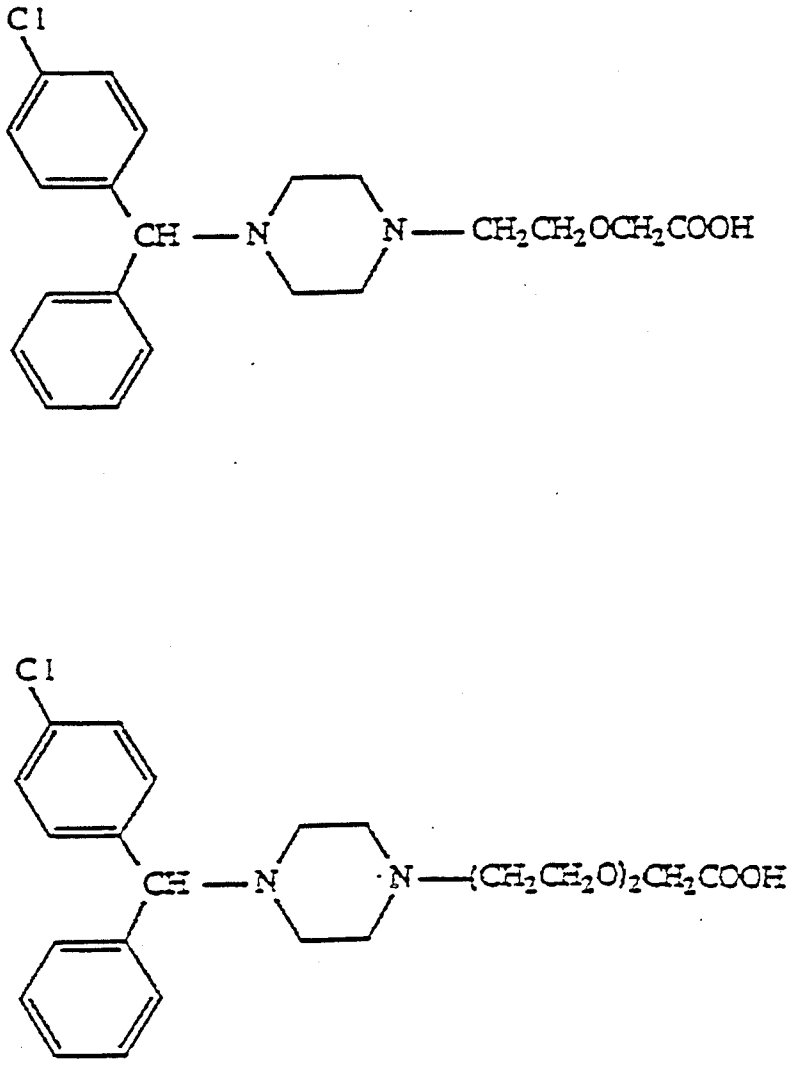

Figure 2b. The chemical structures of cetirizine (upper) and its internal standard P_{265} (below).

the availability of a parenteral formulation.

1.4.2. Pharmacokinetics of hydroxyzine

In a human study, following a single oral dose of hydroxyzine, 0.7 mg/kg, to seven healthy adults, the mean maximum serum hydroxyzine concentration of 72.5 ± 11.1 ng/ml occurred at a mean time of 2.1 ± 0.4 hour. The mean elimination half-life calculated from the terminal linear portion of the serum hydroxyzine concentration vs. time curve was 20.0 ± 4.1 hour. The mean clearance rate was 9.78 ± 3.25 ml/min/kg and the mean apparent volume of distribution was 16.0 ± 3.0 L/kg (19).

1.4.3. Pharmacodynamics of Hydroxyzine

Wheals and flares were significantly suppressed ($p<0.05$) from 1 to 36 hours and 60 hours respectively after the oral administration of hydroxyzine 0.7 mg/kg in young healthy adults. Maximal suppression of the wheals was 80% and the maximal suppression of the flares was 92% (19).

1.5. Cetirizine

1.5.1 Chemistry and pharmacology of cetirizine

Cetirizine, [2-[4-[(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]acetic acid (Figure 2), a piperazine derivative and carboxylic acid metabolite of hydroxyzine, is a potent

histamine H_1-receptor antagonist with anti-allergic properties. It has marked affinity for peripheral histamine H_1-receptors and at the standard dose of 10 mg daily, shows a marked lack of the central nervous system depressant effects of first-generation H_1-receptor antagonists. In addition, it inhibits histamine release and eosinophil chemotaxis during the secondary phase of the allergic response (23).

1.5.2. Pharmacokinetics of cetirizine

Following a single 10 mg oral dose of cetirizine to healthy volunteers, peak plasma concentration of 257 ng/ml occurred within 1 hour of administration, the area under the curve (AUC) was 2.87 mg/l.h. The apparent volume of distribution of cetirizine at steady-state is 30 to 40 L/kg. In animal studies, the peak cetirizine concentration in brain is less than 10% of plasma concentration, which results in minimal CNS adverse effects. The terminal phase elimination half-life is 7 to 10 hours in healthy adults. Approximately 70% of a dose of cetirizine is excreted in the urine, mainly as unchanged drug, although small amounts of unidentified metabolites are found. The total body clearance is 0.04 to 0.05 L/h/kg.

The available evidence suggests that cetirizine is a well tolerated and effective agent for the treatment of allergic rhinitis, rhinoconjunctivitis, chronic idiopathic urticaria

and allergen-induced asthma. Cetirizine does not cause a significant increase in the incidence of any adverse effects in comparison with placebo, specifically sedation (23).

1.5.3. Pharmacodynamics of cetirizine

Pharmacodynamic studies have indicated that cetirizine produces a more effective and longer lasting inhibition of the cutaneous response to histamine than standard therapeutic doses of loratadine. In human studies(33), suppression of the wheal response to intradermally injected histamine were greater with cetirizine 10 mg than with placebo, peaking at 4 to 8 hours and lasting to 24 hours. Moreover, there is some indication that cetirizine may inhibit later-phase histamine, C_4 and PGD_2 release and eosinophil chemotaxis during the allergic response.

1.6. Skin, the target tissue in the animal study

1.6.1. Structure of skin

Mammalian skin is composed of three distinct layers: 1.the epidermis or outer layer, 2. the dermis and 3. the subcutaneous tissue.

The skin's outer layer or epidermis serves to limit free passage of fluid, electrolytes and other substances from the highly hydrated underlying tissue to the outside environment. As well, the epidermis limits the entry of foreign substances

and chemicals from the skin surface to the dermis and subcutaneous tissue.

Directly below the epidermis is a heterogeneous layer known as the dermis. This structure consists primarily of interlacing bundles of collagenous fibrous connective tissue and elastic fibres suspended in a gel of so called ground substance.

The subcutaneous tissue, the third principal layer of the skin, consists of varying amounts of fat tissue and serves primarily as a cushion against trauma and as a pad of insulation around the body.

1.6.2. Route of penetration through the skin

In *vivo*, percutaneous absorption is a complex biological process. The skin is a multilayered biomembrane with unique absorption properties.

Flynn (34) has described the process of percutaneous absorption as follows: "When a drug system is applied topically, the drug diffuses passively out of its carrier or vehicle and into the surface tissue of skin, specifically and most importantly the stratum corneum and the sebum filled pilosebaceous gland ducts. A net mass movement continues through the full thickness of the stratum corneum and ducts into viable epidermal and dermal strata."

It is well known that the barrier function of the skin

for most substances resides principally in the stratum corneum, the outermost horny layer of epidermis. It is also believed that once the stratum corneum has been penetrated, there is little resistance for drugs to penetrate through other layers of the skin until it ultimately reaches the systemic circulation through the capillary network in the papillary layers of the dermis.

Hair follicles and sweat glands of the skin represent potential parallel pathways or shunts for diffusion of drugs through skin. These shunts present a faster penetration route because of their high permeability. However, they present only a very small absorption surface, which is 100 to 10,000 times less than that of stratum corneum (35).

1.7. Liposome, the drug delivery system

1.7.1. Structure and properties of liposome

There are two ways to improve chemotherapy, discover new, better drugs or chemical entities or develop new, controlled, site-specific drug delivery systems. The emphasis in pharmaceutical research is on developing new delivery systems that enhance the efficacy and safety of existing drugs.

The aim of "Drug targeting" is to deliver the drug specifically to the target organ, to the site of action without dispersion throughout the whole body. If local activity is desired, one can expect to achieve drug targeting

by applying the drug directly to these organs. However, in many cases, the drug applied to the surface of those organs may not penetrate enough to reach the effective concentration within the organ or the drug may penetrate the organs with ease, but be quickly removed by the blood and/or lymph systems,which may lead to systemic rather than the desired local action(36).

Liposomes have attracted considerable interest as a drug delivery system in recent years. Liposomes are microscopic vesicles composed of membrane-like lipid layers surrounding aqueous compartments. The lipid layers are made up mainly of phospholipids, which are amphiphilic, having a hydrophilic head and a lipophilic tail. Liposomes contain various domains for encapsulating active drugs. Lipophilic compounds can be integrated into liposome membranes. Proteins, such as antibodies have been embedded in the outer membrane after formation to target liposome to specific organs following systemic administration. Water soluble agents can be captured in the internal aqueous spaces between membranes, and in the core (37).

Liposomes can be prepared in varying size, composition, and surface charges. They are classified as unilamellar if they are composed of a single membrane or multilamellar if the membranes are layered like onion skin. Liposomes range in size from less than 0.05 μm to more than 400 μm in diameter.

1.7.2. Topical application of liposome

Liposomes can be applied topically for different purposes. Their effectiveness depends upon their penetration and mechanism of drug delivery.

Mezei and Gulasekharam (38,39) first reported in 1980 that the corticosteroid triamcinolone encapsulated in liposomes and applied topically to rabbit skin was more concentrated in the epidermis than after "free" drug application. Later, similar results on human skin were reported by using the anesthetic tetracaine (40) and hydrocortisone (41).

Liposomal-encapsulated econazole and progesterone have also been shown to be superior over their conventional dosage forms in the treatment of patients suffering from mycoses and idiopathic hirsutism, respectively (42,43). These observations demonstrated that liposomal formulations may improve the use of potent glucocorticoids considerably by enhancing their therapeutic activity and minimizing their systemic and local side-effects.

In general, liposomes penetrate the skin, carry their content to the skin, can serve as a slow-release dosage formulations and can reduce or increase systemic absorption.

1.8. Toxicity of topical antihistamines

In a discussion of topical treatment, it should be kept

in mind that the target tissue is usually the skin itself, systemic effects by way of the general circulation are not intended. In fact, systemic effects are usually regarded as side-effects of skin treatment.

Topically applied antihistamines have been used for the relief of pruritus and other forms of dermatoses, often in order to avoid the side-effects of systemically administered formulations and to reach higher local drug concentrations.

Damage or abrasion of the skin stratum-corneum barrier generally results in increased absorption. Successive stripping of the layers of stratum corneum increased skin permeability dramatically. There are several reports of contact dermatitis and phototoxicity as a result of the extended use of topical antihistamines in the literature, and most of the antihistamine topical formulations have been withdrawn from the market.

A recent report from a hospital documents the toxicity caused by the excessive use of an antihistamine formulation (44). Diphenhydramine hydrochloride is an antihistamine with anticholinergic properties that is frequently used both orally and topically for temporary relief of pruritus. Significant systemic absorption of diphenhydramine may occur following topical administration in patients with varicella-zoster lesions. Three children with varicella-zoster infection (VZI) developed bizarre behaviour as well as visual and auditory

hallucinations following topical applications of large amounts of diphenhydramine to the majority of skin surface.

1.9. Proposal of the present study

1.9.1. Purpose of the human studies

The pharmacokinetic behaviour of drug absorption and disposition is usually analyzed based on plasma concentration versus time data. However, it is also important in the clinical application of a drug to investigate the drug levels in various tissues or other body fluids to elucidate the relationship between drug concentration in plasma and that in these tissues or fluids, especially if the clinical response is evaluated in such sites.

It is well known, after administration of single oral doses of cetirizine or hydroxyzine, although absorption is rapid and serum concentration peak within 1-2 hours, peripheral H_1-blockade doses not usually become maximal until at least 5 hours post-dose when serum concentrations are waning. Also, peripheral H_1-blockade may persist long after serum H_1-antagonist concentrations have declined below the limits of analytical detection (28,31,33). The concentrations of these H_1-receptor antagonists achieved in human skin after usual doses are unknown and the relationships of skin concentrations to serum concentrations and to peripheral H_1-blockade have not yet been elucidated.

It was hypothesized that cetirizine and hydroxyzine skin concentrations after oral dosing would be higher than serum concentrations, and that skin concentrations would correlate better than serum concentrations with the amount of peripheral H_1-blockade produced. This hypothesis was tested using a randomized, double-blind, parallel-group design in healthy, adult male volunteers.

1.9.2. Purpose of animal studies

Transdermal drug delivery system has been in existence for a long time. In the past, the most commonly applied topical systems were creams and ointments for dermatological disorders. The occurrence of systemic side-effects with some of these formulations is indicative of absorption through the skin to the systemic circulation (45).

The use of liposomes for topical application has received little attention until the 1980's, and since then liposomes have attracted considerable interest and generated many speculative claims concerning their potential utility both as a drug carrier and reservoir for controlled release of drugs within various layers of skin. Now it is generally accepted that drugs topically applied to the skin entrapped in specially designed liposomes can be efficiently delivered into and through the skin (46,47). The results show a greater concentration of the delivered drug in different skin layers

and increased pharmacological effects(53), when the drug is applied to the skin entrapped in liposomes compared to the application of the free drug.

In this study, it was hypothesized that both hydroxyzine and cetirizine liposome formulations would yield higher and longer effects on the suppression of wheal induced by histamine over a 24 hour period, and that liposome formulations would result in lower serum concentrations after topical administration, compared to aqueous formulations. This method of administration should also yield better results in treatment of pruritus by some topical antihistamines, thus avoiding or reducing central nervous system adverse effects such as sedation.

The study was conducted in six healthy rabbits to compare the topical absorption of aqueous hydroxyzine and cetirizine solution with that of liposome formulations. The peripheral H_1-receptor antagonist activity was measured by intradermal histamine administration and assessment of the suppression of the histamine-induced wheals in the skin of the shaved back of the animal in a four-way cross over study design.

CHAPTER II. METHODOLOGY

2.1.Chemicals and equipments

2.1.1. Chemicals

1. Hydroxyzine hydrochloride: Sigma Chemical Co., P.O.Box 14508 St. Louis, MO 63178 USA.
2. Cetirizine dihydrochloride: UCB-Pharmaceutical Sector, Chemin du Roriest-B-1420, Braube-l' alleud, Belgium.
3. Antazoline hydrochloride: Sigma Chemical Co., P.O.Box 14508 St. Louis, MO 63178 USA.
4. P_{265}: UCB-Pharmaceutical Sector,Chemin du Roriest-B-1420, Braube-l' alleud, Belgium.
5. Potassium hydroxide: Fisher Scientific Co., Fair Lawn, NJ 07410, USA.
6. Ethyl Ether: Mallinckrodt Specialty Chemical Co., Paris, KY 40361, USA.
7. Acetonitrile: Mallinckrodt Specialty Chemical Co., Paris, KY 40361, USA.
8. Acetone: Fisher Scientific Co., Fair Lawn, NJ 07410,USA.
9. Phosphoric acid: Fisher Scientific Co., Fair Lawn, NJ 07410, USA.
10. Argon: Praxair Canada Inc., Mississauga, ON L5B 1M2, Canada
11. Sodium citrate dihydrate: Mallinckrodt Specialty Chemical Co., Paris, KY 40361, USA.
12. Citric acid: Fisher Scientific Co., Fair Lawn, NJ 07410,

USA.

13. Ethyl acetate: Fisher Scientific Co., Fair Lawn, NJ 7410, USA.
14. Sodium 1-decanesulfonic acid: Sigma Chemical Co., St. Louis, MO 63178, U.S.A.
15. Ammonium phosphate monobasic: Mallinckrodt Specialty C Chemical Co., Paris, KY 40361, USA.
16. Evans-Blue: Fisher Scientific Co., Fair Lawn, NJ 07410, USA.
17. Histamine phosphate: (U.S.P. 1 mg/ml): Allen & Hanbarys, A Glaxo Canada Limited Co., Toronto, ON, Canada.
18. Sodium chloride (0.9%): Astra Pharma Inc., Mississauga, ON L4Y 1M4, Canada.
19. Heparin sodium injection B.P. (100 i.u./ml): Leo Laboratories Canada Ltd. Ajax, ON, Canada.
20. Egg phosphatidylcholide: Sigma Chemical Co., St. Louis, MO 63178, USA.
21. Cholesterol: Fisher Scientific Co., Fair Lawn, NJ 07410, USA.

2.1.2. Supplies and Equipments

1. Sure-sep II serum plasma separator: Organon Teknika Co., Box 15969 Durham, N. CA 22704-0969, USA.
2. Syringe (5 CC and 1CC): Becton Dickinson & Company Franklin Lakes, NJ 07417-1884, USA.

4. Disposable test tube (16x100 mm): Baxter Healthcare Co., Valencia, CA 91355-8900, USA.
5. I.V. Catheter Placement unit: Critikon Inc., Tampa, FL 33634, USA.
6. Male Adapter Plug-Short: Abbott Hospital Inc., N.Chicago IL 60064, USA.
7. Steri-Pad: Johnson & Johnson Inc., Montreal H1V 2E4, Canada.
8. Needle (23g x 1"): Terumo Medical Co., Elkton, MD 21921, USA.
9. Hair remove lotion (Nair): Carter Products, Div. of Carter-Wallace, N.S. Inc., Mississauga, ON. L5N 1L9, Canada
10. Centrifuge (IEC HN-SII model): International Equipment Company, USA.
11. Refrigerated Centrifuge (Model B-20): International Equipment Co., Needham HTS, MA, USA.
12. pH meter (ZEROMATIC SS-3 model): Beckman Instrument Inc., 2500 Harbar BLV. Fullerton CA, USA.
13. Balance (Mettler AE 160 model): Mettler Instrument AG., Switzerland.
14. Vortex mixer (Thermolyne Maxi Mix™): Fisher Scientific Co., Fair Lawn, NJ 07410, USA.
15. Milli-Q Water system: Millipore Ltd., 3688 Nashua Drive, Mississauga, ON. L4V 1M5, Canada.

16.High performance liquid chromatography system: Water Associates Inc., Millford, MA, USA.

a: a 6000A high pressure pump

b: a U6K injector (manual) and a 710B automatic injector

c: a LC spectrophotometer (Lambda-Max Model 480)

d: a Waters 746 data module

e: a Radial-Pak™ cartridge (5NVC184) and a radial compression module (RCM- 8x10).

2.2. Pharmacokinetic and pharmacodynamic studies of hydroxyzine and cetirizine in humans

2.2.1. Extraction procedure for hydroxyzine:

The method used for extracting hydroxyzine from serum was a slight modification of the method of Simons et al (19,48).

Twenty-five microliters of antazoline solution (1 µg/ml), the internal standard were added to 1 ml of serum along with 250 µl of 10% (w/v) KOH solution and 5 ml of freshly distilled ether. Extraction was achieved by mixing the solution on a vortex mixer for 30 seconds followed by centrifugation for 5 minutes at 2000 rpm. The aqueous portion was frozen in a dry ice/acetone bath and the ether layer was transferred to a clean dry 16x100 mm test tube. The aqueous portion was discarded. Two hundred microliters 0.05% (w/v) H_3PO_4 was added into the test tube containing transferred ether, followed by mixing on a vortex for 30 seconds and centrifugation for 5

minutes at 2000 rpm. The aqueous layer was again frozen in a dry/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. Of the remaining aqueous solution 100 µl was then taken up in a syringe and injected directly into the HPLC.

2.2.2. Extraction procedure for cetirizine:

To 1 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 on a vortex mixer for 1 minute and centrifuged for 15 minutes at 2000 rpm. The organic layer was transferred 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 2000rpm. The ethyl acetate layer was transferred and mixed with the portion from the previous extraction. The aqueous layer was discarded. Two hundred microliters of 1.7% (w/v) H_3PO_4 were added, and the sample was mixed for 1 minute and centrifuged for 5 minutes at 2000 rpm. The organic layer was removed carefully by pipette. The aqueous layer was then exposed to a stream of dry nitrogen to remove remaining traces of ethyl acetate, and 100 µl of the solution were injected onto the column.

2.2.3. Chromatographic separation and quantization of hydroxyzine and cetirizine

The HPLC system included a M-6000A solvent-delivery pump, a U6K injector and a Lambda-Max Model 480 LC Spectrophotometric detector with a wavelength set at 229 nm, a 746 Data Module connected with the detector.

The mobile phase for hydroxyzine was acetonitrile-phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.5) (35:65, v/v). For cetirizine the mobile phase was acetonitrile-phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.9, 0.02M sodium 1-decanesulfonate) (46:54, v/v). The flow rate for both hydroxyzine and cetirizine was set at 0.8 ml/min. The effluent from the C_{18} column was monitored by UV absorption at 229 mn with 0.2 to 0.005 sensitivity settings. The chart speed for the data module was 1.0 cm/min. Peak height ratios of hydroxyzine to antazoline and cetirizine to P_{265} were used for the quantization based on the calibration curves established during the study period. The retention times were 4.23 and 7.25 minutes for antazoline and hydroxyzine, and 4.03 and 6.54 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 hydroxyzine and cetirizine and corresponding internal standards antazoline, and P_{265} were added. All chromatographic separations were carried out at ambient temperature. The serum

concentrations of hydroxyzine and cetirizine were expressed in terms of the free base form only.

2.2.4. Design of human study

2.2.4.1. Subjects

All subjects gave written, informed consent before participation. The study protocol was approved by the University of Manitoba Faculty Committee on the Use of Human Subjects in Research.

Healthy males age 18-40 years with normal skin were recruited. Subjects were excluded if they had a history of any skin disorder, were prone to scar formation, or had a very dark skin precluding accurate visualization and tracing of the flare response. Subjects were also excluded if they were obese or could not tolerate drowsiness or sedation during the day, might need to use an H_1-receptor antagonist other than study medications before or during the study, or had a history of adverse reactions to H_1-antagonist, of intercurrent illnesses within the previous 30 days, or of smoking during the previous year.

2.2.4.2. Procedure

The subjects had a preliminary visit to the Health Sciences Clinical Research center during which they were given the opportunity to become familiar with the test procedures.

On the first study day, they arrived at the Health Sciences Clinical Research Centre at 8 o'clock in the morning, after an 8-hour fast. Baseline skin biopsy, venipuncture and histamine skin tests were performed. They were then randomly allocated to receive either cetirizine tablets 10 mg (Reactine, Pfizer Company LTD., kirkland, Quebec H9R 4V2, Canada) or hydroxyzine capsules 50 mg (Atarax, Pfizer Company LTD., kirkland, Quebec H9R 4V2, Canada), which were encapsulated in opaque hard gelatin capsules to ensure double-blinding. The biopsies, venipunctures, and skin tests were repeated in the same sequence 1,3,6,9, and 24 hours after dosing. The techniques used for these procedures are described below. The subjects received a standardized breakfast consisting of 250 ml apple juice and a muffin, 2 hours after dosing; and a standardized lunch and dinner, 4 and 8 hours after dosing, respectively.

Beginning 36 hours after their initial dose of cetirizine or hydroxyzine, subjects took cetirizine 10 mg or hydroxyzine 50 mg (whichever they had received on the first day) at 21:00 o'clock in the evening for six consecutive days. Medications were administered in the evening in order to minimize daytime somnolence from hydroxyzine and consequent loss of double-blinding (49-51). Skin biopsies,venipunctures, and histamine skin tests were repeated at steady-state, exactly 12 hours after the last H_1-antagonist dose. At all test times, subjects were questioned about the presence of somnolence or other

adverse effects.

2.2.5. Measurement of wheal and flare area

Recently, the mechanisms by which allergens and nonspecific agents can induce skin reactions has been understood considerably. However, studies on this work began more than nearly 70 years ago when the "triple response" of Lewis was described in 1924 (52).

Generally, intradermal injections of histamine can causes a characteristic wheal-and-flare response. At the site of injection, a reddening appears owing to dilatation of small vessels, soon followed by an edematous wheal at the injection site and a red irregular flare surrounding the wheal. The flare is said to be caused by an axon reflex, which involves stimulation of sensory fibres and the passage of antidronic impulses through neighbouring branches, probably by substance P (53). A wheal that is discernible in 1 to 2 minutes occupies the same area as the original small red spot at the injection site. It fades away in about 10 minutes. This is due to the increased permeability of the post-capillary venules. The sensation of itch may also accompany the appearances of these effects.

2.2.6. Techniques for measuring cetirizine and hydroxyzine in human skin and serum samples

2.2.6.1. Skin samples

The skin biopsies were performed on the medial surfaces of the thighs using a 3 mm Accu.Punch (pre-dose baseline) or a 4 mm Accu.Punch (subsequent biopsies), 15 minutes after the biopsy site was infiltrated with lidocaine 1% combined with epinephrine 0.01 mg/ml and sodium bicarbonate 0.1 mEq/ml. A different site was used for each biopsy following a sequence starting as close to the groin as possible in an anterior to a posterior direction. The sequence of sites was the same in each subject. All biopsy samples were blotted free of blood on surgical gauze, transferred to non-absorbent paper, weighed immediately, and then stored and sealed in a polypropylene tube at -20^{0}C until analyzed for cetirizine or hydroxyzine.

Before analysis, samples were thawed, then digested in 0.25 mL 2M sodium hydroxide for 24 hours. Cetirizine and hydroxyzine were extracted from the skin samples using the same procedure, as for serum samples, only the 10% KOH was omitted in the hydroxyzine extraction. H_1-antagonist concentrations were determined in skin using HPLC procedure previously developed in our laboratory for serum samples.

2.2.6.2. Serum samples

Venipunctures for 7 ml blood were performed 30 minutes after application of EMLA^R local anesthetic cream to potential sites. Blood samples were centrifuged at 4^{0}C. Serum was

transferred into appropriately labelled polypropylene tubes with screw caps, frozen promptly in the upright position, and stored at -20°C until analysis, using HPLC methods previously developed in our laboratory (19,28,30). In subjects receiving hydroxyzine, in addition to measurement of hydroxyzine in serum, cetirizine arising from hydroxyzine *in vivo* was also measured.

2.2.7. Skin test in human

Epicutaneous tests were performed with histamine phosphate 1 mg/ml and 10 mg/ml in 0.9% NaCl, sterilized by 0.22 µ filtration. A different site on the volar surfaces of the forearm was used for each test, and the sequence of test sites was then the same in each subject. The wheal and flare circumferences were traced with pen at 10 minutes and the tracings transferred to paper using transparent tape. Wheal and flare areas were measured using a computerized digitizing system (19,28,30,33).

2.3. Pharmacokinetic and pharmacodynamic studies of hydroxyzine and cetirizine in rabbits: the solution and liposome formulations

2.3.1. Extraction procedure for hydroxyzine:

The method used for extracting hydroxyzine from serum was that of Simons et al (19,48) with minimal modifications.

Twenty-five microliters of antazoline solution (1 µg/ml), the internal standard were added to 1 ml of serum along with 250 µl of 10% KOH solution and 5 ml of freshly distilled ether. Extraction was achieved by mixing the solution 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 transferred to a clean dry 16x100 mm test tube. Two hundred microliters 0.05% H_3PO_4 was added into the test tube containing transferred ether, followed by mixing on a vortex for 30 seconds and centrifuging for 5 minutes at 2000 rpm. The aqueous layer was again frozen in a dry/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. Of the remaining aqueous solution 100 µl was then taken up in a syringe and injected directly into the HPLC.

2.3.2. Extraction procedure for cetirizine

To 1 ml serum, 50 µl of P_{256} (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 on a vortex mixer for 1 minute and centrifuged for 15 minutes at 2000 rpm. The organic layer was transferred 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 2000rpm. The ethyl acetate was transferred and combined with the portion from the previous extraction. The aqueous layer was discarded. Two hundred microliters 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 organic layer was removed carefully by pipette. The aqueous layer was then exposed to a stream of dry nitrogen to remove remaining traces of ethyl acetate, and 100 µl of the solution were injected onto the column.

2.3.3. Chromatographic separation and quantization of hydroxyzine and cetirizine

The mobile phase for hydroxyzine was acetonitrile-phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.5) (35:65, v/v). For cetirizine the mobile phase was acetonitrile-phosphate buffer (0.075M $NH_4H_2PO_4$, pH 2.9, 0.02M sodium 1-decanesulfonate) (46:54, v/v). The flow rate for both hydroxyzine and cetirizine was set at 0.8 ml/min. The effluent from the column was monitored by UV absorption at 229 mn with 0.2 to 0.005 a.u.f.s. sensitivity settings. The chart speed for the data module was 1.0 cm/min. Peak height ratios of hydroxyzine to antazoline and cetirizine to P_{265} were used for the quantization based on the calibration curves established during the study period. The retention times were 4.36 and 7.24 minutes for antazoline and hydroxyzine, and 7.02 and

10.02 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 hydroxyzine and cetirizine and corresponding internal standards antazoline, and P_{265} were added. All chromatographic separations were carried out at ambient temperature. The serum concentrations of hydroxyzine and cetirizine were expressed in terms of the free base form only.

2.3.4. Preparation of solution and liposome formulations

2.3.4.1. Solution formulation

Hydroxyzine.2HCl (MW 447.92 g), 119.47mg, dissolved in 10 ml buffer (KCl-HCl buffer, pH 2.0), yields a hydroxyzine solution with concentration 10 mg/ml. One ml of this solution will be applied at each time, containing 10 mg hydroxyzine.

Cetirizine.2HCL (MW 461.89 g), 118.76 mg dissolved in 10 ml buffer (KCl-HCl buffer, pH 2.0), yields a cetirizine solution with concentration 10 mg/ml. One ml of this solution will be applied at each time, containing 10 mg cetirizine.

2.3.4.2. Liposome formulation

The method used for preparation of liposome formulation was based on the Mezei et al (54) with some modifications.

The lipid phase which containing egg phosphatidylcholine (EPC) 420 mg and cholesterol (CHOL) 67.1 mg was dissolved in

chloroform in a round bottom flask. The solvent was then removed by rotary evaporation at approximately 30° C under vacuum so that a thin lipid film was deposited on the wall of the flask. The aqueous phase, 10 ml solution of hydroxyzine dihydrochloride, 50 mg/ml, in pH 2.0 (HCL-KCL buffer) was then added. The concentration of hydroxyzine was confirmed by HPLC analysis. The flask was hand-shaken vigorously for ten minutes, then placed in a 35° C water-bath for two minutes. This process was repeated four times to hydrate the film and form the multilamellar liposome. Then 3 ml of liposome formulation were removed from the round-bottom flask and transferred into a centrifuge tube. The sample was centrifuged at 19,000 rpm for 20 minutes at 20° C. The supernatant was transferred by pasteur pipette into a clean test tube. The pellet was resuspended in 5 ml of buffer, pH 2.0, and the centrifugation at 19,000 rpm for 20 minutes was repeated. The wash supernatant was added to the previous sample in the same test tube. The total volume of the two supernatant aliquots was measured and the concentration was determined by HPLC. The amount of hydroxyzine in the liposome formulation is equal to the total amount in the liposomal suspension before centrifugation, minus the amount measured in the combined supernatant.

The preparation of the cetirizine liposome formulation was by the same process as that of the hydroxyzine but with

the addition of the aqueous phase of 10 ml solution of cetirizine dihydrochloride, 50 mg/ml, in pH 2.0 (HCL-KCL buffer).

In this study, a 10 mg/ml concentration is required in the liposome. If the concentration in the pellet is higher than required, it was diluted with additional buffer (pH 2.0). If the concentration was lower, then it was adjusted by the addition of buffer containing either hydroxyzine or cetirizine.

2.3.5. Study design of animal experiments

2.3.5.1. The use of rabbits in pharmacokinetic studies

This study was approved by University of Manitoba Animal Care Committee.

Rabbits were selected in the study because they are of a size to have sufficient back skin surface area to perform the study and to permit the withdrawal of a number of blood samples which are needed for pharmacokinetic studies. Preliminary studies in our laboratory confirmed that rabbits metabolize hydroxyzine to cetirizine by the same process as human subjects and the pharmacokinetics are well-defined (1).

2.3.5.2. Animals

Six New Zealand white rabbits (five females, one male),

3-5 kg, obtained from the Department of Zoology, Faculty of Science, University of Manitoba, were used in this study. Rabbits were not studied until after a two-week environmental adjustment during which period antibiotics were given against infectious diseases. They were kept individually in metal cages, fitted with wire floors to reduce coprophagy. Food and water were supplied as needed.

2.3.5.3. Procedure

This cross-over study required four treatments: hydroxyzine in solution, hydroxyzine in liposome formulation, cetirizine in solution and cetirizine in liposome formulation. Each rabbit received all the four treatments in a random order with a two-week interval between any two treatments. A dose of 1 ml solution or liposome formulation containing 10 mg of hydroxyzine or cetirizine was applied each time.

For each study, an area around 20 cm^2 (5cm x 4cm) on rabbit back was shaved by electronic shaver, followed by the application of depilatory lotion on the area for 5 to 8 minutes. The lotion was completely removed by carefully washing with warm water. An application of 1 ml liposome or buffer solution which was contained drugs was spread uniformly over the 20 cm^2 hairless area.

The rabbit ears also had the hair removed in a similar manner to permit clear view of the blood vessel on ears. Then

1 ml Evans Blue (1 mg/ml) was injected through the ear vein to permit easier identification of the histamine wheal perimeter. Additional doses of Evans Blue were administered as required.

2.3.6. Techniques of collecting blood samples from rabbit

The rabbit ear artery was dilated by topical application of an alcohol swab. For insertion, the catheter, was held by the needle hub-flush chamber with the bevel in the upright position. At a 25^0 to 30^0 angle, the needle was inserted into skin along the artery. The needle was lowered until it was almost flush with the skin and aimed directly into the artery. A back flow of blood into the flush chamber confirmed the artery entry. The entire unit was advanced into the vessel at least 1/4'' to assure catheter entry into the artery lumen. While holding the needle stable, the catheter was introduced into the artery until almost the whole flush chamber has been inserted. The needle was removed, the catheter secured to the ear with tape and then flushed with Heparin Sodium (100 µg/ml). Blood samples were collected pre-dose and at 0.5,1.0,1.5,2.0,3.0,4.0,6.0,8.0 hours after dose.

The first 1 ml of blood withdrawn was discarded and a 2 to 3 ml blood sample was withdrawn through the catheter, 2 to 3 ml of 0.9% Sodium Chloride were given to flush the catheter immediately followed by 0.01 ml of Heparin (100 µg/ml) to prevent coagulation. The blood samples were collected in clean

16x100 mm glass test tubes without anticoagulants. The blood was allowed to clot and the serum was separated by placing Sure Sep-II separators on the top on the test tubes and centrifuging 15 minutes at 2000 rpm. Serum samples were frozen at -20° C until analyzed.

2.3.7. Skin Test

Skin tests were performed, at the same time schedule as blood samples, pre-dose and at 0.5,1.0,1.5,2.0,3.0,4.0,6.0,8.0 hours after dose.

An intradermal test was used of 0.05 ml of solution containing histamine phosphate (1.0 mg/ml). A different site on the hairless area on the back of rabbits was used for each test. The wheal area was traced 10 minutes after each histamine injection and transferred to a transparent tracing paper using a felt-tipped pen. 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.).

2.4. Data Analysis

2.4.1. Pharmacokinetic data analysis

Data from the human and rabbit studies were analyzed using PKCALC (55) on an IBM-XT compatible. PKCALC is a basic 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, areas under the concentration versus time curves, and mean residence time.

In PKCALC, the elimination half-life was calculated using equation (i):

$$T_{1/2} = 0.693/b \quad \text{(i)}$$

where b 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 infinity according to the formula:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/b \quad \text{(ii)}$$

where C_t is the concentration of hydroxyzine (cetirizine) in the last sample of time t. Results were expressed in terms of ng.hr/ml.

The systemic clearance (Cl_s) was calculated using:

$$Cl_s = \text{Dose} / AUC_{0-\infty} / \text{weight} \quad \text{(iii)}$$

Results were expressed as ml/min/kg.

The apparent volume of distribution for the central compartment, V_b, was calculated using equation (iv):

$$V_b = Cl_s / b \quad \text{(iv)}$$

Results were expressed as l/kg.

2.4.2. Pharmacodynamic data analysis

The efficacy was calculated as the percent suppression of wheal and flare by using equation (v):

$$E = (A_0 - A_t)/A_0 * 100\% \quad (v)$$

where A_0 is the wheal or flare area before drug administration. A_t is the wheal or flare area at time t after hydroxyzine or cetirizine administration.

2.4.3. Statistical analysis

Mean skin cetirizine or hydroxyzine concentrations were compared to serum cetirizine or hydroxyzine concentrations, respectively, over time using ANOVA and the Tukey and Bonferroni Multiple Range Tests (50). Differences were considered significant at $p<0.05$. Mean wheal and flare areas at each time were compared to pre-dose values for cetirizine or hydroxyzine using ANOVA and the Tukey and Bonferroni Multiple Range Tests with differences being considered significant at $p<0.05$. Wheal and flare areas following cetirizine were compared to those values following hydroxyzine using the same statistical tests and level of significance.

Correlations between skin and serum cetirizine or hydroxyzine concentration and degree of suppression of histamine-induced wheals and flares after cetirizine or hydroxyzine were evaluated by linear regression techniques calculating 95% and 99% confidence intervals.

CHAPTER III. RESULTS

3.1. Pharmacokinetic and pharmacodynamic studies of hydroxyzine and cetirizine in humans

3.1.1. HPLC assays of hydroxyzine

Representative HPLC chromatograms for hydroxyzine and internal standard antazoline are shown in Figure 3.

The retention times of hydroxyzine and antazoline were 7.25 and 4.23 minutes respectively. There were no interfering peaks.

3.1.2. Calibration curves for hydroxyzine

The calibration curves for hydroxyzine in human serum were constructed by plotting peak height ratios of hydroxyzine to antazoline versus concentrations of hydroxyzine. Calibration curves were analyzed periodically during the study period using concentrations of hydroxyzine from 2.0 ng/ml to 100 ng/ml over which range the calibration curve was linear. The variability in the calibration curves over a period of 8 months were calculated from six calibration curves, each having six samples at every concentration. The variability is expressed as the coefficient of variation and shown in Table 4, and the curve is shown in Figure 4. The variability is not shown in figure in order to maintain the clarity of the mean data. All the coefficients of variation are less than 10.6%.

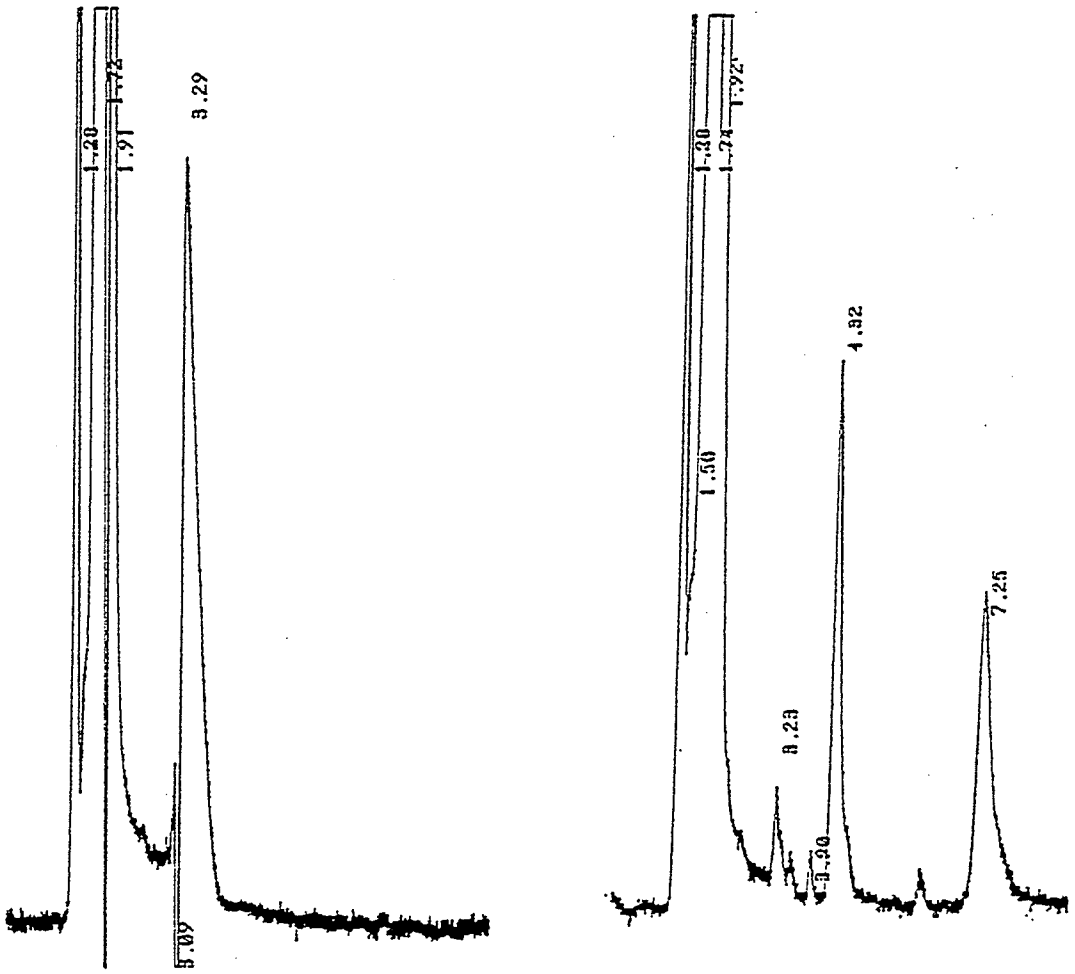

Figure 3. The HPLC chromatograms of hydroxyzine and its internal standard antazoline in human serum (right) compared with that from corresponding blank sample (left). The retention time for hydroxyzine and antazoline are 7.25 minutes and 4.23 minutes respectively.

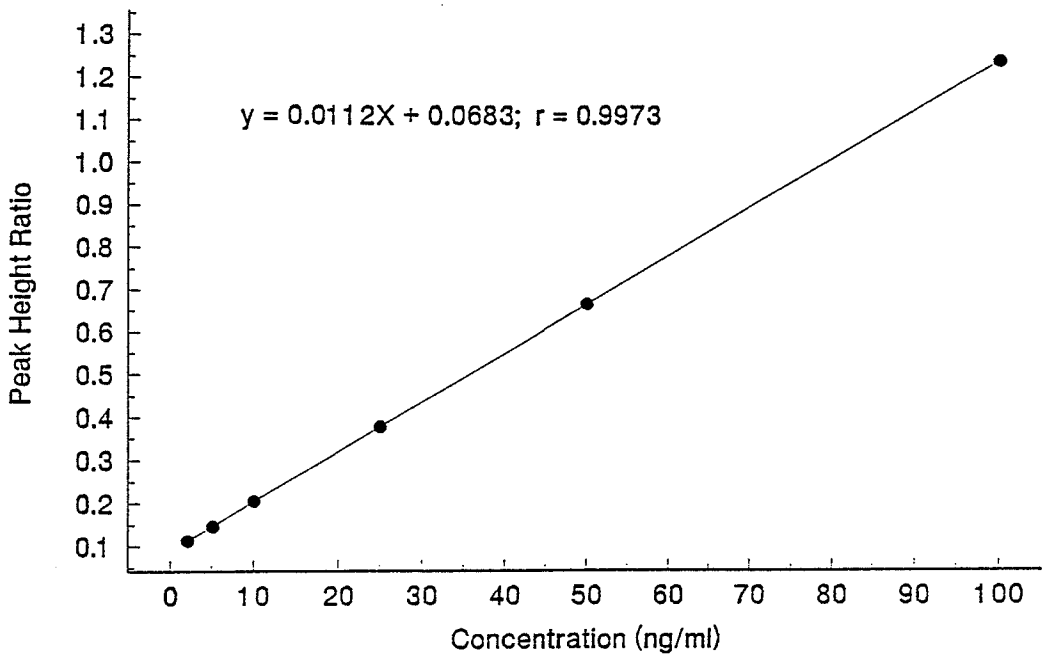

Figure 4. The HPLC calibration curve of hydroxyzine in human serum. The variability was less than 10.6% among six experimental curves performed within eight months.

Table 4. Variability in HPLC calibration curves for hydroxyzine in serum

Concentration (ng/ml)	Peak Height Ratio	C.V.
2.0	0.1130	9.8%
5.0	0.1469	10.6%
10.0	0.2053	4.5%
25.0	0.3780	1.6%
50.0	0.6658	4.0%
100.0	1.2437	3.5%

The calibration curves for hydroxyzine in human skin were constructed using the same procedure as that in serum, but only with the different concentration range of 0.1 ng/ml to 2.0 ng/ml. The variability is shown in Table 5 and the curve is shown in Figure 5. The maximum variability for all the concentrations is 7.6%.

3.1.3. HPLC assay for cetirizine

Representative chromatograms for cetirizine and its internal standard P_{265} in serum is shown in Figure 6.

The retention times of cetirizine and P_{265} are 4.03 and 6.54 minutes respectively. No interfering peaks are observed.

3.1.4. Calibration curves for cetirizine

The calibration curves for cetirizine in human serum were constructed by plotting peak height ratios of cetirizine to P_{265} versus concentrations of cetirizine. The calibration curve was analyzed periodically during the study period using concentrations of cetirizine from 1 ng/ml to 200 ng/ml. The calibration curves were linear over these ranges of concentrations. The variabilities in the serum cetirizine calibration curves over a period of 8 months were calculated from six calibration curves, each having six samples at every

Table 5. Variability in HPLC calibration curves for hydroxyzine in human skin

Concentration (ng/g)	Peak Height Ratio	C.V. (%)
0.10	0.0035	5.6
0.25	0.0068	3.9
0.50	0.0162	7.6
1.00	0.0316	4.8
2.00	0.0564	5.7

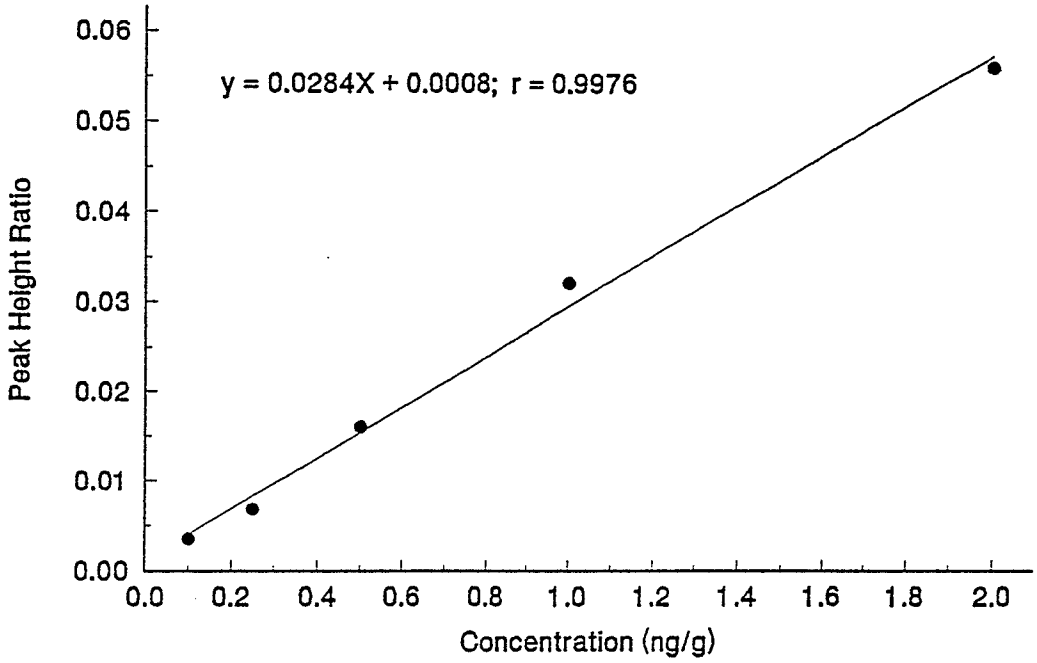

Figure 5. The HPLC calibration curve of hydroxyzine in human skin. The variability was less than 7.6% among six experimental curves performed within eight months.

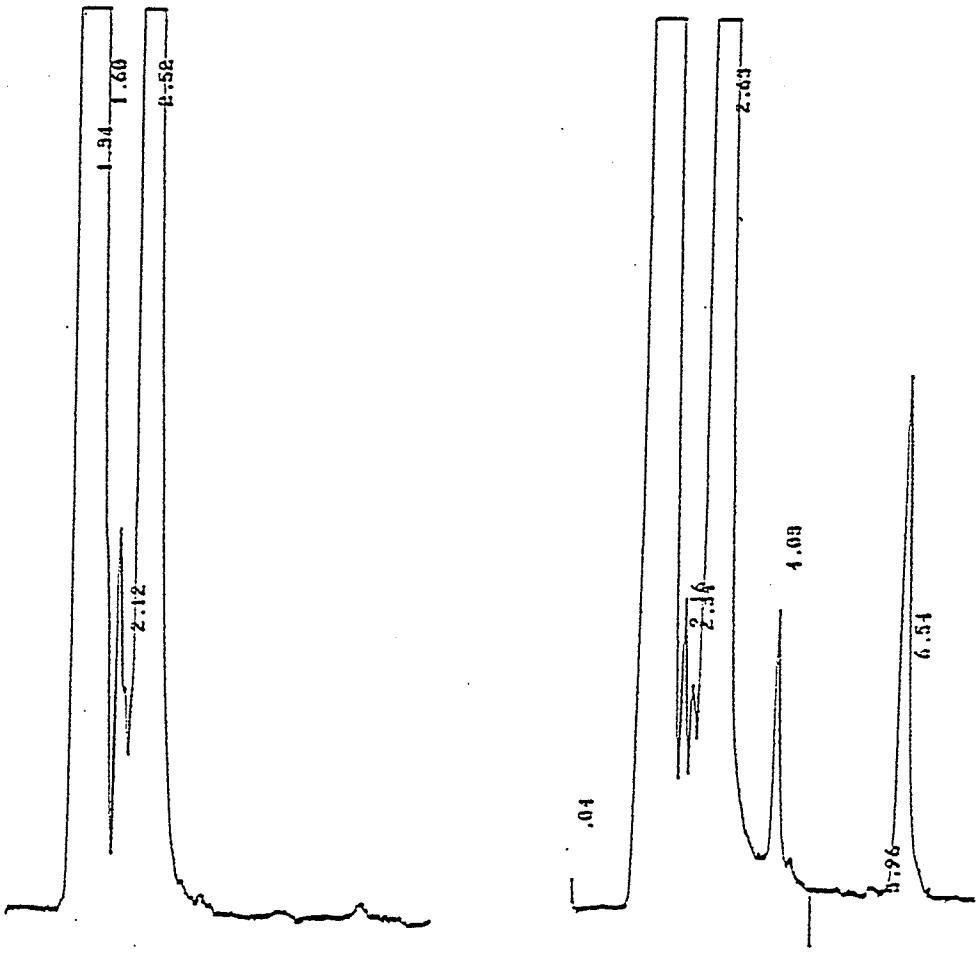

Figure 6. The HPLC chromatograms of cetirizine and its internal standard P_{265} in human serum (right) compared with that from corresponding blank sample (left). The retention time for cetirizine and P_{265} are 4.03 minutes and 6.54 minutes respectively.

Table 6. Variability in HPLC calibration curves for cetirizine in human serum

Concentration (ng/ml)	Peak Height Ratio	C.V.
1.0	0.0052	12.8%
5.0	0.0130	3.5%
25.0	0.0496	5.6%
50.0	0.1138	4.7%
70.0	0.1482	3.0%
100.0	0.1970	1.8%
200.0	0.4201	2.0%

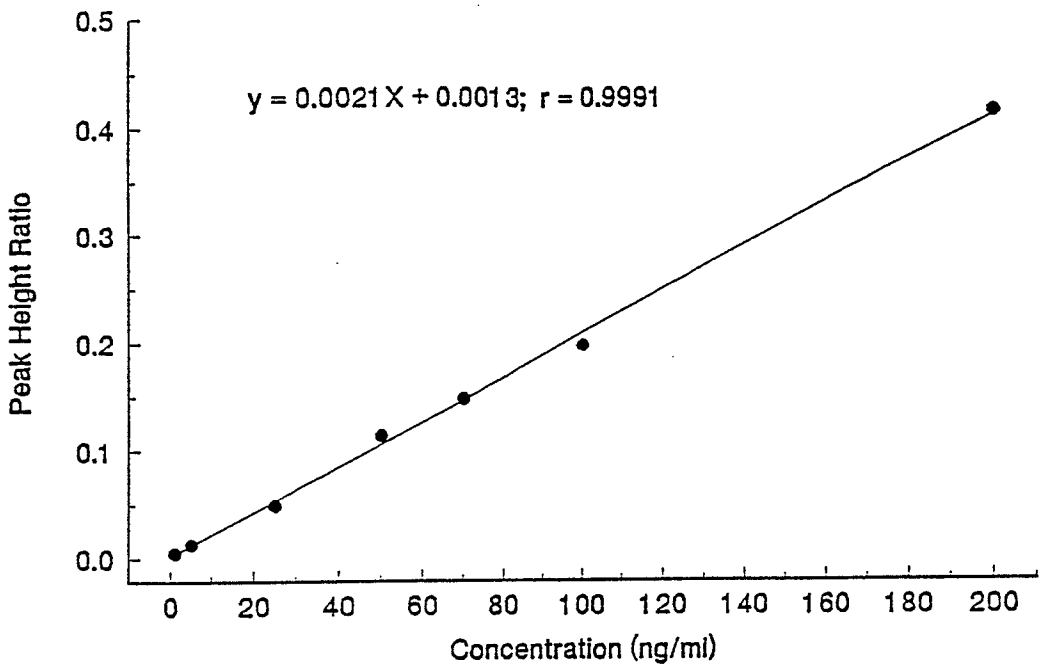

Figure 7. The HPLC calibration curve of cetirizine in human serum. The variability was less than 12.8% among six experimental curves performed within eight months.

concentration. The variability is expressed as the coefficient of variation and shown in Table 6, and the calibration curve is shown in Figure 7. The maximum variability is 12.8%.

The calibration curves for cetirizine in human skin were calculated periodically using the same method as serum with a different concentration range from 0.5 ng/ml to 10.0 ng/ml. The mean peak height ratio and variability is shown in Table 7, and the calibration curve is shown in Figure 8. All the variability is less than 9.1%.

3.1.5. Pharmacokinetic and pharmacodynamic studies of hydroxyzine in humans

Six of the thirteen healthy adult males with normal skin, mean age 26±6 years, mean weight 75±11 kg, completed the study using hydroxyzine. The other seven received cetirizine.

3.1.5.1 The serum and skin concentration of hydroxyzine and serum concentration of cetirizine produced *in vivo*

The serum and skin concentrations of hydroxyzine and the serum concentrations of cetirizine produced *in vivo* after an oral dose of hydroxyzine 50 mg daily in the six subjects are listed in Tables 8-10 and are plotted versus time in Figure 9.

Table 7. Variability in HPLC calibration curves for cetirizine in human skin

Concentration (ng/g)	Peak Height Ratio	C.V. (%)
0.5	0.086	5.8
1.0	0.126	9.1
2.0	0.252	6.3
5.0	0.788	5.8
10.0	1.381	6.5

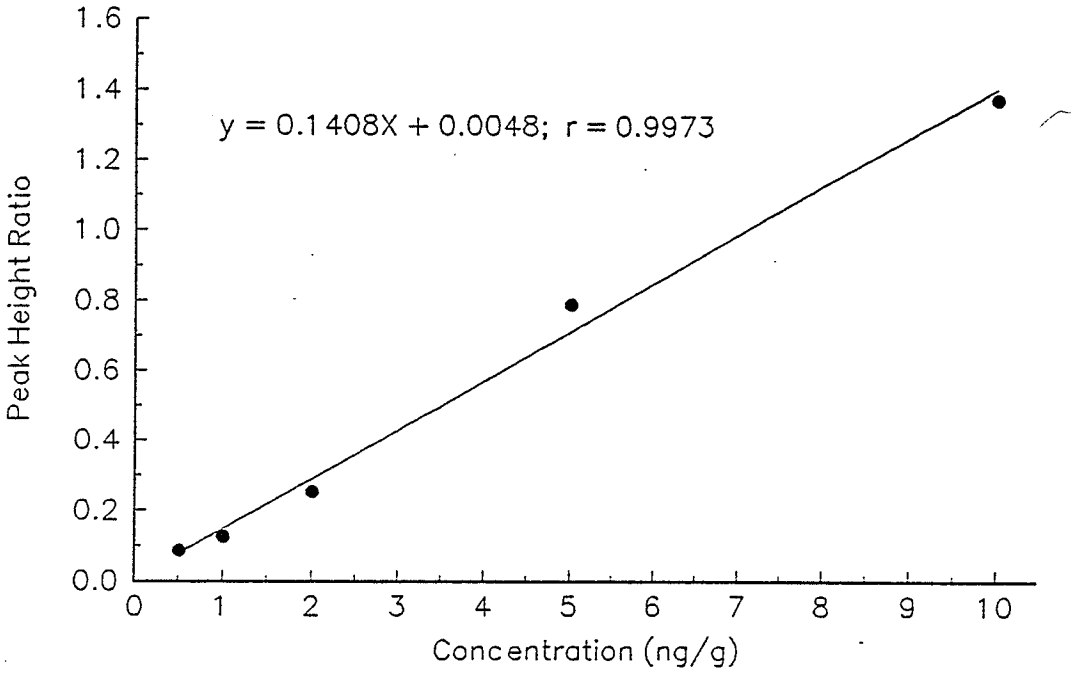

Figure 8. The HPLC calibration curve of cetirizine in human skin. The variability was less than 9.1% among six experimental curves performed within eight months.

Table 8. Hydroxyzine serum concentration (ng/ml) in humans after an oral dose of hydroxyzine 50 mg daily

No.\T	PRE-DOSE	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0	9.7	30.3	24.7	18.7	8.6	23.0
2	0	4.2	15.6	15.5	11.6	N.S.	18.6
3	0	11.9	39.6	28.2	15.5	9.7	20.6
4	0	50.8	66.7	37.1	33.8	12.7	42.0
5	0	39.5	59.9	36.4	20.8	10.2	29.2
6	0	5.9	44.8	81.9	33.4	11.8	77.6
MEAN		20.3	42.8	37.3	22.3	10.6	35.2
SD		18.0	17.2	21.2	8.5	1.5	20.0

Table 9. Hydroxyzine skin concentration (ng/g) in humans after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0	47.01	31.2	10.0	N.S.	N.S.	66.8
2	0	55.4	69.8	41.0	22.4	8.1	101.2
3	0	30.8	54.1	36.9	32.6	11.9	106.8
4	0	50.2	62.1	33.6	45.6	15.3	73.0
5	0	96.7	74.0	52.7	43.0	20.5	96.3
6	0	92.4	105.6	154.0	138.4	N.S.	N.S.
MEAN		62.1	66.1	54.7	56.4	14.0	88.8
SD		24.2	22.6	50.6	41.8	5.3	16.0

*N.S.: no sample available

Table 10. Cetirizine serum concentration (ng/ml) in humans produced *in vivo* from hydroxyzine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0	3.0	6.0	9.0	24.0	168.0
1	0	8.1	157.2	165.9	127.1	70.7	171.2
2	0	102.9	2314	257.8	222.1	106.7	430.2
3	0	213.9	260.3	254.1	223.0	113.8	424.7
4	0	247.6	328.5	346.2	299.1	165.6	704.0
5	0	331.6	322.3	241.6	248.2	8.8	309.9
6	0	N.D.	196.3	179.9	172.5	93.1	479.0
MEAN		150.6	249.3	240.9	215.3	105.3	419.7
SD		135.2	68.3	64.6	59.7	33.5	178.0

N.D.: not detectable.

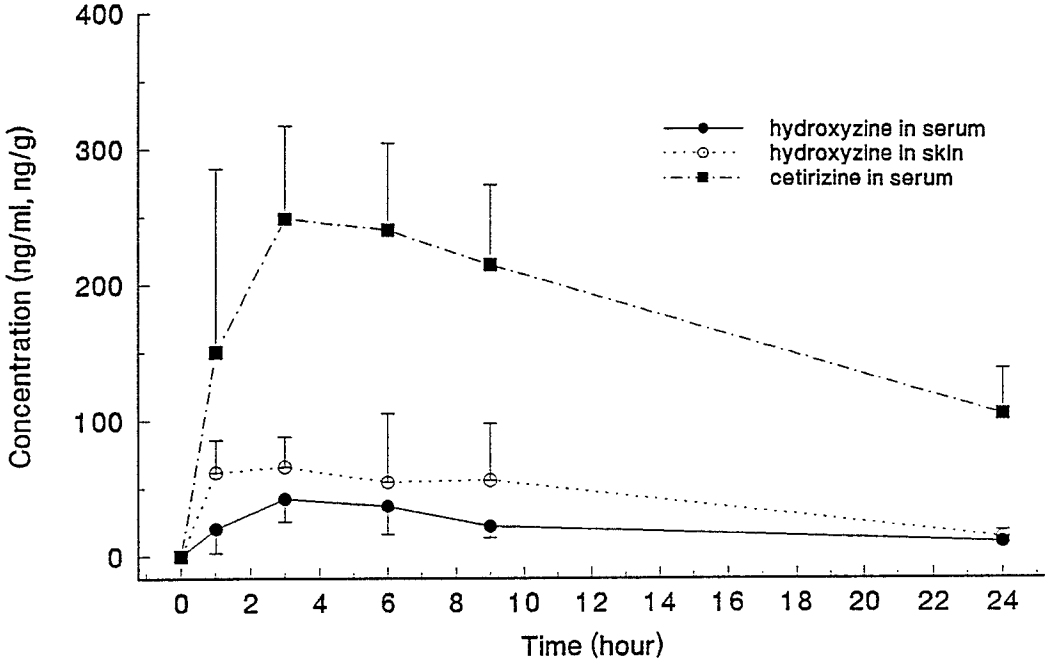

Figure 9. Mean hydroxyzine concentrations in serum and skin and mean cetirizine concentration in serum arising *in vivo* in human after an oral dose of 50 mg hydroxyzine (N=6, mean±SD).

3.1.5.2. Pharmacodynamic studies of hydroxyzine after an oral dose of hydroxyzine 50 mg daily in healthy males

The results of the efficacy tests calculated as the percent suppression of wheals and flares induced by the intradermal injection of 1 mg/ml and 10 mg/ml histamine are listed in Tables 11-14, and are plotted in Figure 10 and Figure 11. The wheal and flare areas are also listed in Tables 15-18, and are plotted in Figures 12, 13. The mean wheal suppressions induced by 10 mg/ml histamine after an oral dose of 50 mg hydroxyzine are plotted against the serum and skin concentrations of hydroxyzine in Figure 14.

3.1.5.3. Curve fitting, pharmacokinetic and pharmacodynamic parameters

The mean serum hydroxyzine concentrations versus time plot after an oral dose of hydroxyzine 50 mg daily is best described by a triexponential equation, corresponding to an absorption phase, a distribution phase and an elimination phase. The skin hydroxyzine concentrations versus time plot after the administration is best described by either biexponential or triexponential equation. Pharmacokinetic parameters of hydroxyzine in human serum are listed in Table 19. The systemic clearance and the apparent volume of distribution for the central compartment of hydroxyzine were

Table 11. The percent suppression of wheals in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0.0	0.0	59.9	100.0	100.0	59.3	100.0
2	0.0	100.0	100.0	100.0	100.0	100.0	100.0
3	0.0	7.6	89.4	100.0	100.0	100.0	100.0
4	0.0	100.0	100.0	100.0	100.0	100.0	100.0
5	0.0	48.0	100.0	100.0	100.0	100.0	100.0
6	0.0	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
MEAN		51.1	89.8	100.0	100.0	91.9	100.0
SD		43.1	15.6	0.0	0.0	16.3	0.0

Table 12. The percent suppression of wheals in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0.0	7.0	53.2	79.3	100.0	65.0	89.6
2	0.0	23.6	100.0	69.2	100.0	100.0	100.0
3	0.0	16.7	89.0	80.2	100.0	89.4	100.0
4	0.0	25.8	100.0	100.0	100.0	100.0	100.0
5	0.0	60.2	64.6	100.0	100.0	46.6	100.0
6	0.0	0.0	26.6	77.5	83.5	100.0	100.0
MEAN		22.2	72.4	84.4	97.3	83.5	98.3
SD		19.2	26.9	11.6	6.1	20.6	3.9

N.S.: no sample available

Table 13. The percent suppression of flares in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0	0.0	75.7	87.8	95.0	81.5	91.2
2	0	86.0	78.9	95.0	92.5	92.9	90.8
3	0	0.0	80.7	92.1	86.6	86.6	91.1
4	0	94.1	95.7	93.8	96.5	92.4	93.0
5	0	43.1	84.0	89.1	88.4	78.3	85.7
6	0	0.0	0.0	0.0	0.0	0.0	0.0
MEAN		44.7	83.0	91.5	91.8	86.3	90.4
SD		40.4	6.9	2.7	3.8	5.8	2.4

Table 14. The percent suppression of flares in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0.0	29.1	93.9	95.6	96.5	93.5	94.7
2	0.0	71.9	95.7	93.8	95.8	92.5	96.0
3	0.0	0.0	88.3	86.1	87.1	90.7	91.6
4	0.0	0.0	82.1	78.0	85.0	73.0	78.5
5	0.0	25.5	88.9	89.3	91.6	83.6	88.9
6	0.0	0.0	27.0	48.1	61.6	53.1	31.3
MEAN		22.5	79.3	81.8	91.2	81.1	0.2
SD		18.6	23.8	16.1	4.6	14.3	22.6

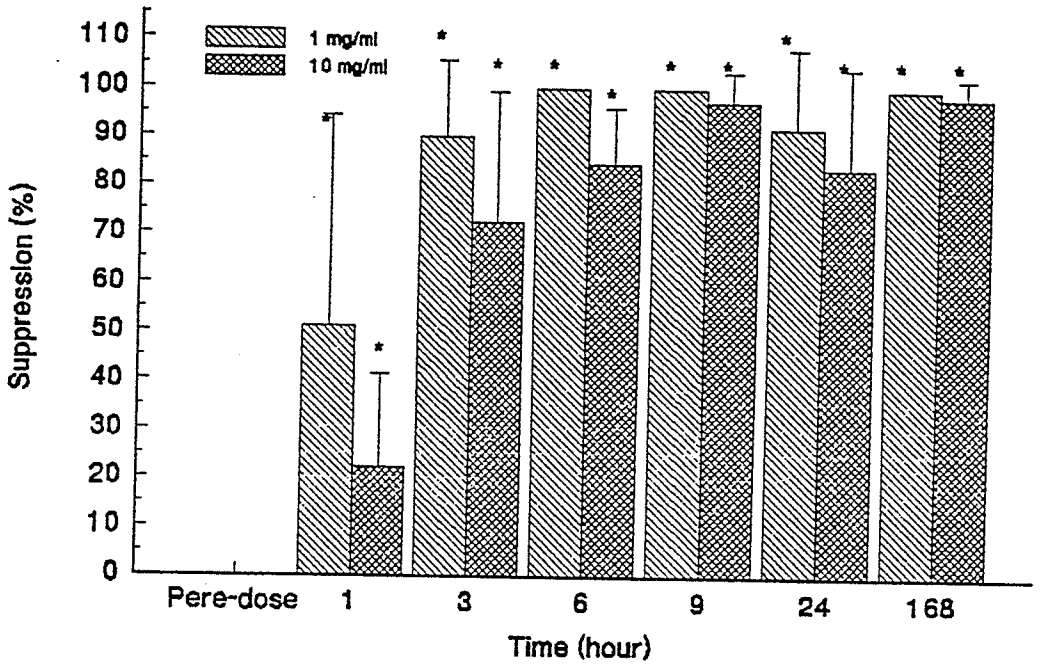

Figure 10. Mean suppressions of wheals in humans induced by intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 50 mg hydroxyzine daily for seven days (N=6, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

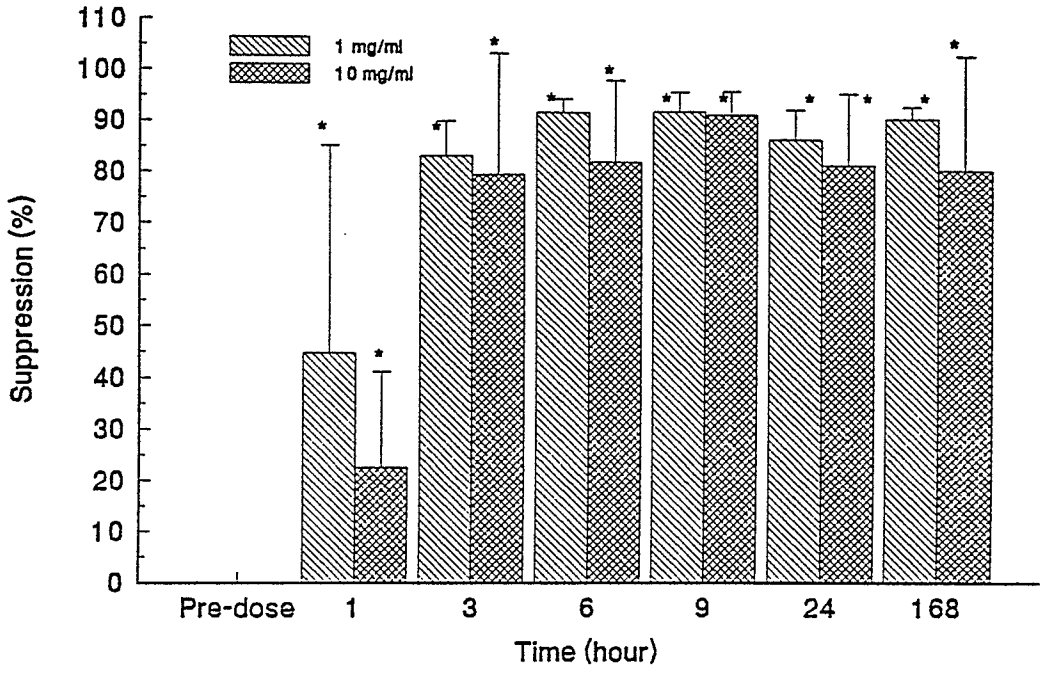

Figure 11. Mean suppressions of flares in humans induced by intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 50 mg hydroxyzine daily for seven days (N=6, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

Table 15. The wheal areas (cm^2) in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0.127	0.218	0.051	0.000	0.000	0.051	0.000
2	0.097	0.000	0.000	0.000	0.000	0.000	0.000
3	1.97	0.182	0.020	0.000	0.000	0.000	0.000
4	0.185	0.000	0.000	0.000	0.000	0.000	0.000
5	0.142	0.074	0.000	0.000	0.000	0.000	0.000
6	0.000	0.107	0.093	0.000	0.000	0.000	0.000
MEAN	0.125	0.097	0.027	0.000	0.000	0.009	0.000
SD	0.071	0.091	0.038	0.000	0.000	0.021	0.000

Table 16. The wheal areas (cm^2) in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0.260	0.242	0.122	0.054	0.000	0.091	0.026
2	0.284	0.217	0.000	0.087	0.000	0.000	0.000
3	0.321	0.267	0.035	0.063	0.000	0.034	0.000
4	0.252	0.187	0.000	0.000	0.000	0.000	0.000
5	0.232	0.092	0.082	0.000	0.000	0.124	0.000
6	0.143	0.256	0.105	0.032	0.023	0.000	0.000
MEAN	0.249	0.210	0.057	0.039	0.004	0.042	0.004
SD	0.060	0.065	0.053	0.035	0.009	0.054	0.011

Table 17. The flare areas (cm^2) in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	0.967	0.985	0.235	0.118	0.048	0.179	0.085
2	1.017	0.142	0.214	0.051	0.076	0.072	0.093
3	0.937	1.045	0.181	0.073	0.125	0.126	0.083
4	1.346	0.079	0.058	0.083	0.046	0.102	0.094
5	0.888	0.505	0.142	0.097	0.103	0.192	0.126
6	0.108	0.350	0.277	0.163	0.123	0.155	0.111
MEAN	0.877	0.518	0.185	0.098	0.087	0.138	0.098
SD	0.411	0.414	0.077	0.040	0.036	0.046	0.017

Table 18. The flare areas (cm^2) in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of hydroxyzine 50 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
1	4.580	3.245	0.281	0.199	0.159	0.298	0.244
2	2.307	0.647	0.098	0.142	0.097	0.171	0.093
3	1.916	3.271	0.223	0.266	0.247	0.178	0.161
4	0.628	1.578	0.112	0.138	0.094	0.169	0.135
5	2.499	1.863	0.227	0.267	0.209	0.409	0.276
6	0.551	3.370	0.402	0.286	0.211	0.258	0.379
MEAN	2.080	2.329	0.232	0.215	0.170	0.247	0.215
SD	1.48	1.130	0.115	0.070	0.063	0.102	0.110

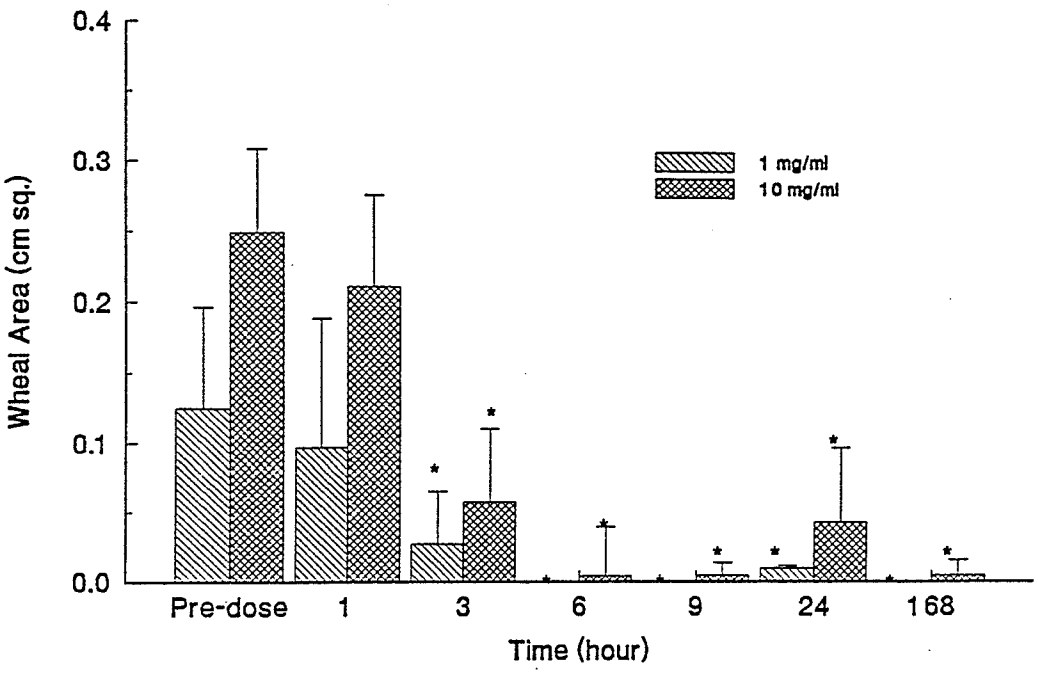

Figure 12. Mean wheals in humans induced by the intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 50 mg hydroxyzine daily for seven days (N=6, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

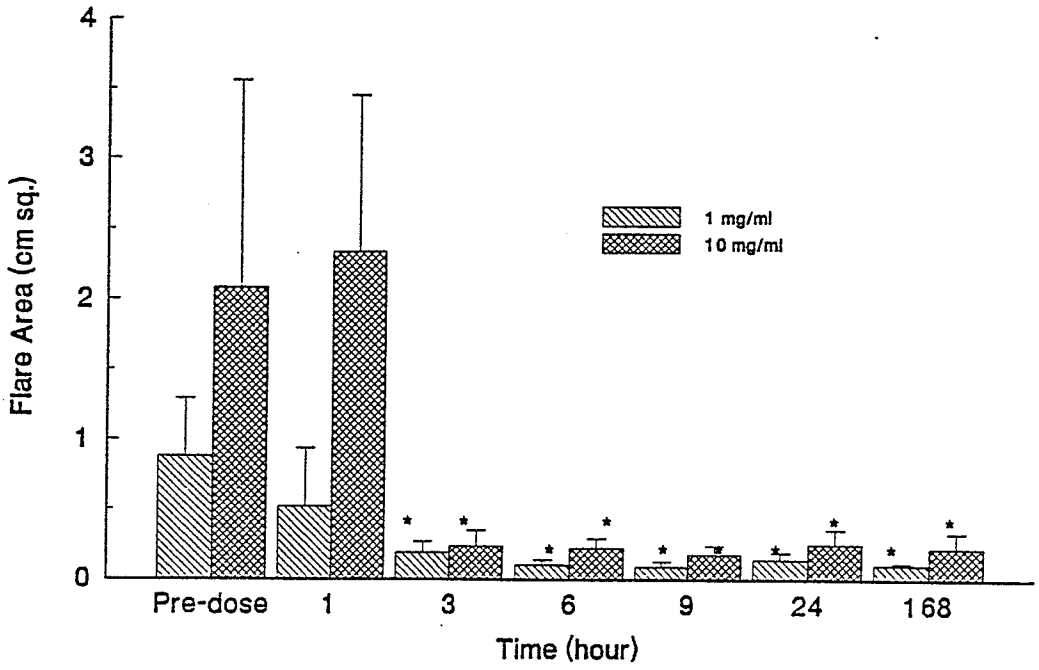

Figure 13. Mean flares in humans induced by intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 50 mg hydroxyzine daily for seven days (N=6, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

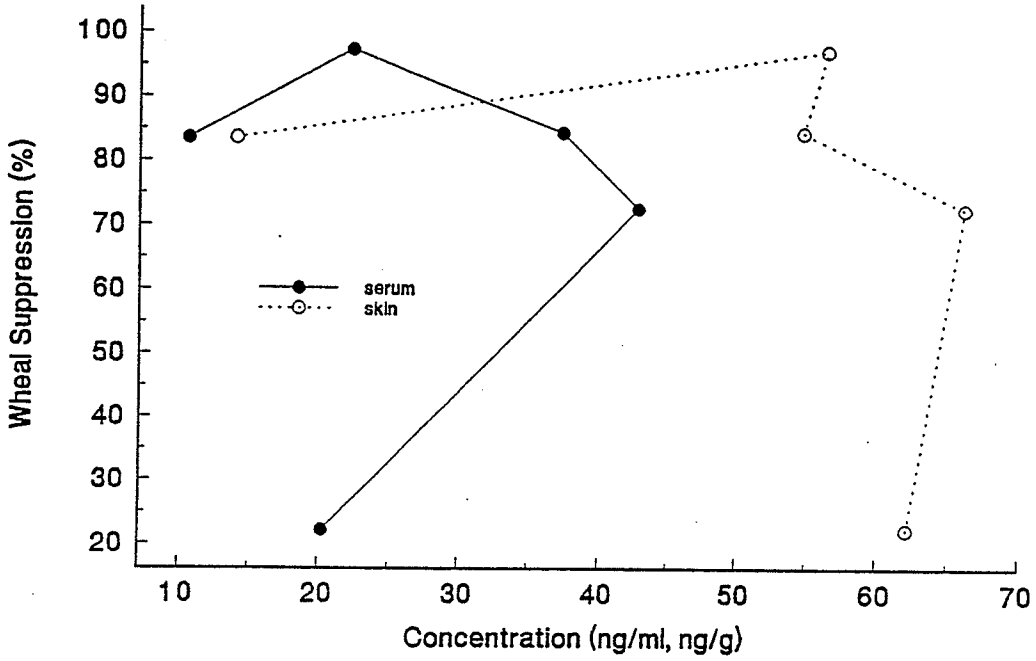

Figure 14. The effect-concentration curve in serum and skin in humans after the intradermal injection of 10 mg/ml histamine phosphate and an oral dose of 50 mg hydroxyzine.

Table 19. Pharmacokinetic parameters of hydroxyzine in human serum after an oral dose of hydroxyzine 50 mg daily

Subject	C_{max} ng/ml	T_{max} hour	$T_{1/2}$ hour	$AUC_{0-\infty}$ ng.hr/ml	Cl_s ml/min/kg	V_β l/kg
1	30.3	3.0	11.8	540	19.8	20.5
2	15.6	3.0	14.0	355	29.6	37.5
3	39.6	3.0	11.3	557	19.6	19.1
4	66.7	3.0	9.6	923	13.4	10.7
5	59.9	3.0	9.0	701	18.0	13.4
6	81.9	6.0	7.3	872	10.4	7.0
MEAN	49.0	3.5	10.5	658	16.8	18.0
SD	24.8	1.2	2.4	197	7.2	9.8

calculated by assuming that hydroxyzine administered orally was totally absorbed (F=1).

Pharmacokinetic parameters of hydroxyzine in human skin are listed in Table 20.

3.1.6. The serum and skin concentrations of cetirizine

Seven of the thirteen healthy adult males with normal skin, mean age 26±6 years, mean weight 75±11 kg, completed the study with cetirizine. The others received hydroxyzine.

3.1.6.1. Serum and skin concentration of cetirizine in human

The serum and skin concentrations of cetirizine in seven healthy males after an oral dose of cetirizine 10 mg daily are listed in Table 21 and Table 22, and the mean serum and skin concentrations of cetirizine are plotted versus time and shown in Figure 15.

3.1.6.2. Pharmacodynamic studies of cetirizine in healthy males after and oral dose of 10 mg cetirizine daily

The results of the efficacy tests calculated as the percent suppression of wheals and flares induced by the intradermal injection of 1 mg/ml and 10 mg/ml histamine are listed in Tables 23,24 and Tables 25,26, and the mean percent

Table 20. Pharmacokinetic parameters of hydroxyzine in human skin after an oral dose of hydroxyzine 50 mg daily

Subject (No.)	C_{max} (ng/ml)	T_{max} (hour)	$T_{1/2}$ (hour)	$AUC_{0-\infty}$ (ng.hr/ml)
1	47.0	1.0	1.8	190
2	69.8	3.0	7.2	720
3	54.1	3.0	10.5	845
4	62.1	3.0	11.5	856
5	96.7	1.0	12.0	1374
6	154.0	6.0	19.4	4959
MEAN	80.6	2.83	12.0	1490
SD	36.4	1.67	4.53	1588

Table 21. Cetirizine serum concentration (ng/ml) in humans after an oral dose of cetirizine 10 mg daily

NO.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0	270.0	179.7	114.9	82.7	16.2	134.0
8	0	349.1	304.9	185.6	138.4	41.2	219.4
9	0	N.S.	287.2	209.9	172.3	58.9	116.3
10	0	324.0	272.5	175.3	120.8	22.1	187.0
11	0	383.5	268.1	145.8	113.4	79.4	192.9
12	0	321.7	261.8	180.1	139.2	53.4	233.2
13	0	521.9	359.9	219.6	153.5	49.3	235.9
MEAN		320.9	276.3	175.9	131.5	45.8	188.4
SD		147.8	50.1	33.4	26.9	20.1	43.8

Table 22. Cetirizine skin concentration (ng/g) in humans after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0	80.5	102.4	71.8	129.7	54.5	238.4
8	0	277.0	128.7	127.4	108.9	35.3	298.4
9	0	108.5	126.3	97.3	53.1	N.S.	128.6
10	0	94.6	241.8	180.5	N.S.	47.2	194.8
11	0	158.7	79.3	84.1	98.4	102.6	182.2
12	0	51.9	83.6	N.S.	31.1	N.S.	42.0
13	0	67.2	47.0	27.5	N.S.	37.6	186.4
MEAN		119.8	115.6	98.1	84.2	55.6	181.5
SD		71.6	57.9	47.5	36.5	24.6	75.0

N.S.: no sample available

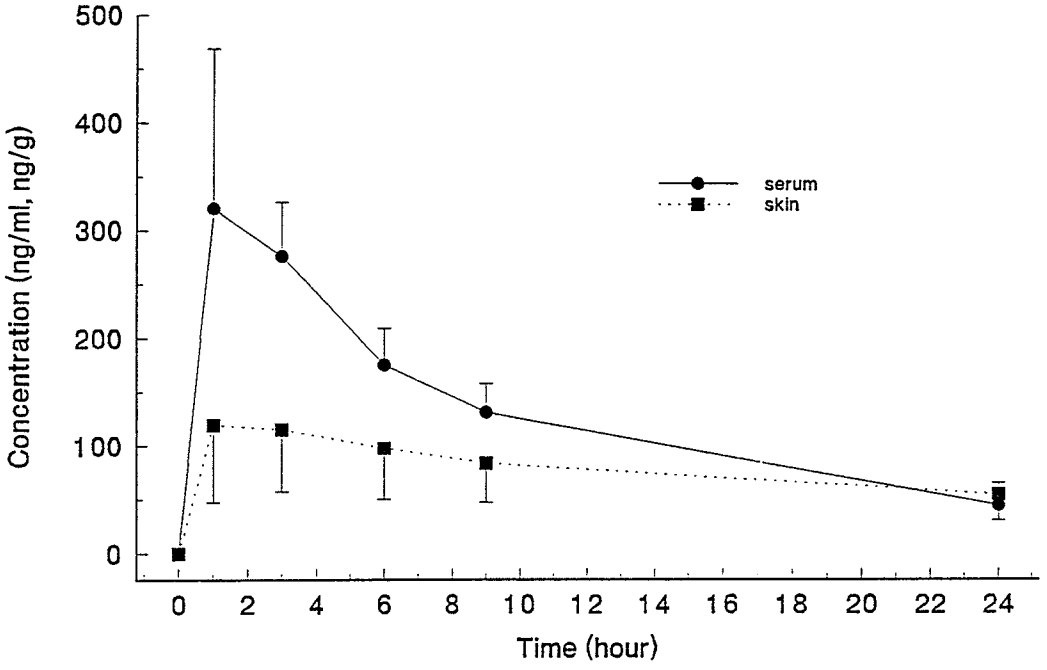

Figure 15. Mean cetirizine concentrations in serum (ng/ml and skin (ng/g) in humans after an oral dose of 10 mg cetirizine (N=7, mean±SD).

Table 23. The percent suppression of wheals in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0.0	33.7	33.7	100.0	100.0	53.0	84.4
8	0.0	100.0	100.0	100.0	100.0	100.0	100.0
9	0.0	43.3	43.3	100.0	68.3	74.0	84.5
10	0.0	100.0	100.0	78.3	100.0	44.1	100.0
11	0.0	47.2	47.2	100.0	100.0	100.0	100.0
12	0.0	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
13	0.0	51.7	51.7	100.0	100.0	79.7	100.0
MEAN		62.6	62.6	96.4	94.7	75.1	94.8
SD		27.0	27.0	8.1	11.8	21.3	7.3

Table 24. The percent suppression of wheals in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0.0	75.9	93.2	89.9	100.0	70.3	80.1
8	0.0	66.7	75.2	87.4	84.0	67.7	81.9
9	0.0	0.0	39.0	73.4	66.8	69.8	72.7
10	0.0	2.7	73.0	76.8	79.0	44.5	71.0
11	0.0	34.1	65.5	81.3	76.5	55.1	72.3
12	0.0	45.1	67.4	100.0	100.0	43.5	100.0
13	0.0	26.1	33.7	100.0	100.0	62.5	100.0
MEAN		35.8	63.9	87.0	86.6	59.0	82.8
SD		27.2	19.3	9.8	12.5	10.7	11.5

N.S.: no sample available

Table 25. The percent suppression of flares in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0.0	78.5	95.8	99.2	97.4	83.3	96.0
8	0.0	96.3	97.0	98.6	96.3	95.4	95.5
9	0.0	65.4	86.9	96.5	93.1	96.6	95.1
10	0.0	44.9	89.2	74.9	72.3	45.3	73.7
11	0.0	0.0	74.0	84.0	89.4	87.7	76.5
12	0.0	0.0	30.2	20.8	27.5	11.7	32.6
13	0.0	69.9	95.2	95.8	92.9	92.6	95.6
MEAN		67.2	81.2	81.4	81.3	70.0	80.7
SD		33.2	22.0	26.1	23.3	31.2	21.6

Table 26. The percent suppression of flares in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-Dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0.0	78.0	94.6	94.2	96.8	88.7	89.2
8	0.0	86.0	91.7	97.0	94.3	86.9	94.6
9	0.0	0.0	71.7	91.1	87.5	86.2	81.8
10	0.0	42.9	76.1	76.5	75.2	29.6	70.9
11	0.0	60.3	90.4	95.3	94.6	91.4	93.3
12	0.0	0.0	78.7	91.4	90.7	80.2	91.6
13	0.0	80.8	85.5	95.9	91.9	93.8	96.3
MEAN		50.0	84.1	91.6	90.2	79.5	88.3
SD		34.4	8.1	6.5	6.7	20.8	8.4

suppressions of wheals and flares in these subjects are plotted versus time in Figure 16 and Figure 17. The mean percent suppressions of wheals induced by 10 mg/ml histamine after 10 mg cetirizine orally are plotted against the mean concentrations of cetirizine in serum and skin and shown in Figure 18. The wheal and flare areas are also listed in Tables 27,28 and Tables 29,30, and plotted in Figures 19,20.

3.1.6.3. Curve fitting, pharmacokinetic and pharmacodynamic parameters

The serum cetirizine concentrations versus time plot after an oral dose of cetirizine 10 mg daily is best described by a triexponential equation, corresponding to an absorption phase, a distribution phase and an elimination phase. The skin cetirizine concentrations versus time plot after the administration is best described by either a biexponential or triexponential equation. Pharmacokinetic parameters of cetirizine in human serum are listed in Table 31. The systemic clearance and the apparent volume of distribution for the central compartment of cetirizine were calculated by assuming that cetirizine administered orally and was totally absorbed (F=1).

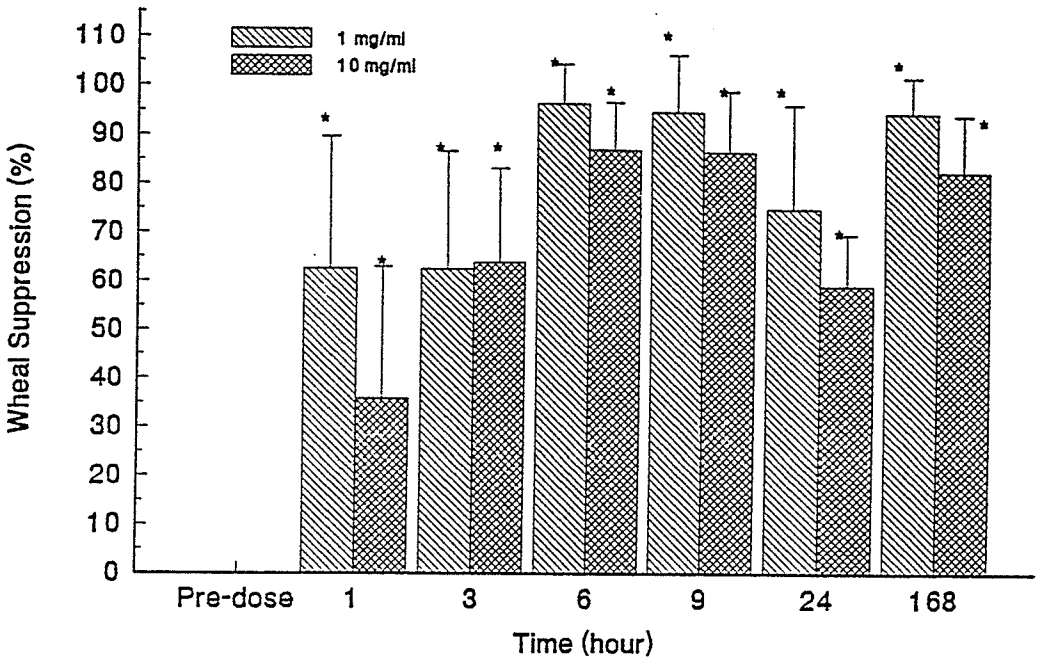

Figure 16. Mean suppressions of wheals in humans induced by intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 10 mg cetirizine daily for seven days (N=7, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

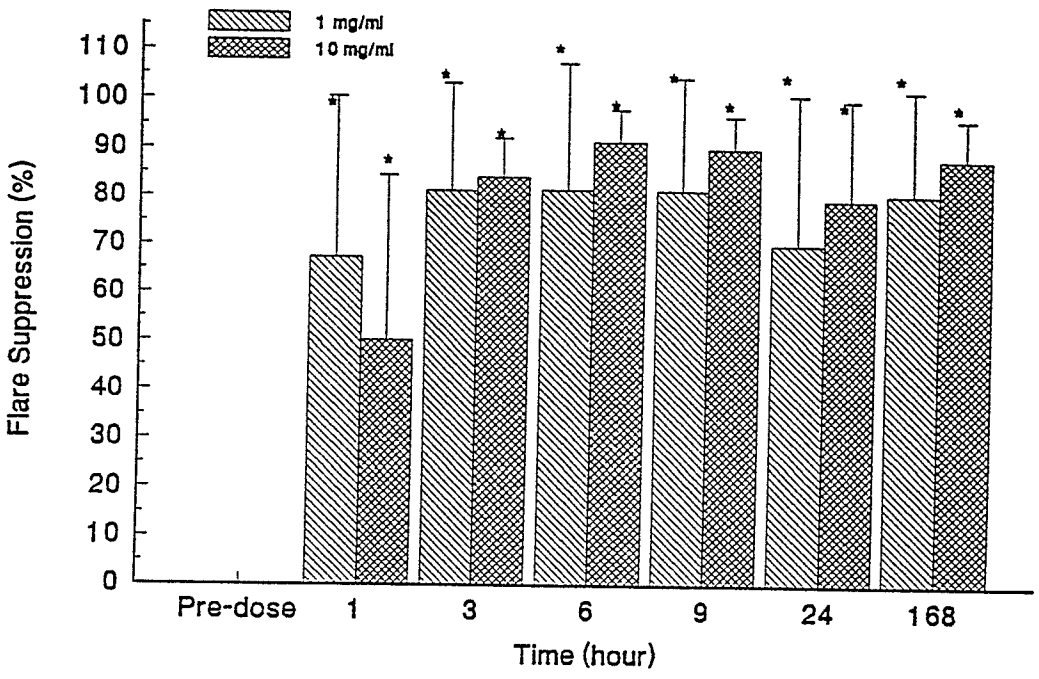

Figure 17. Mean suppressions of flares in humans induced by intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 10 mg cetirizine daily for seven days (N=7, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

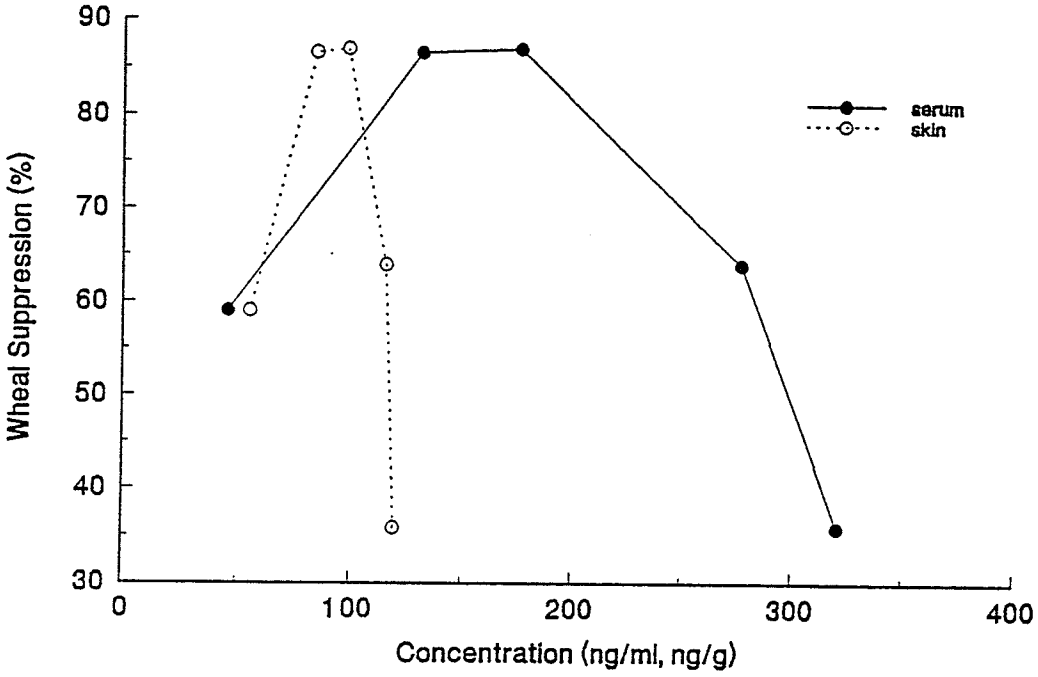

Figure 18. The effect-concentration curve in human serum and skin after the intradermal injection of 10 mg/ml histamine phosphate and an oral dose of 10 mg cetirizine.

Table 27. The wheal areas (cm^2) in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0.217	0.144	0.000	0.000	0.000	0.102	0.033
8	0.257	0.000	0.000	0.000	0.000	0.000	0.000
9	0.179	0.102	0.071	0.000	0.057	0.046	0.027
10	0.147	0.000	0.000	0.032	0.000	0.082	0.000
11	0.153	0.081	0.000	0.000	0.000	0.000	0.000
12	0.000	0.126	0.000	0.000	0.000	0.000	0.000
13	0.281	0.136	0.000	0.000	0.000	0.057	0.000
MEAN	0.176	0.081	0.010	0.005	0.008	0.041	0.008
SD	0.093	0.061	0.027	0.012	0.020	0.042	0.015

Table 28. The wheal areas (cm^2) in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	0.572	0.137	0.038	0.057	0.000	0.169	0.113
8	0.446	0.148	0.110	0.056	0.071	0.144	0.080
9	0.253	0.340	0.154	0.067	0.084	0.076	0.066
10	0.229	0.223	0.062	0.053	0.048	0.127	0.066
11	0.288	0.190	0.099	0.054	0.067	0.129	0.079
12	0.220	0.120	0.071	0.000	0.000	0.124	0.000
13	0.282	0.208	0.187	0.000	0.000	0.105	0.000
MEAN	0.330	0.195	0.103	0.041	0.038	0.125	0.058
SD	0.135	0.074	0.053	0.028	0.037	0.029	0.043

Table 29. The flare areas (cm^2) in humans induced by intradermal injection of 1 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	2.090	0.449	0.088	0.016	0.054	0.350	0.083
8	2.778	0.102	0.082	0.039	0.102	0.129	0.126
9	2.671	0.924	0.350	0.093	0.184	0.091	0.130
10	0.320	0.176	0.034	0.080	0.088	0.175	0.084
11	0.788	0.799	0.204	0.126	0.083	0.097	0.184
12	0.145	3.536	0.101	0.115	0.105	0.128	0.098
13	2.917	0.878	0.141	0.122	0.205	0.217	0.126
MEAN	1.673	0.988	0.143	0.076	0.117	0.170	0.118
SD	1.217	1.171	0.106	0.046	0.056	0.091	0.035

Table 30. The flare areas (cm^2) in humans induced by intradermal injection of 10 mg/ml histamine after an oral dose of cetirizine 10 mg daily

No.\T	Pre-dose	1.0 (h)	3.0 (h)	6.0 (h)	9.0 (h)	24.0 (h)	168.0 (h)
7	4.307	0.862	0.233	0.251	0.135	0.485	0.463
8	5.812	0.815	0.485	0.175	0.328	0.761	0.313
9	2.058	5.968	0.581	0.184	0.257	0.285	0.373
10	1.005	0.574	0.240	0.236	0.249	0.707	0.292
11	4.459	1.771	0.428	0.211	0.240	0.384	0.299
12	1.840	4.397	0.391	0.158	0.171	0.364	0.154
13	4.968	0.954	0.718	0.204	0.403	0.309	0.184
MEAN	3.493	2.192	0.439	0.203	0.255	0.471	0.297
SD	1.832	2.126	0.175	0.033	0.091	0.191	0.105

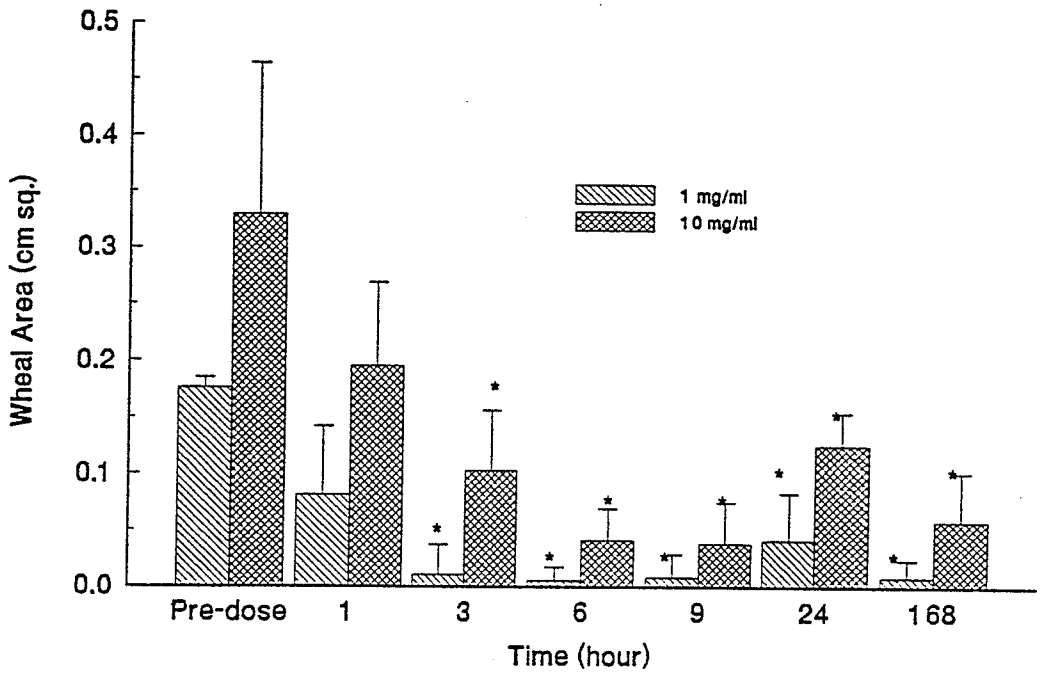

Figure 19. Mean of wheals in humans induced by intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 10 mg cetirizine daily for seven days (N=7, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

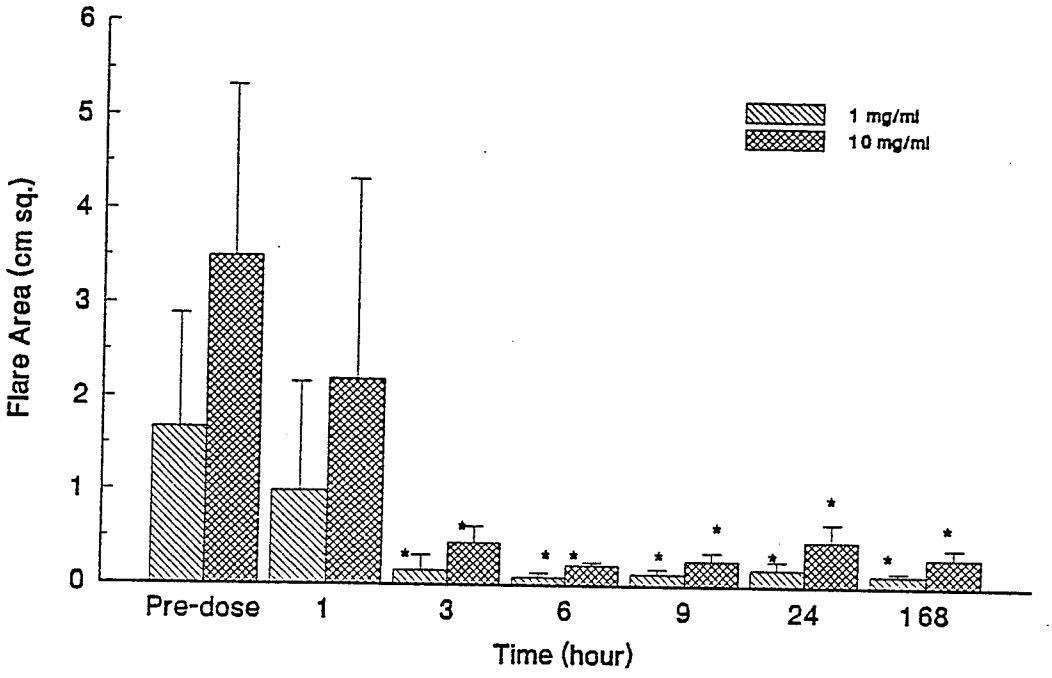

Figure 20. Mean flares in humans induced by intradermal injection of 1 mg/ml and 10 mg/ml histamine phosphate after an oral dose of 10 mg cetirizine daily for seven days (N=7, mean±SD, *significant difference at $P<0.05$ compared to pre-values).

Table 31. Pharmacokinetic parameters of cetirizine in human serum after an oral dose of cetirizine 10 mg daily

Subject (No.)	C_{max} ng/ml	T_{max} hour	$T_{1/2}$ hour	$AUC_{0-\infty}$ ng.hr/ml	Cl_s ml/min/ kg	V_β l/kg
7	270.0	1.0	6.4	2212	0.89	0.41
8	349.1	1.0	8.6	3907	0.64	0.42
9	287.2	3.0	9.8	4315	0.41	0.35
10	324.0	1.0	5.9	3129	0.91	0.43
11	383.5	1.0	15.6	4815	0.45	0.53
12	321.7	1.0	9.6	4046	0.50	0.39
13	521.9	1.0	7.8	4615	0.61	0.37
MEAN	351.1	1.28	9.1	3862	0.63	0.41
SD	77.9	0.70	3.2	843	0.19	0.05

3.1.7. Statistical tests of pharmacodynamic parameters in human study

Mean wheal and flare areas in human subjects induced by 1 mg/ml and 10 mg/ml histamine after the administration of an oral dose of 50 mg hydroxyzine or 10 mg cetirizine were compared by Tukey's Studentized Range Test (α=0.05) to measure whether there was a significant difference among the different test times. The results are listed in Table 32.

3.2. Pharmacokinetic and pharmacodynamic studies of hydroxyzine and cetirizine in rabbits

3.2.1. HPLC assays of hydroxyzine

Representative HPLC chromatograms for hydroxyzine and internal standard antazoline in rabbit serum are shown in Figure 21.

The retention times of hydroxyzine and antazoline are 7.24 and 4.36 minutes respectively. There were no interfering peaks.

3.2.2. Calibration curves for hydroxyzine

The calibration curves for hydroxyzine in rabbit serum were constructed by plotting peak height ratios of hydroxyzine

Table 32. Tukey's Studentized Range (HSD) Test (α=0.05) for mean wheals and flares in humans induced by 1 & 10 mg/ml histamine after an oral dose of 50 mg hydroxyzine and 10 mg cetirizine

Part I: Mean Wheal Area

Time (hour)	Hydroxyzine		Cetirizine	
	Group 1	Group 2	Group 1	Group 2
Pre-dose	A	A	A	A
1.0	B	A	B	B
3.0	B	B	B	B
6.0	C	B	C	C
9.0	C	B	B	C
24.0	C	B	B	B
168.0	C	B	B	C

Part II: Mean Flare Area

Time (hour)	Hydroxyzine		Cetirizine	
	Group 1	Group 2	Group 1	Group 2
Pre-dose	A	A	A	A
1.0	B	A	B	B
3.0	B	B	B	B
6.0	C	B	B	C
9.0	C	B	B	C
24.0	B	B	B	B
168.0	C	B	B	C

* Group 1: 1 mg/ml histamine; Group 2: 10 mg/ml histamine

** Groups with the same letter are not significantly different

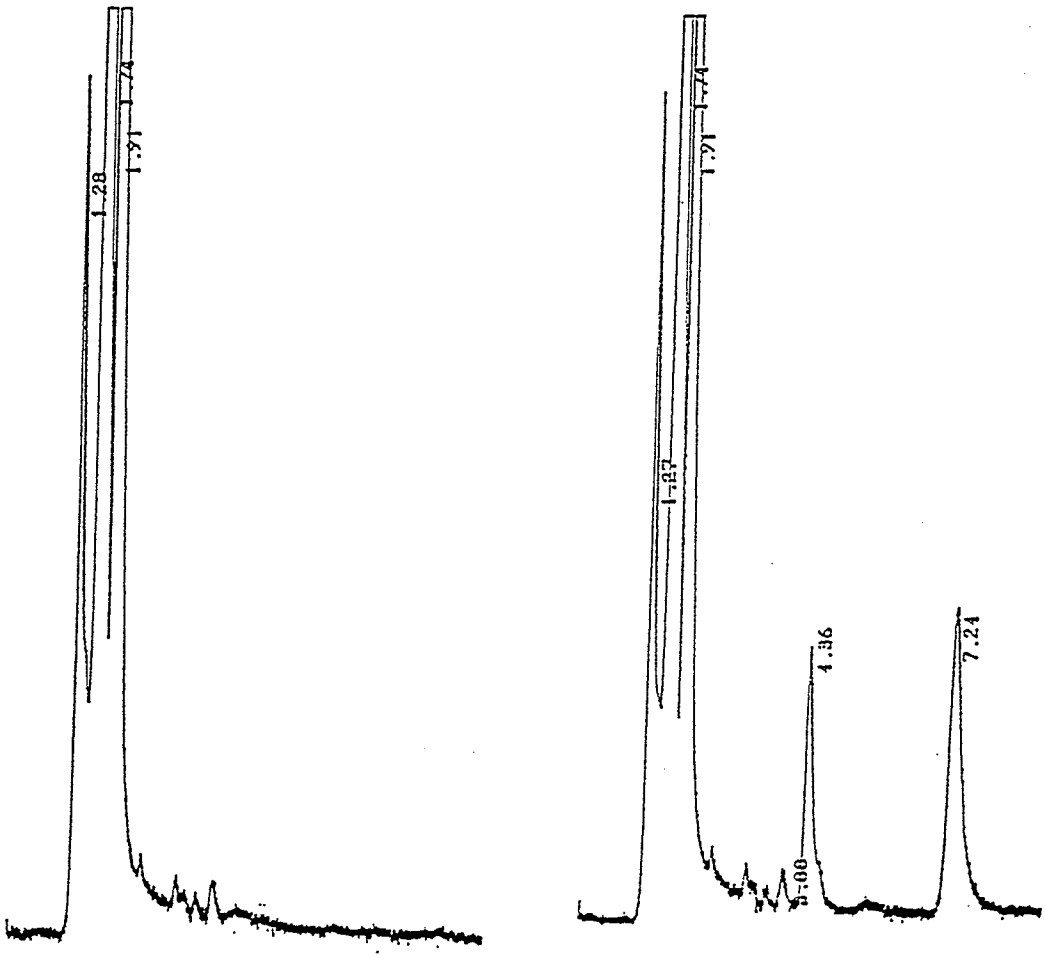

Figure 21. The HPLC chromatograms of hydroxyzine and its internal standard antazoline in rabbit serum (right) compared with that from corresponding blank sample (left). The retention times for hydroxyzine and antazoline are 7.24 minutes and 4.36 minutes respectively.

to antazoline versus concentrations of hydroxyzine. Calibration curves were analyzed periodically during the study period using concentrations of hydroxyzine from 2.0 ng/ml to 100 ng/ml over which range the calibration curve is linear. The variability in the calibration curves over a period of 8 months were calculated from four calibration curves, each having four samples at every concentration. The variability is expressed as the coefficient of variation and shown in Table 33, and the curve is shown in Figure 22. The variability is not shown in Figure in order to maintain the clarity of the mean data. All variances are provided in the corresponding tables.

3.2.3. HPLC assay for cetirizine

Representative chromatograms for cetirizine and its internal standard P_{265} in rabbit serum are shown in Figure 23.

The retention times of cetirizine and P_{265} are 7.02 and 10.02 minutes respectively. No interfering peaks were observed.

3.2.4. Calibration curves for cetirizine

The calibration curves for cetirizine in rabbit serum

Table 33. Variability in HPLC calibration curves for hydroxyzine in rabbit serum

Concentration (ng/ml)	Peak Height Ratio	C.V (%)
2.0	0.0659	12.3
5.0	0.0764	5.6
10.0	0.2246	9.2
25.0	0.4118	2.8
50.0	0.8426	3.1
100.0	1.1948	6.6

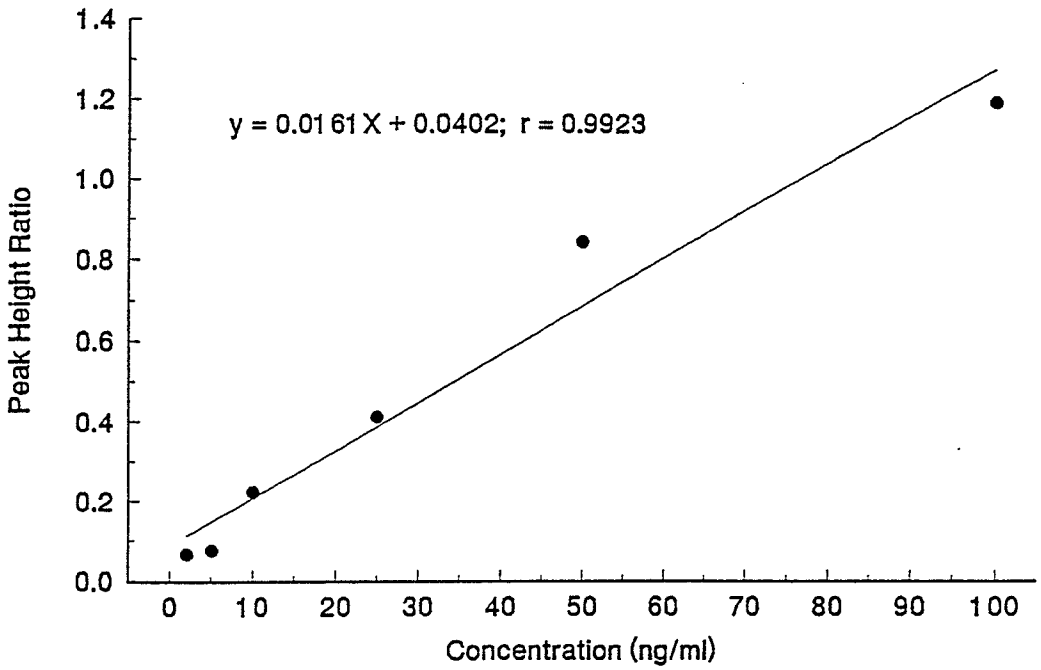

Figure 22. The HPLC calibration curve of hydroxyzine in rabbit serum. The variability was less than 12.3% among four experimental curves performed within eight months.

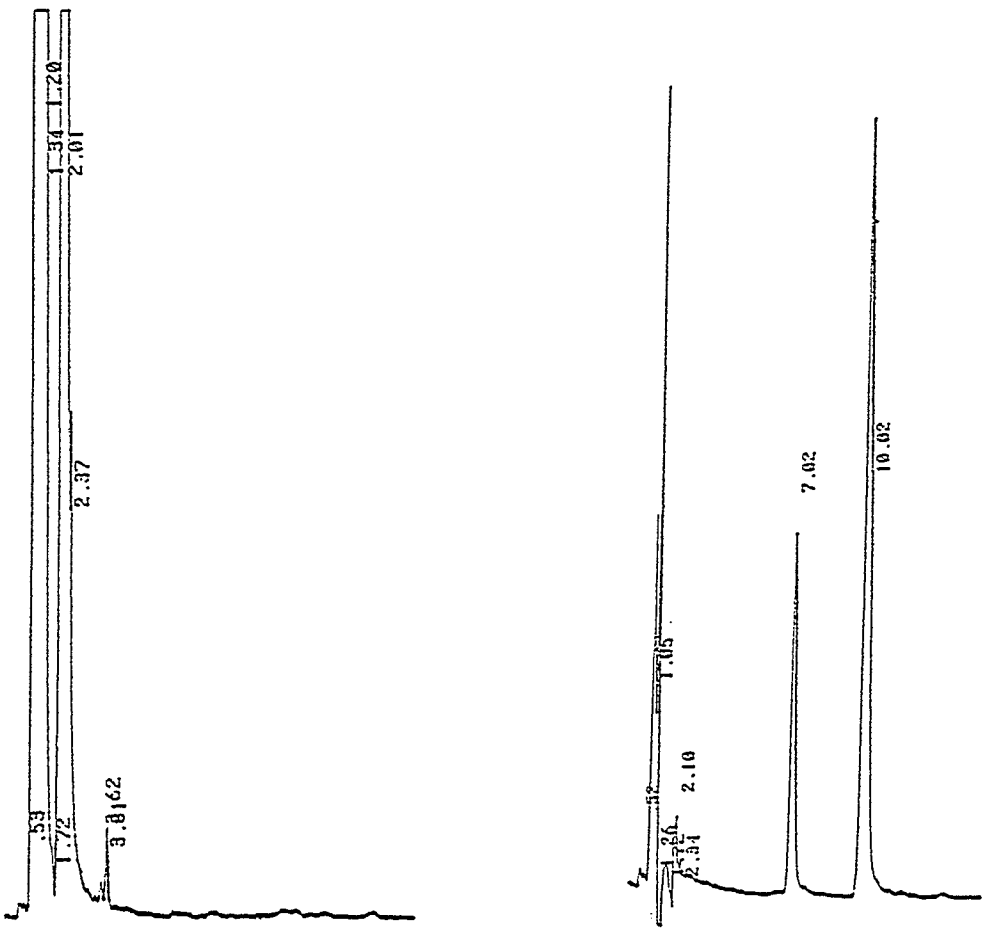

Figure 23. The HPLC chromatograms of cetirizine and its internal standard P_{265} in rabbit serum (right) compared with that from corresponding blank sample (left). The retention times for cetirizine and P_{265} are 7.02 minutes and 10.02 minutes respectively.

were constructed by plotting peak height ratios of cetirizine to P_{265} versus concentrations of cetirizine. The calibration curve was analyzed periodically during the study period using concentrations of cetirizine from 5 ng/ml to 100 ng/ml. The calibration curves were linear over these ranges of concentrations. The variabilities in the serum cetirizine calibration curves over a period of 8 months were calculated from six calibration curves, each having six samples at every concentration. The variability is expressed as the coefficient of variation and shown in Table 34, and the calibration curve is shown in Figure 24.

3.2.5. Pharmacokinetics and pharmacodynamics of hydroxyzine and cetirizine in rabbits: the effect of different formulations

3.2.5.1. Serum concentrations of hydroxyzine in solution and in liposome formulations

Serum concentrations of hydroxyzine following topical application of 10 mg hydroxyzine in solution or in liposome formulations on rabbit back skin are listed in Tables 35, 36.

Table 34. Variability in HPLC calibration curves for cetirizine in rabbit serum

Concentration (ng/ml)	Peak Height Ratio	C.V (%)
5.0	0.0572	2.9
10.0	0.0984	1.8
25.0	0.1663	7.6
50.0	0.3758	3.5
70.0	0.5697	2.7
100.0	0.7285	1.6

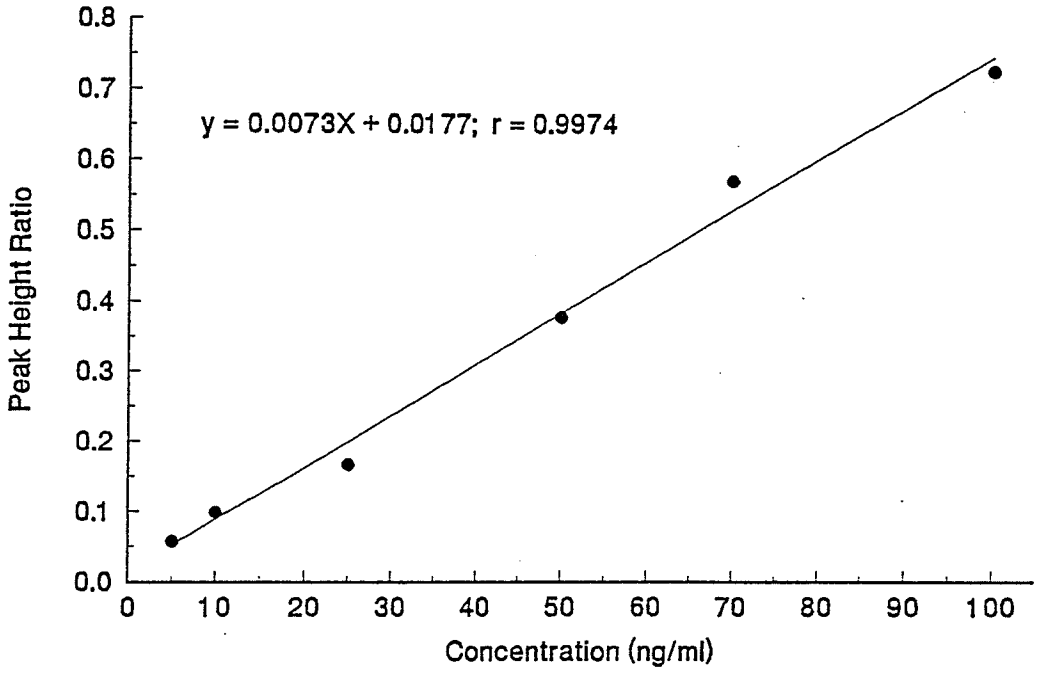

Figure 24. The HPLC calibration curve of cetirizine in rabbit serum. The variability was less than 7.6% among four experimental curves performed within eight months.

Table 35. Serum concentration (ng/ml) of hydroxyzine after topical application of 10 mg hydroxyzine in solution on rabbit skin

N.\T	Pre-dose	0.5 (h)	1.0 (h)	1.5 (h)	2.0 (h)	3.0 (h)	4.0 (h)	6.0 (h)	8.0 (h)
1	N.D.	6.3	5.1	4.6	6.3	5.0	5.0	5.1	N.D
2	N.D.	3.2	4.5	5.1	3.9	N.S.	N.S.	N.S.	N.S
3	N.D.	7.3	6.8	6.3	5.1	4.9	N.S.	N.S.	N.S
4	N.D.	6.0	2.7	1.6	2.8	N.S.	N.S.	N.S.	N.S
5	N.D.	2.9	3.9	2.8	1.6	N.D.	N.D.	N.D.	N.D
6	N.D.	3.0	2.8	N.S.	N.S.	N.S.	N.S.	N.S.	N.S
MEAN		4.8	4.4	4.1	3.9	3.3	2.6	2.0	0.0
SD		1.0	1.4	1.9	1.9	2.8	3.6	2.8	0.0

Table 36. Serum concentration (ng/ml) of hydroxyzine after topical application of 10 mg hydroxyzine in liposome on rabbit back skin

N.\T	Pre-dose	0.5 (h)	1.0 (h)	1.5 (h)	2.0 (h)	3.0 (h)	4.0 (h)	6.0 (h)	8.0 (h)
1	N.D	N.D	N.D	6.3	N.S	3.9	2.8	3.9	2.7
2	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
3	N.D	N.D	1.6	2.7	N.S	N.S	N.S	N.S	N.S
4	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
5	N.D	N.D	N.D	N.D	2.9	5.1	2.7	2.3	N.D
6	N.D	2.8	5.1	5.1	4.6	3.9	5.1	5.0	N.D
mean		0.5	1.1	2.4	1.9	2.6	2.1	2.2	0.5
SD		1.1	2.1	2.8	2.3	2.4	2.1	2.2	1.2

N.D.:not detectable; N.S.: no sample available

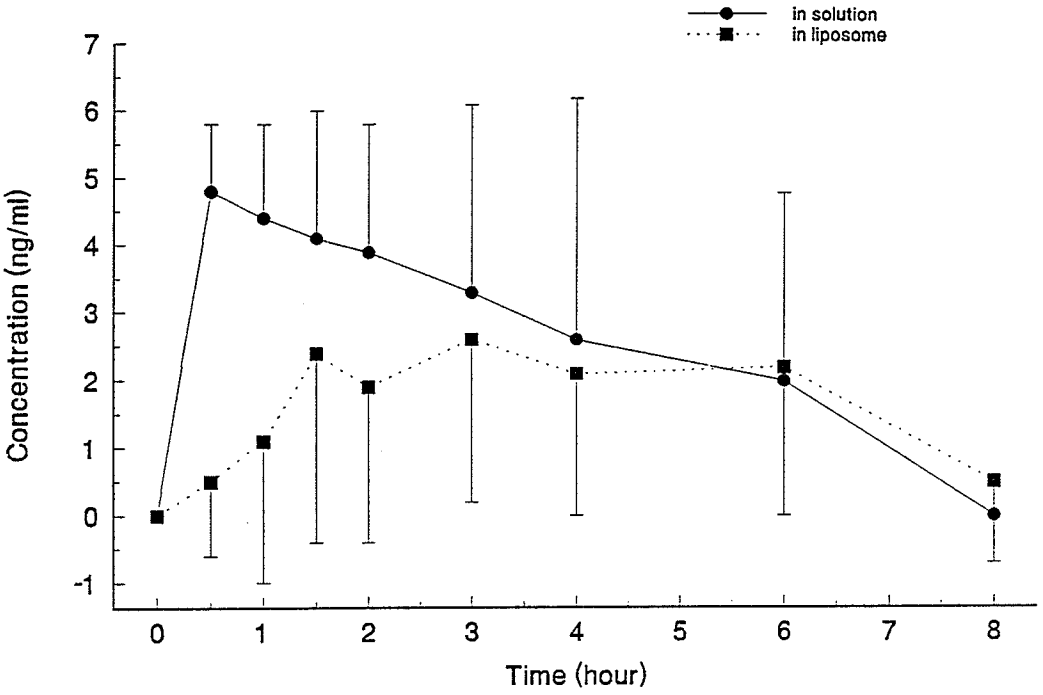

Figure 25. Mean hydroxyzine serum concentrations in rabbit after the topical application of 10 mg hydroxyzine in solution and liposome (N=6, mean±SD).

The mean serum concentrations of hydroxyzine in solution and in liposome are plotted versus time and shown in Figure 25

3.2.5.2. Serum concentrations of cetirizine in solution and in liposome formulations

Serum concentrations of cetirizine after topical application of 10 mg cetirizine in solution or in liposome formulations on rabbit back skin are listed in Table 37 and Table 38. The mean serum concentrations of cetirizine in solution and in liposome are plotted against time and shown in Figure 26.

3.2.5.3. Pharmacodynamic studies of hydroxyzine and cetirizine in rabbit

Results of the efficacy tests calculated as the percent suppression of wheals induced by the intradermal injection of 1 mg/ml histamine after the topical application 10 mg of hydroxyzine in solution and liposome formulations, and 10 mg of cetirizine in solution and liposome formulations are listed in Tables 39,40,41,42. The mean wheal suppressions after the topical application of 10 mg hydroxyzine in solution and in liposome are plotted against time and shown in Figure 27. The mean wheal suppressions after the topical administration of 10 mg cetirizine in solution and in liposome are plotted against

Table 37. Serum concentration (ng/ml) of cetirizine after topical application of 10 mg cetirizine in solution on rabbit back skin

No.\ T	Pre-dose	0.5 (h)	1.0 (h)	1.5 (h)	2.0 (h)	3.0 (h)	4.0 (h)	6.0 (h)	8.0 (h)
1	N.D	3.8	4.3	3.9	3.3	N.D	N.D	N.D	N.D
2	N.D	19.8	15.6	11.2	9.9	6.6	4.2	ND	ND
3	N.D	11.7	7.7	4.0	4.0	N.D	N.D	N.D	N.D
4	N.D	6.6	N.D	8.6	N.D	5.1	N.D	N.D	N.D
5	N.D	N.S	4.8	4.3	4.3	N.D	N.D	N.D	N.D
6	N.D	N.D	N.D	3.2	N.D	N.D	N.D	N.D	N.D
MEAN		8.4	5.4	5.9	3.6	1.9	0.7	0.0	0.0
SD		7.7	5.8	3.3	3.7	3.0	1.7	0.0	0.0

Table 38. Serum concentration (ng/ml) of cetirizine after topical application of 10 mg cetirizine in liposome on rabbit back skin

#\T	Pre-dose	0.5 (h)	1.0 (h)	1.5 (h)	2.0 (h)	3.0 (h)	4.0 (h)	6.0 (h)	8.0 (h)
1	N.D	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
2	N.D	21.0	N.D	1.0	N.D	N.D	N.D	N.D	N.D
3	N.D	16.3	56.3	N.S	N.S	N.S	N.S	N.S	N.S
4	N.D	18.8	16.2	30.7	10.4	12.9	5.6	N.D	N.D
5	N.D	N.D	N.D	N.D	N.D	N.D	N.D	2.7	N.D
6	N.D	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
MEAN		14.1	18.1	10.6	3.4	3.4	4.3	0.9	0.0
SD		9.5	26.6	17.5	5.9	5.9	3.8	1.6	0.0

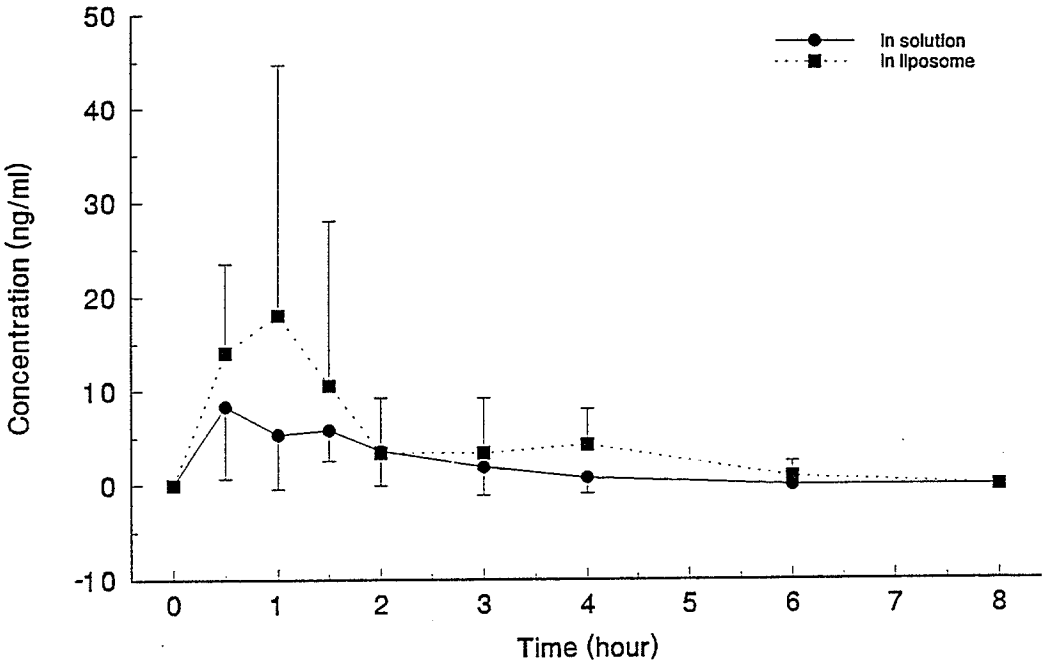

Figure 26. Mean cetirizine serum concentrations in rabbit after the topical application of 10 mg cetirizine in solution and liposome (N=6, mean±SD).

Table 39.The percent suppression of wheals on rabbit back skin induced by intradermal injection of 1 mg/ml histamine after topical application of 10 mg hydroxyzine in solution

T\No. (h)	1	2	3	4	5	6	mean	SD
Pre	0.0	0.0	0.0	0.0	0.0	0.0		
0.5	31.4	33.2	29.0	28.3	27.1	6.9	26.0	9.6
1.0	47.6	38.6	35.3	27.7	30.9	18.2	33.1	10.0
1.5	41.1	58.0	19.7	31.5	24.0	38.2	35.4	13.7
2.0	43.3	50.3	43.3	57.1	46.1	53.3	48.9	5.6
3.0	36.6	45.0	47.5	46.2	38.2	54.1	44.6	6.4
4.0	62.6	51.4	37.1	47.5	40.8	66.4	51.0	11.7
6.0	35.8	37.0	26.4	30.4	34.5	44.5	34.8	6.1
8.0	13.3	39.6	31.2	4.6	13.2	31.6	20.6	15.5
24.0	3.7	3.8	1.5	2.5	0.0	5.9	2.9	2.1

Table 40.The percent suppression of wheals on rabbit back skin induced by intradermal injection of 1 mg/ml histamine after topical application of 10 mg hydroxyzine in liposome

T\No. (h)	1	2	3	4	5	6	mean	SD
Pre	0.0	0.0	0.0	0.0	0.0	0.0		
0.5	55.7	46.9	35.8	45.1	41.8	44.1	44.9	6.5
1.0	66.6	33.1	47.9	62.6	47.4	44.8	50.4	12.3
1.5	67.0	33.8	64.9	74.2	84.4	46.7	61.8	18.5
2.0	75.7	24.3	53.2	85.0	86.2	58.8	63.9	23.5
3.0	83.1	76.2	82.9	95.2	94.9	41.0	78.9	20.0
4.0	55.8	58.0	78.1	95.1	58.1	48.4	65.6	17.5
6.0	69.6	44.9	75.8	98.4	87.3	45.1	70.2	21.8
8.0	48.4	30.3	68.2	57.8	80.2	25.3	51.7	21.4
24.0	19.5	38.5	50.1	55.3	40.6	26.7	38.4	13.6

Table 41.The percent suppression of wheals on rabbit back skin induced by intradermal injection of 1 mg/ml histamine after topical application of 10 mg cetirizine in solution

T\No. (h)	1	2	3	4	5	6	mean	SD
Pre	0.0	0.0	0.0	0.0	0.0	0.0		
0.5	51.6	43.0	31.6	31.7	51.8	17.6	37.9	13.4
1.0	37.3	45.1	2.6	60.8	71.5	46.7	52.2	13.7
1.5	37.0	37.8	15.3	68.5	71.0	49.3	46.5	21.1
2.0	36.1	46.1	47.8	47.0	49.5	30.9	42.8	7.5
3.0	51.7	51.1	45.3	29.0	40.0	33.1	41.7	9.3
4.0	13.6	63.0	38.0	36.4	32.0	28.6	35.1	15.8
6.0	11.2	45.0	21.6	38.6	25.5	22.5	27.4	12.3
8.0	6.8	28.4	4.4	34.6	18.5	12.8	17.6	12.0
24.0	8.0	13.5	1.5	5.9	10.9	7.4	7.9	4.2

Table 42.The percent suppression of wheals on rabbit back skin induced by intradermal injection of 1 mg/ml histamine after topical application of 10 mg cetirizine in liposome

T\No. (h)	1	2	3	4	5	6	mean	SD
Pre	0.0	0.0	0.0	0.0	0.0	0.0		
0.5	13.3	46.7	15.3	34.6	29.4	69.0	34.7	20.9
1.0	38.3	57.6	34.8	50.9	49.5	42.0	45.5	8.6
1.5	42.7	62.3	52.0	45.3	55.6	54.7	52.1	7.2
2.0	44.3	68.6	18.9	66.8	68.5	61.5	54.7	19.8
3.0	32.9	51.8	51.5	62.6	69.1	73.9	56.9	15.0
4.0	24.2	75.4	55.3	74.7	54.4	71.3	59.2	19.5
6.0	34.9	78.4	66.0	82.0	43.9	45.7	58.5	19.7
8.0	29.1	38.9	46.2	61.8	44.7	32.2	42.1	11.7
24.0	34.9	53.9	45.7	32.0	29.9	41.4	39.6	9.1

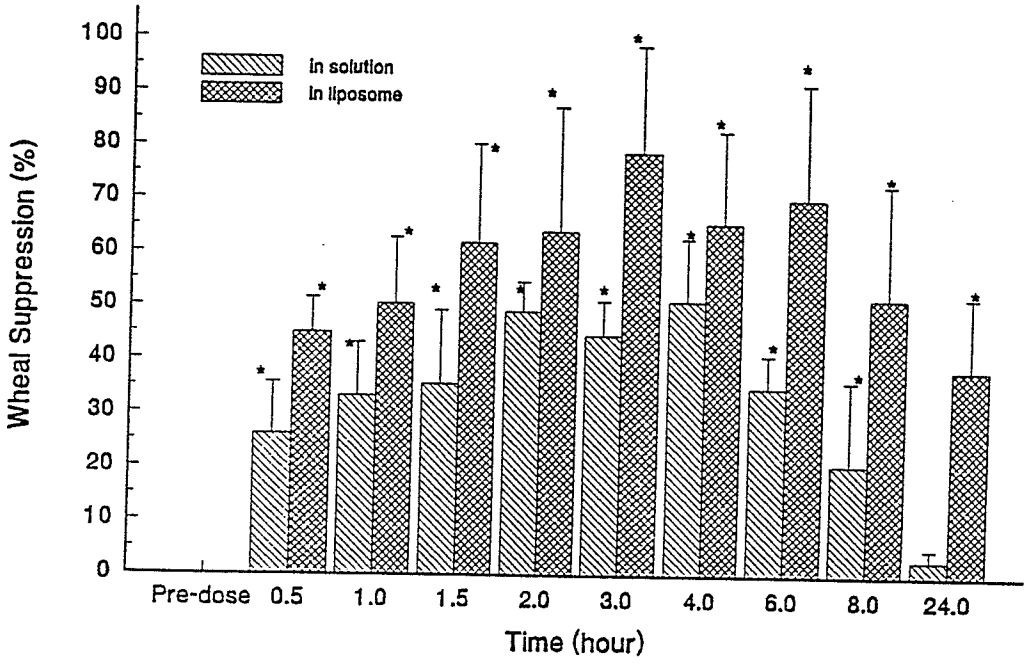

Figure 27. Mean wheal suppressions of hydroxyzine in rabbit induced by 1 mg/ml histamine phosphate after the topical application of 10 mg hydroxyzine in solution and liposome (N=6, mean±SD, *significant difference at $p<0.05$ compared to pre-values).

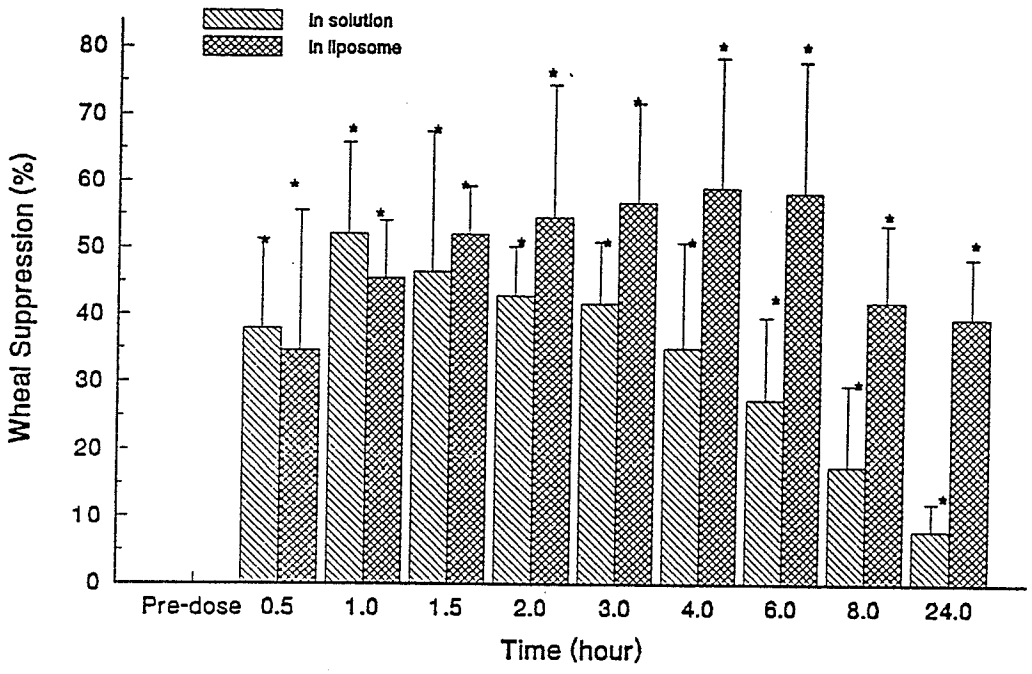

Figure 28. Mean wheal suppressions of cetirizine in rabbit induced by 1 mg/ml histamine phosphate after the topical application of 10 mg cetirizine in solution and liposome (N=6, mean±SD, *significant difference at $p<0.05$ compared to pre-values).

Table 43. The wheal areas (cm^2) on rabbit skin induced by 1 mg/ml histamine after topical application of 10 mg hydroxyzine in solution

T\No. (h)	1	2	3	4	5	6	MEAN	SD
Pre-	0.727	0.903	0.878	0.809	0.861	0.840	0.840	0.060
0.5	0.499	0.603	0.623	0.580	0.627	0.802	0.622	0.100
1.0	0.381	0.554	0.568	0.585	0.595	0.704	0.560	0.100
1.5	0.428	0.379	0.705	0.554	0.654	0.532	0.540	0.120
2.0	0.412	0.449	0.498	0.347	0.464	0.402	0.420	0.050
3.0	0.461	0.497	0.461	0.435	0.532	0.395	0.460	0.050
4.0	0.272	0.439	0.552	0.425	0.510	0.289	0.410	0.110
6.0	0.467	0.569	0.646	0.563	0.564	0.478	0.550	0.070
8.0	0.630	0.545	0.604	0.772	0.747	0.591	0.649	0.089
24.0	0.700	0.869	0.865	0.789	0.863	0.810	0.816	0.066

Table 44. The wheal areas (cm^2) on rabbit skin induced by 1 mg/ml histamine after topical application of 10 mg hydroxyzine in liposome

T\No. (h)	1	2	3	4	5	6	MEAN	SD
Pre-	0.805	0.659	1.001	0.818	0.667	0.956	0.820	0.140
0.5	0.357	0.350	0.643	0.449	0.388	0.534	0.450	0.120
1.0	0.269	0.441	0.522	0.306	0.351	0.528	0.400	0.110
1.5	0.266	0.436	0.351	0.211	0.104	0.510	0.310	0.150
2.0	0.196	0.499	0.468	0.123	0.092	0.394	0.300	0.180
3.0	0.136	0.157	0.171	0.039	0.034	0.564	0.180	0.200
4.0	0.356	0.277	0.219	0.040	0.279	0.493	0.280	0.150
6.0	0.245	0.363	0.242	0.013	0.085	0.525	0.250	0.190
8.0	0.415	0.459	0.318	0.345	0.132	0.714	0.397	0.191
24.0	0.648	0.405	0.500	0.366	0.396	0.701	0.503	0.141

Table 45. The wheal areas (cm^2) on rabbit skin induced by 1 mg/ml histamine after topical application of 10 mg cetirizine in solution

T\No. (h)	1	2	3	4	5	6	MEAN	SD
Pre-	0.789	0.843	0.813	0.734	0.769	0.689	0.771	0.052
0.5	0.382	0.480	0.556	0.501	0.371	0.568	0.476	0.084
1.0	0.495	0.463	0.792	0.288	0.219	0.367	0.437	0.203
1.5	0.497	0.524	0.689	0.231	0.223	0.349	0.419	0.183
2.0	0.504	0.454	0.424	0.389	0.388	0.476	0.439	0.047
3.0	0.381	0.412	0.445	0.521	0.461	0.461	0.447	0.047
4.0	0.682	0.312	0.504	0.467	0.523	0.492	0.500	0.118
6.0	0.701	0.464	0.637	0.451	0.573	0.534	0.560	0.097
8.0	0.735	0.604	0.777	0.480	0.627	0.601	0.637	0.106
24.0	0.726	0.729	0.801	0.691	0.685	0.638	0.711	0.054

Table 46. The wheal areas (cm^2) on rabbit skin induced by 1 mg/ml histamine after topical application of 10 mg cetirizine in liposome

T\No. (h)	1	2	3	4	5	6	MEAN	SD
Pre-	0.653	0.736	0.615	0.746	0.768	1.030	0.758	0.140
0.5	0.566	0.392	0.521	0.488	0.542	0.319	0.471	0.100
1.0	0.403	0.312	0.401	0.366	0.388	0.597	0.411	0.100
1.5	0.374	0.277	0.295	0.408	0.341	0.467	0.360	0.070
2.0	0.364	0.231	0.499	0.248	0.242	0.397	0.330	0.110
3.0	0.438	0.355	0.298	0.279	0.237	0.269	0.313	0.070
4.0	0.495	0.181	0.275	0.189	0.350	0.296	0.296	0.120
6.0	0.425	0.159	0.209	0.134	0.431	0.559	0.320	0.180
8.0	0.463	0.450	0.331	0.285	0.425	0.698	0.442	0.140
24.0	0.425	0.339	0.334	0.507	0.538	0.604	0.457	0.110

time and shown in Figure 28. The wheal areas of above data are also listed in Tables 43,44,45,46, and shown in Figures 29,30.

3.2.6. Statistical tests of pharmacokinetic parameters

Mean wheal and flare areas at each time, which were induced by 10 mg/ml histamine phosphate after topical treatment by both hydroxyzine and cetirizine in either solution or liposome formulation were compared by using Tukey's Studentized Range Tests (α=0.05) to measure whether there were significant differences. The results are listed in Table 47.

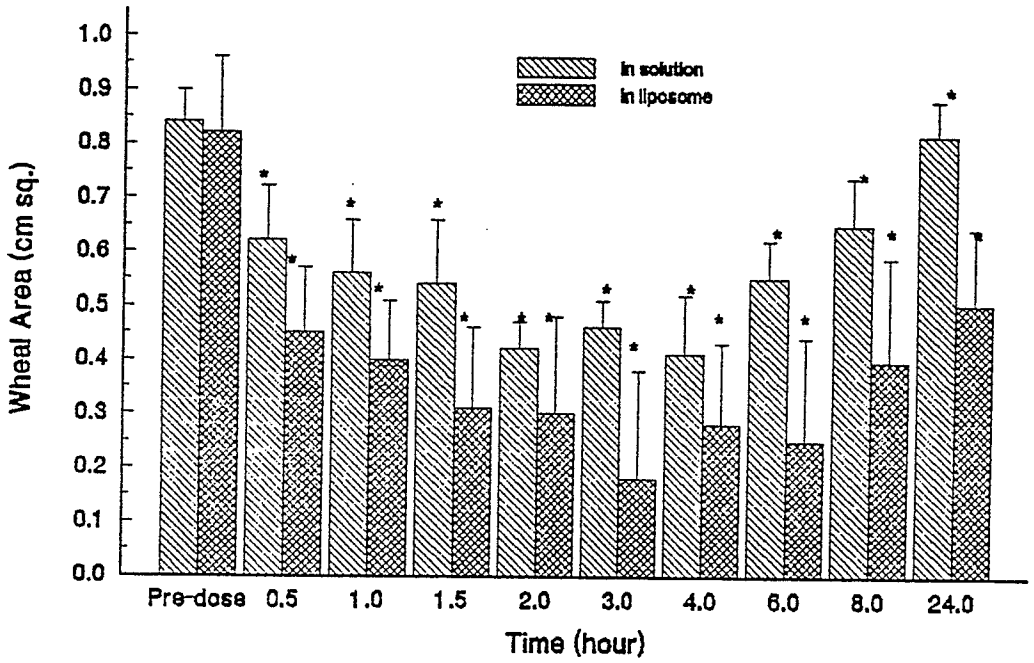

Figure 29. Mean wheals of hydroxyzine in rabbit induced by 1 mg/ml histamine phosphate after the topical application of 10 mg hydroxyzine in solution and liposome (N=6, mean±SD, *significant difference at $p<0.05$ compared to pre-values).

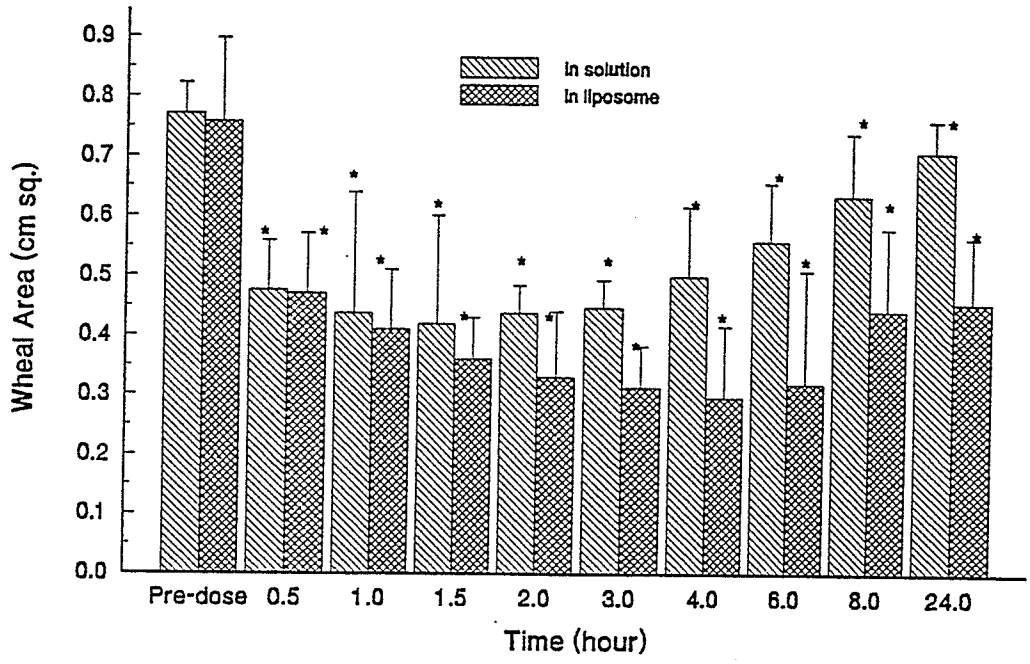

Figure 30. Mean wheals of cetirizine in rabbit induced by 1 mg/ml histamine phosphate after the topical application of 10 mg cetirizine in solution and liposome (N=6, mean±SD, *significant difference at $p<0.05$ compared to pre-values).

Table 47. Tukey's Studentized Range (HSD) Test (α=.0.5) for wheal areas comparison of pre-dose and after doses in rabbit induced by 10 mg/ml histamine after topical application of antihistamines in different formulations

Time (h)	Hydroxyzine (mean)		Cetirizine (mean)	
	solution	liposome	solution	liposome
Pre-dose	A	A	A	A
0.5	B	B	C	B
1.0	B	B	C	B
1.5	B	B	C	B
2.0	C	B	C	B
3.0	C	B	C	B
4.0	C	B	C	B
6.0	B	B	B	B
8.0	B	B	B	B
24.0	A	B	B	B

* Group with the same letter are not significantly different

CHAPTER IV. DISCUSSION

4.1. Pharmacokinetic and pharmacodynamic studies of hydroxyzine and cetirizine in human serum and skin

4.1.1. HPLC assays

HPLC methods, developed in our laboratory (19,48,56), for measuring the serum and skin concentrations of hydroxyzine were modified for these studies. A Nova-pak C_{18} Radial-pak 0.8cm X 10cm cartridge was used, composed of a flexible casing packed with particles 4 μm in diameter. The structure of the Radial Compression column results in a more homogeneously packed structure, reduces the void volume and increases the column efficiency. Additionally, the Nova-pak C_{18} packing is less hydrophilic than that of C_{18} μBondapak, which was used for hydroxyzine HPLC analysis previously. The retention times of hydroxyzine and its internal standard were reduced resulting in a shorter analysis time.

Mobile phases of different compositions were evaluated to optimize the degree of resolution. The mobile phase finally selected for hydroxyzine in this study was acetonitrile-phosphate buffer (0.075M $NH_4H_2PO_4$, pH = 2.5) (33:67, v/v). The retention times for hydroxyzine and its internal standard antazoline were 7.25 and 4.23 minutes respectively. No

interfering peaks were observed.

Within the concentration range of 2.0 ng/ml to 100 ng/ml, the hydroxyzine-antazoline peak height ratios versus hydroxyzine concentrations were linear when extracted from serum. The coefficient of variation was less than 10.6% within the experimental period.

The calibration curves for hydroxyzine extracted from skin over the concentration range of 0.1 ng/g to 2.0 ng/g was also found to be linear. The coefficient of variation was less than 7.6% during the experimental period.

The extraction of hydroxyzine from both serum and skin samples was relatively simple and efficient. The recovery of a known quantity of hydroxyzine in serum using the present method was 80%, according to previous studies in our laboratory.

As to the cetirizine serum and skin samples, the same HPLC system and column as those of hydroxyzine were used except a different mobile phase was selected composed of acetonitrile-phosphate buffer (0.075M $NH_4H_2PO_4$, pH = 2.9), with 1 M sodium decanesulfonate (46:54, v/v).

The limitation of sensitivity of this system for both cetirizine and hydroxyzine was 1-2 ng/ml in serum and 0.1 to 0.5 ng/g in skin.

Within the calibration curve concentration range which was from 1 ng/ml to 200 ng/ml in serum, the peak height ratios of cetirizine and its internal standard P_{265} were found to be linear. The coefficient of variation calculated over the test period was less than 12.8% for all concentrations.

The calibration curves for cetirizine in skin with the range of 0.5 ng/g to 10 ng/g was found linear. The coefficient of variability was less than 9.1% for all concentrations.

The extractions of cetirizine from both serum and skin samples were more complicated and less efficient than those of hydroxyzine. Cetirizine is an oxidative derivative of hydroxyzine with a carboxylic acid group instead of a hydroxyl group at the end of its side-chain, which allows it to be ionized easily in aqueous solution. The pH of the buffer added to serum and skin samples at the beginning of the extraction is critical. The citrate buffer of pH =5.0 gives the highest efficiency of cetirizine extraction and the recovery of a known quantity of cetirizine in serum was around 55%, according to previous studies performed in this laboratory.

Another problem experienced during the cetirizine extraction was that serum samples emulsified very easily during vortexing, which made the separation difficult after centrifugation. From experience, the simple solution to this problem was to repeat the vortex-centrifuge step as many times

as necessary.

4.1.2. Pharmacokinetic and pharmacodynamic studies of hydroxyzine in healthy human subjects: the difference of hydroxyzine concentration in serum and skin after an oral dose of 50 mg hydroxyzine daily

It is a well-known fact that body mass distribution, e.g. fat/muscle ratio, mean body weight, etc., is different between male and female subjects. Data obtained only from male subjects in this study may be different from data which would have been obtained from females. In spite of these differences, female subjects were not used in this study for several reasons. Firstly, concern about potential pregnancy and teratogenicity, because female subjects would be in child bearing age range 18-40 years. Secondly, since the most reliable methods of contraception would be oral contraceptives, there is the possibility of drug interaction between the oral contraceptives and the test drugs. Thirdly, from a cosmetic point of view, testing may leave scars on the inner thighs and this may not be acceptable to female subjects.

4.1.2.1 Pharmacokinetics of hydroxyzine in human

serum and skin

Figure 9 shows the hydroxyzine serum concentration versus time after an oral dose of 50 mg hydroxyzine. The curve is best described by a biexponential equation, corresponding to an absorption phase and an elimination phase.

Pharmacokinetic analysis is based on fitting multiple exponential equations to the serum concentration versus time data. The best fit was selected based on the minimization of the sum of squares. Up to 5 exponential were evaluated for each data set. Due to the small number of samples in the skin study, it was not possible to fit more than a bi- or triexponential equation. This resulted in a minimal number of pharmacokinetic parameters, so it was not possible to do meaningful multi- compartmental modelling.

After a single oral dose of 50 mg hydroxyzine, the mean maximum serum concentration of 49.0 ± 24.8 ng/ml occurred at 3.5 ± 1.2 hours post-dose. The mean elimination half-life calculated from the terminal linear portion of the serum concentrations of hydroxyzine vs time curve was 10.5 ± 2.4 hours. The mean clearance rate was 16.8 ± 7.2 ml/min/kg and the mean apparent volume of distribution was 18.0 ± 9.8 L/kg. The AUC was 658 ± 197 ng.h/ml (Table 19).

The short t_{max} reflected a rapid absorption at the beginning after the administration. A relative short half-life

was obtained in present study, compared to previous studies in which Simons et al.(19) reported an elimination half-life of 20.0 ± 4.1 hour after a single oral dose of hydroxyzine syrup, 0.7 mg/kg (mean 39.0 ± 5.4 mg). In previous studies (19,30,57), samples were collected for 48 hours and up to 7 days after a single dose.

Following oral administration, there is always the possibility that some absorption and distribution is still occurring at 6-8 hours after dosing and the resulting serum concentrations at these times may not reflect a true post-absorption/distribution elimination phase. In addition, samples at times greater than 24 hours were not available in the present study since the multiple dose portion of the study was started at that time. Since samples at earlier times may be affected by continuing absorption and/or distribution, and no samples after 24 hours were collected, the small number of data points used to calculated the terminal elimination half-life may be skewed, resulting in an underestimation of the true elimination half-life value.

Again a lower mean maximum concentration observed in the present study of 49.0 ± 24.8 ng/ml compared to the literature value of 72.7 ± 11.1 ng/ml reported after a similar oral dose of hydroxyzine syrup (19). In the study reported from the literature, samples were collected hourly from 0-12 hours, so

there is a good possibility of determining a valid C_{max}. In the present study, samples were collected only at 0,1,3,6 and 9 hours, so it is possible that a true C_{max} value was not observed.

The other pharmacokinetic parameters were not significantly different from the data shown in other studies (19,30,57).

The concentrations of cetirizine generated *in vivo* after the single oral dose of hydroxyzine 50 mg were also measured (Table 10). A mean maximum concentration of cetirizine of 259.7 ± 65.1 ng/ml occurred at 4.1 ± 1.9 hours after hydroxyzine was given. It was much higher than that of its parent compound, hydroxyzine of 49.0 ± 24.8 ng/ml observed at 3.5 ± 1.2 hours post-dose. Concentrations of cetirizine were approximately 12 times higher than that of hydroxyzine (419.7 ± 178.0 ng/ml vs 35.2 ± 20.0 ng/ml) at steady-state after 50 mg oral dose of hydroxyzine daily for seven days.

The pharmacokinetic parameters of cetirizine produced *in vivo*, as an active metabolite of hydroxyzine, following hydroxyzine administered orally may be quite different from those after administration of cetirizine alone (58,59). According to Gibaldi and Perrier (58), at least five exponential terms are needed to describe the time course of the metabolite of a drug displaying multiple compartments

properties. Therefore, it is difficult to describe pharmacokinetic processes of cetirizine arising in the body after the oral administration of hydroxyzine. Using the cetirizine concentrations following hydroxyzine adminstration, the serum cetirizine concentration versus time curve is best described by a biexponential equation describing a formation phase and an elimination phase.

The mean skin concentration versus time curve of hydroxyzine after the dose was similar to that in serum, but with higher concentration at test points, 1,3,6,9,24 hours post-dose (Figure 9). The curve is also best described by a biexponential equation: an absorption phase and an elimination phase. At steady-state, 168 hours post-dose, the mean hydroxyzine skin concentration of 88.8 ± 16.0 ng/g was more than two times higher than that in serum of 35.0 ± 20.0 ng/ml (Tables 8,9).

The concentrations of cetirizine attained *in vivo* in skin after the administration of hydroxyzine 50 mg was not studied due to the limited skin samples available in this study.

The mean skin hydroxyzine concentrations in this study was significantly higher than that found in the serum, which were concomitantly measured in the subjects throughout the dosing intervals after the oral administration of the drug. This data confirmed other earlier studies in rabbits (60,61)

in which it was found that skin concentrations of the H_1-receptor antagonists hydroxyzine, cetirizine, diphenhydramine, and chlorpheniramine following single intravenous doses were as high, or even higher than those in serum.

The mean elimination half-life of hydroxyzine in humans after an oral dose of 50 mg hydroxyzine 10.5 ± 2.4 hours in serum and 12.0 ± 4.53 hours in skin (Table 20). Although these values are shorter than previous literature values (19), there was no significant difference between the two values, indicating the possibility of similar elimination profile in serum and in skin.

The AUC of hydroxyzine in skin was 1490 ± 1588 ng.h/ml, which was much higher than that in serum.

4.1.2.2. The pharmacodynamics of hydroxyzine in humans

The histamine skin test is a well-standardized quantitative test of antihistamine efficacy (15,62,63). The marked wheal and flare areas were measured by digitizer connected to a personal computer with good accuracy and efficient reproducibility in this study (15).

The mean wheal area suppressions after the intradermal injection of 0.01 ml, 1 mg/ml and 10 mg/ml histamine, wre all

significantly different from the pre-dose values ($p<0.05$) from 1 to 24 hours (Figure 10). At 168 hours, 12 hour after the final 50 mg oral dose in the multiple doses study of hydroxyzine, significant suppression was still present.

Maximum wheal suppression was 100% and 98.3% for 1 mg/ml and 10 mg/ml histamine, occurring at 6 hours and at the 168 hour time interval, respectively. The mean wheal suppression ranges for 1 mg/ml histamine injection was 51.1% ± 43.1% to 100%, and 22.2% ± 19.2% to 98.3% ± 3.9% for the 10 mg/ml histamine dose. No significant difference was found between these two histamine doses (Tables 11,12).

The flare areas were significantly suppressed from 1 hour to 24 hour and at 168 hour, after the intradermal injection of both 1 mg/ml and 10 mg/ml histamine, compared to the pre-dose value ($p<0.05$) (Figure 11). The maximum mean flare suppressions after the injection of the two histamine doses were similar (91.8% ± 3.8% vs 91.2% ± 4.6%) occurring at 9 hours post-dose. The flare suppression ranges for the 1 mg/ml and 10 mg/ml histamine injection were 44.7% ± 40.4% to 91.8% ± 3.8% and 22.5% ± 18.6% to 91.2% ± 4.6% respectively (Tables 13,14). There were also no significant differences between these two doses.

The mean hydroxyzine serum & skin concentration-effect relationship curve is shown in Figure 14. Pharmacodynamic

analysis of the serum concentration of hydroxyzine versus percent suppression of histamine induced wheals resulted in loops of hysteresis indicating a delay in onset of response relative to drug concentration (64). The lag in onset of maximum response is probably not due to the delay in the drug distribution to the skin, since hydroxyzine concentrations in skin measured at the same testing point were always higher than those in serum, which was confirmed by this and an earlier study (60). The delay in onset may be due to the time required for the drug to reach a sufficient concentration at the receptor site and bind to the receptor in order to elicit a response.

A similar pharmacodynamic analysis calculated with the skin concentrations of hydroxyzine. The plot showed smaller loop of hysteresis, indicating a stronger correlation between H_1-blockade and skin concentration of H_1-receptor antagonists in skin, compared to the serum data (Figure 14).

4.1.3. Pharmacokinetic and pharmacodynamic studies of cetirizine in healthy humans

4.1.3.1. Pharmacokinetics of cetirizine in human serum and skin

Seven healthy volunteers received an oral dose of 10 mg

cetirizine daily for seven days. Serum and skin samples were collected at pre-dose, and 1,3,6,9,24,168 pose-dose.

Serum and skin concentration versus time curves after an oral dose of 10 mg cetirizine daily are shown in Figure 15. The curves could be best described by either triexponential or biexponential phases, corresponding to an absorption phase, an elimination phases for the biexponential equation, with an extra distribution phase for the triexponential equation.

In serum, a mean maximum concentration of cetirizine of 351.1 ± 77.9 ng/ml occurred at 1.28 ± 0.7 hours after administration. The half-life calculated from the terminal linear portion was 9.1 ± 3.2 hours after single oral dose of cetirizine 10 mg. The AUC was 3862 ± 843 ng.hr/ml with this dose. The apparent volume distribution of cetirizine was 0.41 ± 0.05 L/kg in this study and the apparent clearance is 0.63 ± 0.19 ml/min/kg (Table 31). All these pharmacokinetic parameters were consistent with data reported in the literature (23), in which the peak plasma concentration was 257 ng/ml within 1 hour after administration of 10 mg oral dosage to healthy volunteers. The volume of distribution of cetirizine at steady-state was 30-40 L/kg. The terminal phase elimination half-life is 7 to 10 hours and the apparent total body clearance is 0.67 to 0.83 ml/min/kg.

The mean maximum cetirizine concentrations of 351.1 ±

77.8 ng/ml in serum and 152.3 ± 73.7 ng/g in skin, indicated that cetirizine was absorbed and distributed quickly. The maximum concentration of cetirizine in serum was nearly two times as high as that in skin, suggesting that partition of cetirizine into the skin was less than for hydroxyzine. This may be due to the fact that cetirizine is more hydrophillic than hydroxyzine.

The elimination half-life of cetirizine in serum and in skin were 9.1 ± 3.2 hour and 10.6 ± 8.4 hour respectively. There was no significant difference between each other, which also indicated parallel elimination profile from the skin as from the serum for cetirizine. A study conducted by Schafer-Korting et al. (65) showed that the terminal half-life of 9.3 hours calculated from plasma level for griseofulvin after a single oral dose was similar to that of 9.2 hours and 9.8 hours calculated from suction blister fluid and cantharides blister fluid in skin respectively.

The systemic clearance of cetirizine in serum after an oral dose of 10 mg daily was 0.63 ml/min/kg, which confirmed the results obtained from the study conducted by Lefebvre et al(66), in which an apparent oral clearance of 0.64 ml/min/kg was reported.

The cetirizine concentration at steady-state was 188.4 ± 43.8 ng/ml in serum and 181.5 ± 75.0 ng/g in skin after

multiple doses of 10 mg/day cetirizine orally (Tables 21,22). In another similar part of this investigation (Table 48) in which only three subjects were chosen from the same 15 volunteers, cetirizine concentrations arising from hydroxyzine in serum at steady-state after multiple doses of 50 mg/day hydroxyzine orally were 481.6 ± 85.0 ng/ml, while only 39.7 ± 27.1 ng/g was found in skin.

If it is assumed that about 55% of a dose of hydroxyzine is metabolized to cetirizine *in vivo*, then the resulting estimated dose of 27.5 mg cetirizine is obtained. Because cetirizine displays linear kinetics after oral administration, the serum concentrations shown in the two studies could be expected to relate to the doses. On the other hand, the mean skin concentration of cetirizine, as an active metabolite, arising after hydroxyzine administration was far lower than that after given cetirizine alone at steady-state.

For most drugs, metabolism occurs in the hepatocytes, but metabolism can occur in the gut, in the kidney, in the lung and even in the skin. If metabolism of hydroxyzine to cetirizine takes place in a more peripheral site, e.g. skin, the extent of distribution of cetirizine into the skin may be reduced, since cetirizine may partition back into the systemic circulation peferentially. In addition, cetirizine is more polar (lipophobic) and does not distribute into the skin as

Table 48.Serum and skin concentration of hydroxyzine and cetirizine produced *in vivo* in human at steady-state after a multiple dose of hydroxyzine 50 mg/day

Subject (No.)	Cetirizine(HDZ) in skin (ng/g)	Serum (ng/ml)	
		Hydroxyzine	Cetirizine
CM-4	42.0	19.05	396.8
NJ-7	11.6	78.9	480.6
CF-8	65.6	44.7	567.5
MEAN	39.7	47.6	481.6
SD	27.1	30.0	85.4

readily as hydroxyzine. Cetirizine arising from hydroxyzine may be eliminated at a different rate to cetirizine administered alone. Although serum concentrations of cetirizine from hydroxyzine are higher than from cetirizine alone, exposure to sites of partition into skin may differ because of the site of generation of cetirizine from hydroxyzine. Also the presence of hydroxyzine present may have an effect on the distribution of cetirizine into skin. If active transport is involved, both similar molecules may compete and hydroxyzine may be distributed more readily than cetirizine.

4.1.3.2. Pharmacodynamics of cetirizine in human

The mean wheal areas after epicutaneous injection of histamine, 1 mg/ml and 10 mg/ml, were significantly suppressed ($P<0.05$) at all testing times, 1,3,6,9, and 24 hours post-dose after single dosing, and at 168 hours after multiple doses of 10 mg/day cetirizine orally, compared to the base-line ($p<0.05$) (Figure 16). No significant difference was found between the two histamine doses. The range of wheal suppression was 62.6% ± 27.0% to 94.7% ± 11.8% for 1 mg/ml histamine challenge and 35.8% ± 27.2% to 87.0% ± 9.8% for 10 mg/ml dose (Tables 23,24).

The mean flare areas after 1 mg/ml and 10 mg/ml histamine injection were significantly suppressed ($P<0.05$) at all

testing times, compared to the pre-dose values (Figure 17). Values of 67.2% ± 33.2% to 81.4% ± 26.1% and 50.0% ± 34.4% to 91.6% ± 6.5% were the ranges of flare suppression after 1 an 10 mg/ml histamine injection respectively (Tables 25,26). No significant difference was observed between the two histamine dose.

Cetirizine and hydroxyzine are the most effective H_1-receptor antagonists available for wheal-and-flare suppression. Cetirizine will even suppress wheal and flare responses produced by epicutaneous test with extremely high concentration of histamine, as high as 500 mg/ml (67). The most effective serum concentration of H_1-receptor antagonists required to suppress the wheal and flare response are unknown. The serum and skin concentration-effect curves is shown in Figure 18, in which skin concentration-effect curve showed a smaller loop of hysteresis than that of serum.

In the skin biopsy study, the smallest number of samples possible were drawn due to the invasive nature of the study. There was therefore minimal data with which to evaluate the pharmacodynamics of hydroxyzine and cetirizine mathematically. Even at 168 hour, steady state, there was no simple linear relationship between H_1 antagonist serum concentration and effect as monitored by suppressions of histamine induced wheal and flare areas.

4.2. Pharmacokinetic and pharmacodynamic study of hydroxyzine and cetirizine in rabbit: the effect of different formulations

4.2.1. HPLC assays

The HPLC assay for measuring hydroxyzine and cetirizine concentrations in rabbit serum was same as that used for human serum. The slight difference of retention times of hydroxyzine and its internal standard antazoline, cetirizine and its internal standard P_{265} caused by the efficacy of the column.

Within the concentration range of 2 ng/ml to 100 ng/ml for hydroxyzine and 5 ng/ml to 100 ng/ml for cetirizine, the calibration curves were found to be linear and the coefficients of variation at all selected points were less than 12.3% for hydroxyzine and 7.6% for cetirizine during the whole experimental period (Figures 22,24).

4.2.2 The preparation of liposome formulation

Liposomes have increasing applications as models of biological membranes and as drug delivery vesicles for *in vivo* applications in recent years.

Most phospholipids spontaneously adopt a bilayer organization when dispersed in water, and form closed bilayer "liposome" structure. These liposomes may be large or small; multilamellar or unilamellar depending upon the method of dispersal. The simplest liposome to construct is obtained by mechanically dispersing lipid in water. The resulting

multilamellar vesicles (MLV) consist of concentric bilayers of lipid with water entrapped between the bilayers, and these are of relatively large diameter (>400nm).

As drug delivery vesicles, the technique of drug entrapment must satisfy demands such as high trapping efficiency and reasonably long retention times. Another important consideration in the behaviour of liposomes *in vivo* is the sensitivity to vesicle size and composition. Workers in several laboratories have demonstrated that vesicles composed only of phospholipid were rendered leaky in serum or plasma, but this effect can be reduced by the incorporation of cholesterol into the liposomal bilayer. Techniques for the efficient entrapment of a large variety of lipophilic and hydrophilic agents inside vesicles of differing size and lipid compositions are now available (68).

Topical administration of liposomes is generally employed where the slow-release property of liposome-encapsulated drug is desired. Under these conditions, the retention of the vesicles at the site of application is preferred. Large MLVs appear most suitable for this purpose since they remain localized at the site of application much longer than the small vesicles do (69).

Since the efflux of water-soluble drug from liposomes is often dictated by their membrane permeability, MLVs can exhibit maximal drug retention time due to the number of

lamellae. There are now a large variety of techniques for generating multilamellar liposomal system. The procedures for MLV that result in increased trapped volume and equilibrium solute distribution include hand-shaken MLVs with variation of lipid composition, dehydration-rehydration procedures and freeze-thawing. Previous studies have demonstrated that the multiple freeze-thaw step completed directly on MLV dispersions results in a 10-fold or greater increase in the aqueous trapped volume (70). Trapping efficiencies as high as 88% for these systems can be achieved by utilizing high lipid concentrations. Encapsulation efficiencies approaching 50% for MLVs can also be obtained by dehydration-rehydration procedures (71).

In this study, the lipid was dissolved in an organic solvent, which was subsequently evaporated under vacuum to form a thin film. The film was hydrated with buffer containing hydroxyzine or cetirizine. The lipid aggregates into the bilayer liposomal system as the hydrophobic nature of their environment decreases. Clearly, equilibrium solute distributions are more likely to be achieved by this hydration procedure, which can also lead to higher trapping efficiency for drugs present in the aqueous buffer. The limitations of this method are that lipids may have some solubility in the organic solvent employed, as well as the problem of the removal of traces of the solvent from the final preparation.

Hydroxyzine and cetirizine, available as the dihydrochloride salts are two water-soluble lipophilic drugs (72), which can be encapsulated into the MLVs system by the preparation of "hydration from organic solvent" technique used in our study. A stable desired concentration of the drugs encapsulated in the liposome was achieved according the results of HPLC analysis of these formulations.

4.2.3. Pharmacokinetic and pharmacodynamic study of hydroxyzine in rabbit after the topical administration of 10 mg hydroxyzine solution & liposome formulations

The mean hydroxyzine serum concentration versus time curves after the topical application of 10 mg hydroxyzine solution and liposome are shown in Figure 25. Both 10 mg doses of solution formulation and liposome formulation resulted in a significantly lower concentration in serum compared to the 10 mg intravenous dose (1), with a maximum concentration of 4.8 ± 1.0 ng/ml and 2.6 ± 2.4 ng/ml respectively (Tables 35,36). However, although relatively higher concentrations were found following the aqueous solution formulation, no significant difference was found between the two formulations.

Hou et al. reported (73) that after the topical application of aqueous lidocaine and liposome lidocaine on forty rabbit vessels, serum levels of lidocaine were

significantly lower. The peaks in concentration appeared later after the use of liposome lidocaine than after the use of aqueous formulation. This was reported to be the result of the local accumulation of lidocaine entrapped in liposome and the slow release of lidocaine from the liposome formulation.

The calculation of different pharmacokinetic parameters was not possible due to the insufficient test points during the late elimination phase. A significant number of test points were also below the limitation of detection, which reduced the reliability of our pharmacokinetic parameters calculated by traditional curve-fitting methods.

In contrast, the efficiency tests provide some interesting results. First, the wheal areas on rabbit back skin induced by the intradermal injection of 1 mg/ml histamine were significantly suppressed ($p<0.05$) from 0.5 hours to 24 hours after a 10 mg hydroxyzine dose either in solution or in liposome, compared to the pre-dose value (Figure 27), although the extent of suppression at 24 hours after the solution may not be clinically relevant. The percent suppression ranges of 20.6% ± 15.5% to 51.0% ± 11.7% for solution were significantly less than values of 44.9% ± 6.5% to 78.9% ± 20% for the liposome formulation (Tables 30, 40). At 24 hours after administration, hydroxyzine liposome formulation still yielded 38.4 ± 13.6% suppression when only 2.9 ± 2.1% was found for the solution formulation, however both values were

significantly different from pre-dose values ($p<0.05$). These results indicated that liposome formulation increased and prolonged the duration of the effect of the drug following topical administration when compared to the solution formulation. The elimination half-life in rabbits of hydroxyzine after intravenous administration is 2 hours, so 10 mg dose iv will have a minimal effect at 8-10 hours post-dose (1).

Another study of various formulations evaluating *in vitro* diffusion in hairless mouse skin (74) showed that the accumulation of drugs in stratum corneum was in the following order: 'skin lipid' liposomes > 'phospholipid' liposomes > emulsion > hydroalcoholic solution, suggesting that topically applied liposomes, particularly those prepared from lipid mixtures having compositions similar to the stratum corneum, may provide sustained, enhanced levels of drug in the stratum corneum (the reservoir) while minimizing high levels in the strata associated in blood and lymph supplies. This was also found in the present study as serum hydroxyzine concentrations were lower after the liposome formulation, yet yielded superior wheal suppressions.

4.2.4. Pharmacokinetic and pharmacodynamic study of cetirizine in different formulations after topical administration of 10 mg cetirizine on rabbit skin

The mean serum cetirizine concentration versus time curves, after the topical application of 10 mg cetirizine either in solution or in liposome, are shown in Figure 26. The maximum concentration was 18.1 ± 26.6 ng/ml for the liposome formulation, occurring at 1.0 hour while 8.4 ± 7.7 ng/ml for the solution formulation observed at 0.5 hours (Tables 37,38). Cetirizine from liposomes resulted in slightly higher serum concentrations than that from solution.

Compared to its parent drug, hydroxyzine, cetirizine has a carboxylic acid group at the end of its side-chain, which makes it more lipophobic. For less polar molecules, the lipid-saturated stratum corneum can be a sink for topically applied lipotropic materials (75). The lower concentration of hydroxyzine detected in the blood after topical administration is probably due to the holding ability of the stratum corneum, compared to cetirizine. On the other hand, liposome enhances the penetration into the deep layer of skin, so cetirizine, the more hydrophilic molecule, carried by the liposome, penetrated into the deep layer of skin which contains blood vessels, resulting in a slightly higher serum concentration of cetirizine from liposome formulation.

It is known that the skin pocesses many of the same

enzymes that the liver does. Cutaneous enzymes are active and, in the epidermal layer, may be equal or even exceed the activity of hepatic drug metabolizing enzymes (76) and if we assume that these enzymes are contained in the epidermal layer, hydroxyzine may be metabolized by skin enzymes, it is not surprising that less hydroxyzine got into the systemic circulation. It was not possible to measure cetirizine concentrations following the topical administration of hydroxyzine.

The insufficient test points during the late elimination phase, as well as numbers of undetectable concentrations made it impossible to calculate reliable pharmacokinetic parameters.

According to the wheal suppression data, induced by 1 mg/ml histamine, significant suppression was found from 0.5 hours to 24 hours after the topical administration of 10 mg cetirizine either in solution or liposome, compared to the base-line (Tables 41,42). No significant difference existed between the two formulations as the result of statistical evaluation, corresponding to the similar maximum of percent suppression of 52.2% ± 13.7% in solution occurring at 1.0 hour versus 59.2% ± 19.5% in liposome observed at 4.0 hours, respectively. The delay of onset effect of the drug and relatively higher suppression at 24 hours for the liposome formulation, compared to the solution formulation (39.6% ±

9.1% vs 7.9% ± 4.2%) indicated that liposome also prolonged the effect of cetirizine like hydroxyzine.

Liposomes are macromelecular complexes having a hydrophillic surface, they can control the delivery and localization of drug at skin surface and in this way might provide a route of administration that would ameliorate undesirable systemic adverse-effects or toxicity, following oral administration. There should be no sedation following the topical administration of hydroxyzine since serum levels were very low. It is also expected that longer retention times of drug from MLVs (liposome) will occur compared to that for aqueous solution due to their molecular size.

The permeability barrier of the stratum corneum consists of a hydrophobic lipid lamellae. Consequently, drugs used topically with good effect tend to have hydrophobic characteristics that enable them to diffuse through the barriers. Some studies in the literature have investigated whether liposomes can promote the percutaneous absorption of more water-soluble drugs. Methotrexate was readily encapsulated in the aqueous core, and liposomal methotrexate has been shown to be retained in the skin of hairless mice longer than free drug (77). In this study, both hydroxyzine and cetirizine, which were water-soluble and encapsulated in the liposomal aqueous core, were shown to have variable lag times in the efficacy test from half an hour to three hours,

compared to the solution formulation.

CHAPTER V. SUMMARY AND CONCLUSION

There were two objectives of the present study. First, an evaluation of the correlation of serum and skin concentrations with the amount of peripheral H_1-blockade produced following single and multiple oral doses of hydroxyzine and cetirizine in human subjects. Second, an evaluation of pharmacokinetics and pharmacodynamics of hydroxyzine and cetirizine in different formulations after topical administration on rabbit back skin.

Human studies were carried out on 13 healthy male adults, among which, 6 received an oral dose of 50 mg hydroxyzine capsules and 7 received an oral dose of 10 mg cetirizine tablets for seven days respectively. Animal studies were performed on six New Zealand white rabbits, each received a topical administration of 10 mg hydroxyzine or cetirizine either in solution or in liposome. Blood samples were collected at pre-scheduled times after drug administration, followed by skin tests induced by an intradermal injection of histamine. HPLC systems were used to measure serum and skin concentrations of hydroxyzine and cetirizine. A computer connected digitizer was used to calculate the areas of wheal and flare induced by intradermal injection of histamine.

Pharmacokinetic data from the human studies were calculated by using PKCALC computer program. Various

pharmacokinetic parameters, such as C_{max}, t_{max}, the elimination half-life, the apparent volume of distribution, the systemic clearance and $AUC_{0-\infty}$, were calculated. The pharmacodynamic data were calculated as percent suppression of wheal and flare areas after the skin efficacy tests. Statistical differences among the data from different studies were tested by statistical programs of either ANOVA or Tukey or Boniferroni Multiple Range Test.

In the human study, it was found that skin hydroxyzine concentrations were higher than serum concentrations during 24 hours after the initial dose, and at steady-state after multiple dosing. However, skin cetirizine concentrations were lower than serum concentrations during the first 9 hours after dosing, but at 24 hours and at steady-state, skin concentrations were equal to or higher than the serum concentrations. There was no significant difference between the mean elimination half-life values of hydroxyzine in serum and in skin, which indicated the possibility of similar elimination profile in serum and in skin. The apparent volume of distribution of cetirizine, e.g., 0.4 L/kg, was considerably smaller than that of hydroxyzine, e.g., 16 L/kg. This could be due to the slightly lower penetration of cetirizine into the skin compartment due to its more lipophobic characteristics.

Wheal and flare areas were significantly suppressed from

3-24 hours, inclusive, and at steady-state after epicutaneous test with 1 mg/ml and 10 mg/ml histamine, compared to the pre-dose values after oral administration of hydroxyzine and cetirizine in human studies. Correlations between H_1-blockade and serum H_1-receptor antagonist concentrations were weak, but correlations between H_1-blockade and skin H_1-receptor antagonist concentrations were somewhat stronger.

In animal studies, the serum concentrations following topical application of hydroxyzine or cetirizine in either solution or liposome were found to be very low compared to those values following other routes of administration with the same dose reported in the literature. No pharmacokinetic parameters were calculated.

In the skin efficacy tests, wheal areas were significantly suppressed after all the treatments, compared to pre-dose values. Significant differences were found in hydroxyzine application with suppression ranges of 20.6% to 51% for solution and 44.9% to 78.9% for liposome. At 24 hours, liposome formulation still showed 38% suppression while only 2.9% was found in solution. A similar maximum suppression (52.2% vs 59%) was found in the cetirizine study, but a significant difference was observed at 24 hours which showed 39.6% suppression with liposome and only 7.9% for solution.

Only a limited number of skin biopsy samples were obtained due to the invasive nature of the technique. This

resulted in difficulties in data analysis using standard pharmacodynamic models such as the E_{max} model. However, the slightly better correlations obtained using skin H_1-receptor antagonist concentration data, provides support for the hypothesis that measurement of H_1-receptor antagonist values closes to the site of action may provide better information regarding the optimum concentration of medication required to provide the maximum therapeutic effect. Once these values are established, it may be possible to correlate these concentratios with serum concentrations which can be measured more easily resulting in the definition of obtained serum H_1-receptor antagonist concentrations.

In summary, liposome formulation of either hydroxyzine or cetirizine exhibited a greater suppression of wheal areas than its solution, particularly at 24 hours after topical administration. Also, topical application of both solution and liposome formulation yielded extremely low serum concentrations, indicating minimal systemic absorption.

The topical administration of H_1-receptor antagonists in the treatment of allergic skin disorders is not recommended in many text books (78). This restriction is based on one or two clinical reports of adverse effects after topically administered H_1-receptor antagonists in cases where severe skin abrasion was present (44). Although the rabbit skin was shaved and treated with a depilatory, there was minimal

systemic H_1-receptor antagonist absorption from the site of application of the formulations used in this study.

In the treatment of allergic skin disorders, the topical route of application provides a source of drug close to the site where the effect is required. The minimal absorption observed following these topical administration studies results in lower serum concentrations, and thus should reduce the possibility of central adverse effects such as sedation. Based on the results of our research, the topical route of administration of H_1-receptor antagonists should be re-considered, especially in cases of treatment of allergic skin disorders.

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