EVALUATION OF THE DISPOSITION AND EFFICACY OF HYDROXYZINE IN LIPOSOME FORMULATIONS APPLIED TOPICALLY TO RABBITS

BY

Mohammad Jehad Bawati

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MOHAMMAD JEHAD BAWATI

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Glossary

C.V.:	Coefficient of Variation
S.D.:	Standard Deviation
SEM:	Standard Error of the Mean
HPLC:	High Performance Liquid Chromatography
EPC:	Egg Phosphatidylcholine
Chol:	Cholesterol
MLV:	Multilamellar Vesicles
LUV:	Large Unilamellar Vesicles
SUV:	Small Unilamellar Vesicles
EIM:	Ethanol Injection Method Liposomes

CHAPTER I

INTRODUCTION

1.1 Allergy and Allergic Diseases:

1.1.1. Epidemeology of Allergic Diseases:

Evidence is accumulating that supports an increase in the prevalence of allergic diseases in many countries of the world. The incidence of allergic diseases is about 35% of the general population (1). The prevalence of hay fever in 9 European countries is in the range of 6-17% (2). Recent data from Europe reveal that the current asthma incidence in school children ranges from 2.5% in Finnish children to 13% in children in the United Kingdom. The cumulative European incidence of atopic dermatitis ranges from 10-12 % with a slightly greater prevalence in females compared with males (ratio 1.4:1) (3). The incidence of allergic contact dermatitis in the general population is estimated at 1% (1).

1.1.2. Types of Allergies:

There are numerous types of allergic disorders which include allergic reactions in the eye and ear, allergies to stinging insects, urticaria, angioedema, food allergy, atopic dermatitis, drug allergy, contact dermatitis, sinusitis, allergic rhinitis, asthma, hypersensitivity pneuomonitis and Stevens-Johnson syndrome, but the most common allergic diseases are: asthma, allergic rhinitis, urticaria, contact dermatitis and atopic dermatitis (5).

Asthma, a complex inflammatory disease is characterized by hyperresponsivness of the bronchi to various stimuli, an increase in airway resistance and changes in lung volume and expiratory flow rates, with symptoms of wheezing, dyspnea, cough and shortness of breath.

Allergic rhinitis is the most common allergic disorder which involves inflammation of the nasal membranes characterized by sneezing, increased nasal discharge and congestion and itching of eyes, nose and palate.

One of the most common cutaneous inflammatory diseases, urticaria, is often precipitated by the ingestion of substances to which atopic individuals are allergic. There are also a number of physical causes of urticaria such as pressure, heat or cold. Urticaria is characterized by the appearance of transient pruritic, edematous, erythematous papules or wheals in the skin.

Contact dermatitis refers to a vast range of skin reactions following contact with external agents like poison ivy, hair dyes, nickel compounds, rubber, cosmetics and perfumes. Acute skin lesions are often red, blistered and oozing with itching being the primary symptom (11).

Atopic dermatitis, commonly referred to as eczema, is a chronic inflammatory pruritic dermatosis with a majority of patients having family or personal history of allergic disease. It is characterized by a thickening of the skin, weeping erosions, and vesicles as well as excoriated reddened scaling papules resulting from scratching (5).

1.1.3. Treatment of Allergic Diseases:

Allergic reactions result from an antigen-antibody interaction, followed by the release of mediators and cytokines which affect target organs (5). Three principal means are available to treat allergic diseases. These are avoidance of allergens, immune therapy and pharmacological therapy.

If exposure to the allergen can be avoided, no antigen-antibody interaction takes place and no allergic manifestations occur. Complete avoidance of allergen is a crucial part of the treatment. This can be accomplished readily in some patients, for example if an individual is allergic to certain types of food or drugs, the individual should avoid ingesting those agents.

Immune therapy is the term used to describe the mechanism by which multiple injections of increasing amounts of allergen, to which the patient has shown hypersensitivity over time, result in desensitization to that allergen. The patient will then be able to tolerate exposure to the offending allergen with minimal symptoms. Immune therapy is considered more effective in treating patients with allergic rhinitis or extrinsic asthma than other allergic disorders.

Pharmacological therapy differs according to the type of allergy. This will be discussed separately under common types of allergy.

1.1.3.1 Pharmacologic Management of Asthma

For asthmatic patients, several lines of therapy have proven effective in controlling the disease and achieving prevention of physiological and physical

impairment. These include β_2-agonists, corticosteroids, theophylline, anticholinergics, mast cell stabilizers and antihistamines (5).

Adrenergic drugs, especially the peripheral β_2-agonists like salbutamol, terbutaline and pirbuterol, are effective if taken orally, by aerosol or subcutaneously. The most common side effects are related to β_1 stimulation resulting in cardiac symptoms as seen with epinephrine, and the paradoxical response of increased bronchial obstruction seen in patients using inhalers.

Corticosteroids, given to patients orally, are the most effective drugs for treating the underlying inflammatory component of asthma. They reduce symptoms like cough, wheezing and dyspnea, and they decrease mucous production and decrease the need for β_2-adrenergic agonists and theophylline. For chronic asthma, patients can be managed adequately using an alternate day prednisone schedule; a shorter 3-5 day therapy is effective for treating occasional acute asthmatic attacks. However the suppressive effect of the corticosteroids on the hypothalamus pituitary axis and the occurrence of other systemic side effects limit their oral use. Topically-active corticosteroids including beclomethasone dipropionate, budesonide, fluticasone and triamcinolone acetonide have been used by inhalation for the out-patient treatment of asthma.

The bronchodilatory effect of oral theophylline is now used infrequently in adults to treat asthma. The 24-hour once-daily dosing theophyllines (Uni-phyl®, Uni-dur®) provide greater convenience for patients, and improves compliance.

Anticholinergic drugs like ipratropium bromide yield bronchodilation in a synergistic fashion, when coadminstered with inhaled β_2-adrenergic agonists.

Mast cell stabilizers like cromolyn sodium were shown to be an effective means for treating asthma, as well as allergic rhinitis, due to their inhibitory effect on Ig-E initiated histamine and other mediators release from mast cells, but these compounds have no intrinsic bronchodilatory or anti-histaminic properties.

H_1-antagonists given concurrently with other medications to asthmatic patients may provide some protection against bronchospasm induced by multiple factors such as histamine, exercise, and hyperventilation of cold or dry air, or allergens. The amount of protection varies with the H_1-antagonist, the stimulus used and the dose, which is generally higher than that used for allergic rhinitis (19).

1.1.3.2 Pharmacologic Management of Allergic Rhinitis

Antihistamines remain the mainstay in allergic rhinitis therapy. The extent of relief of nasal or eye symptoms, and the likelihood of adverse effects, vary somewhat from drug to drug. Antihistamines reduce the symptoms of sneezing, itching and clear nasal discharge but they have minimal effect on nasal congestion. The most troublesome adverse effect of the first-generation antihistamines is sedation, to which most patients may develop tolerance within about 5 days of continuous use. Second-generation antihistamines like fexafenadine, astemizole and loratadine lack the CNS adverse effects encountered with first-generation antihistamines (6).

Oral decongestants such as the α_1-adrenergic agonists pseudoephedrine and phenylpropanolamine, constrict nasal vascular tissue, decreasing the resistance to nasal airflow. Topically applied decongestants such as oxymetazoline are quite effective, but after 3 days of continuous use they cause rebound congestion (6).

Treatment of allergic rhinitis has been improved with the introduction of intranasal corticosteroids such as beclomethasone, budesonide, flunisolide and triamcinolone acetonide. They are the most effective medications available for regular use, relieving the entire range of symptoms of allergic rhinitis (6).

1.1.3.3. Pharmacologic Management of Atopic Dermatitis

For patients with eczema, the goal of treatment is directed towards breaking the itch-scratch cycle. Patients should avoid irritants such as harsh soaps, scratchy clothing like wool, and extremes of temperatures and humidity which are likely to induce significant sweating or alternatively, dryness. Although the exact mechanism of itching associated with atopic dermatitis remains unknown, histamine is certainly involved to some extent because the concentration of histamine is increased in the skin and in the plasma of these patients. First-generation antihistamines have been found to be more effective in relieving itching than the second-generation antihistamines. The second-generation of antihistamines should not therefore automatically replace the existing first-generation agents in the treatment of atopic dermatitis (5).

Topical application of low to medium potency corticosteroids e.g. hydrocortisone and triamcinolone acetonide has proven to be effective in moderately severe cases of dermatitis. High potency topical corticosteroids can only be used for a short period of time to control acute flare up conditions. However the relief produced is due to their anti- inflammatory and immunosuppressive properties rather than anti-pruritic effects. Even topical corticosteroid therapy can cause skin atrophy and hypothalamic pituitary axis suppression during long-term administration (9).

1.1.3.4. Pharmacologic Management of Urticaria

In patients with chronic urticaria, H_1-antagonists have proven to be effective in relieving pruritis and in reducing the number, size and duration of urticarial lesions. First-generation antihistamines such as diphenhydzramine and the potent hydroxyzine are very beneficial against pruritis. However they block H_1 receptors both in the peripheral and in the central nervous system. As a result, somnolence is a common adverse effect encountered with this class of antihistamines. Second-generation antihistamines, such as fexofenadine, astemizole and loratadine, are probably as effective as the first-generation and they lack the sedative adverse effects. In some patients with urticaria refractory to treatment with an H_1-antagonist, concurrent administration of H_2-antagonists such as cimetidine provides an effective relief of pruritis. This could be due in part to the inhibitory effect of some H_2 antagonists on the liver enzymes responsible for the metabolism of H_1 antagonists, leading to an increase in plasma and skin concentrations of H_1 antagonists (4,19).

Other formulations used for the treatment of pruritis may contain rubefacients such as camphor or menthol, anesthetics such as phenol or benzocaine or astringents and protectants such as calamine or zinc oxide. Camphor, which in low concentrations provides a feeling of "cold" that inhibits itching, is also a mild anaesthetic. Phenol, in low concentrations, has antipruritic properties due to its anaesthetic action, but it can be absorbed percutaneously so is not recommended for children less than 6 months old, pregnant women or individuals with renal diseases. Application of phenol-containing products should be avoided in areas where the skin is more sensitive to its irritating effect (10).

Local anesthetics such as benzocaine and lidocaine are available in topical creams and ointments. They act by stabilizing the neuronal membrane of cutaneous nerve endings, and preventing the generation of nerve impulses thus blocking the sensation of itching. However benzocaine is a well-recognized sensitizing agent and its topical use should be discouraged (10).

Emollients and moisturizers are sometimes used to treat mild pruritis, alone, or as an adjunct in more severe cases. They are used to replace natural skin oils and provide a soothing protective film. If applied immediately after bathing they slow evaporation of moisture from the skin and maintain hydration. They are available as ointments, gels, creams or lotions. Most of these products are either oil in water or water in oil emulsions. However, patient acceptability of water in oil products is less, because the greasy texture increases discomfort. In addition, a greasy application is unsuitable for an oozing skin dermatitis. In contrast, evaporating water from oil in water formulations produces an additional cooling effect, which helps to alleviate pruritis (11).

Hydrating agents are products that contain a humectant in addition to emollient base. They have hygroscopic properties that enable them to draw water into the *stratum corneum* and hydrate the skin. Examples of such agents include glycerin, propylene glycol, urea, lactic acid and phospholipids (11).

1.1.4. Histamine Production in Allergic Diseases:

Dale and Laidlaw identified histamine as a potent vasoactive substance in 1911, while Reily and West discovered the association between histamine and mast cells

in 1953 (4). Histamine is stored preformed, in cytoplasmic granules of mast cells and basophils.

In humans, the mast cell is found in the connective tissues of all organs, especially around blood vessels, nerves and lymphatics. Mast cells are found in the skin, the mucosa of upper and lower respiratory tract, the gastrointestinal tract and the reproductive tract.

There are three different subclasses of histamine receptors, H_1, H_2 and H_3 receptors. Most of the histaminic effects important in allergic disorders are mediated through the H_1 receptors. These include smooth muscle contraction, increased vascular permeability, pruritis, prostaglandin generation, decreased A-V node conduction, vagal reflexes activation and increased cGMP production. The H_2 receptor-mediated effects include gastric acid secretion, increased airway mucous secretion, increased cAMP, esophageal contraction and inhibition of basophils (4). The H_3 receptors are thought to be involved in arousal, energy metabolism, cerebral circulation, cardiovascular reflexes, pituitary hormone release and body temperature regulation.

Histamine is the only proven mediator of pruritis, a prominent symptom in urticaria, anaphylaxis, eczema and allergic rhinitis. The mechanism of histamine-induced pruritis is indirect and involves stimulation of sensory nerve endings.

The allergic response involves two phases, a phase of sensitization followed by a clinical disease phase. Allergic patients develop IgE antibodies to allergen after the initial exposure. These antibodies have a high affinity for mast cells and basophils. Further exposure to antigen, which binds to specific IgE on the mast cells, results in degranulation causing the release of multiple inflammatory mediators including

histamine. Histamine is in part responsible for bronchial and vascular smooth muscle contraction with subsequent vasodilation, edema, through an increase in vascular permeability, inflammation, and increased mucous secretion (4).

1.1.5. Antihistamines

1.1.5.1 Oral Agents

Antihistamines, H_1-receptor antagonists, first introduced in the 1940's remain the mainstay in rhinitis and urticarial therapy (9). The pharmacological effects of H_1-receptor antagonists derive primarily from competitive inhibition of histamine activity at the H_1-receptors. Most H_1-receptor antagonists bind competitively to H_1-receptors, in a reversible manner. *In vitro*, many H_1 antagonists also prevent the release of mediators of inflammation from human basophils and mast cells. However these effects vary with the stimulus for mediator release, the concentration of H_1 antagonist and the mediator being measured. *In vivo*, antihistamines reduce sneezing, itching and clear nasal discharge. Their efficacy in either allergic rhinitis or urticaria differs somewhat from agent to agent. The most troublesome adverse effect encountered with the first-generation of antihistamines is sedation, which occurs in 20% of patients. However tolerance to the effect often develops within 5 days of continuous use. Common anticholinergic adverse effects may include urinary retention and dry mouth (6).

Since the 1980's, a new generation of antihistamines has appeared, they include the non-sedating agents fexafonadine, astemizole and loratadine and the low sedating cetirizine. These drugs are just as effective as the potent first-generation antihistamines yet they lack the CNS and anticholinergic adverse effects seen with older

drugs because they have minimal penetration of the blood brain barrier, and they have greater specificity for the H_1-histamine receptor. The second-generation antihistamines also offer twice daily and once daily dosage schedules which are more convenient than the two to four times daily schedule of the first-generation compounds. The second generation H_1-receptor antagonists should therefore be considered as the first line of treatment for chronic urticaria (7.8).

1.1.5.2. Topical Administration of H_1-Receptor Antagonists:

Mast cell activation and subsequent mediator release have been reported in chronic urticaria, with a demonstrated increase in the number of mast cells and monocytes in the skin as well as an increase in the skin histamine content. In patients with urticaria, there is an increase in the skin reactivity to histamine suggesting that the target tissue in allergic skin diseases is the skin itself. Systemic effects are regarded as adverse effects of the treatment of the allergic skin reactions. Topical application of antihistamines is intended to achieve higher local drug concentrations and to avoid the adverse effects of systemically administered formulations.

The problem with a topically applied drug is that when it is applied to the surface of the skin, it may not penetrate rapidly or extensively enough to achieve effective concentrations within the skin. In other cases the drug may penetrate the skin, but be just as quickly removed by the blood or lymphatic systems. This may lead to systemic effects, in addition to the desired local actions (12).

In the 1960s, many non-prescription formulations designed for the topical administration of antihistamines were available and widely used. However, the discovery

that these compounds were associated with a high incidence of contact sensitization and cross-reactivity has all but eliminated their use in dermatology. Some topical antihistamine formulations are still available but should be used with caution because any benefit may be overshadowed by the risk of inducing an allergic contact dermatitis (9).

1.2. Factors Controlling Dermal Drug Delivery:

1.2.1 Skin the Target Organ Anatomy and Physiology:

In pharmaceutics research one area of the specialty is devoted to developing new, controlled, site-specific drug delivery systems. With conventional vehicles there is no effective way to limit or control percutaneous absorption. Progress in pharmaceutical research has provided novel dermatological vehicles designed to control the release and, to some extent, the penetration and pharmacokinetic fate of the active ingredients applied topically. In contrast with currently available topical dosage forms, especially if penetration promoting agents are included in the formula, percutaneous absorption may lead to unintentional systemic and possibly toxic effects (12).

The skin in an adult human body covers an area of about 2 square meters and receives about one third of the total blood supply (14). On average, every one square centimeter of skin has about 10 hair follicles, 12 nerves, 15 sebaceous glands, 100 sweat glands, 3 blood vessels and 3 million cells (13). The skin is referred to as a multilayered organ that can be distinguished microscopically into three major parts, epidermis, dermis and hypodermis (15).

The epidermis is a continuous stratified keratinizing epithelium which upon differentiation gives rise to the outermost flattened, dehydrated and dead keratinized layer, the *stratum corneum*, which can be represented by a "brick-and-mortar model" (13,15). The bricks consist of anucleate corneocytes filled with a matrix of keratin filaments surrounded by sulfur-rich proteins, composed mainly of involucrine (16). Corneocytes contain gamma-hydroxylacyl sphingosines in the lipid portion of the envelope (13). The intercellular spaces, representing the mortar, are filled by multiple lipid lamellae that unlike other biological membranes do not contain phospholipids (16). Instead they contain ceramides, cholesterol, fatty acids and cholesteryl esters which represent an efficient barrier to the penetration of many hydrophilic substances, as well as provide a barrier against water loss from the body (13,16).

The *stratum corneum* results from the differentiation of the epidermis cells particularly the keratinocytes. Microscopically different layers of epidermis each representing a unique stage of differentiation can be observed, which can be classified from the dermis to the surface as follows: *basal layer*, *stratum spinosum* and *stratum granulosum* (13).

Beneath the epidermis resides a moderately dense fibroelastic connective tissue known as the dermis (15). It consists of collagen and elastin fibers forming a network within a matrix of mucopolysaccharides (13). The dermis plays a role in the control of passage of nutrients, it also serves as a mechanical support for the epidermis as well as a physiologic support, via the blood vessels, the nerve endings and the lymphatic system embedded within it (13,16).

1.2.2 Mechanism of Drug Penetration Through the Skin:

Penetration of the *stratum corneum* lipid lamellae represents the rate-limiting step governing drug transport through the skin; it has been shown that hydrophilic compounds are absorbed 1000 times more rapidly when *stratum corneum* is damaged (16). Even with intact skin, a ten thousand-fold difference was noted for a range of different types of drugs, depending on the structure and physiochemical properties of both the drug and the penetrating vehicle (15).

Two major routes for drug penetration have been postulated, the intercellular route and the transcellular route (13,17). The intracellular route is the route of penetration of small molecules via the intercorneocyte lipid rich region of the *stratum corneum*, while penetration through the corneocytes represents the transcellular pathway. These account for about 99% of the transepidermal routes. Shunt pathways are defined as penetration through skin appendages, the transfollicular and transsebaceous routes, which account for less than 1%, while the transsecrine route does not exceed 0.1% (17).

Depending on the physiochemical properties of the drug molecules, in particular the diffusion coefficient and the water/octanol partition coefficient, compounds may penetrate the *stratum corneum* to the epidermis aqueous environment, and then distribute to the dermis and the blood stream. Compounds that are extremely hydrophilic would not penetrate the skin, while extremely lipophilic ones would be retained in the epidermis and would not penetrate deeper into the dermis (13).

Liposomes have been demonstrated to improve penetration of certain drugs by means of drug targeting either for dermal and in some cases for transdermal delivery. However these studies have been conducted mainly in normal, healthy skin. Many

diseases affecting the skin may alter its permeability. For example hyperproliferation of the *stratum corneum* was found to increase permeability due to reduced barrier properties (13,15).

1.3. Liposomes

1.3.1. Liposomes Structure and Composition:

Among the variety of new drug delivery systems, liposomes seem to have the best potential to accommodate both water and lipid soluble compounds, to protect the liposome-encapsulated drug from metabolic degradation and to act as a delivery mechanism, releasing active ingredients slowly and in a controlled manner.

Liposomes first described in 1965 by Bangham *et al* can be defined as "lyotropic liquid crystals composed predominantly of amphiphilic bilayers " (18). Phospholipids, the cornerstone of the liposome lipid bilayer, usually extracted from egg yolk or soy bean oil, consist of a hydrophilic head portion, covalently attached to two hydrocarbon tails representing the lipophilic portion. Aggregation in a bilayer structure occurs by orientation of the hydrophilic head groups towards the aqueous environment, while keeping the lipophilic hydrocarbon chains sequestered inside. Formation of such a configuration provides the vesicle with the lowest potential energy state through solvation of the polar head groups and hydrophobic interactions of the lipid chain(20).

1.3.2. Classification of Liposomes:

There is no single universal method for classifying liposomes. One well-accepted method of classifying liposomes is dependent on the bilayer arrangement and the size of the vesicles. These fall into two main groups: unilamellar vesicles, which are further subclassified into small unilamellar vesicles (SUV) which have a size range between 25 and 50 nm, large unilamellar vesicles (LUV) which have a size of up to 200 nm, and multilamellar vesicles (MLV) comprised of many concentric bilayers, of variable size and uniformity ranging from 1,000 nm to 10,000 nm (Figure 1). Another way to classify liposomal formulations is according to the method of preparation, such as: reverse phase evaporation vesicles (REV), frozen and thawed multilamellar vesicles (FATMLV) and large unilamellar vesicles obtained by extrusion techniques (LUVET).

1.3.3. Composition and Physiochemical Properties of Liposomes for Dermal Application:

Natural phosphatidylcholine extracted from egg yolk or soy bean oil or its semisynthetic derivatives represents the main constituent in various liposomal formulations. The chemical structure of naturally occurring phosphatidylcholine has a glycerol moiety attached to two acyl chains which may be saturated or unsaturated. Each may have between 10 to 24 carbon atoms, together forming the hydrophobic (lipophilic) portion of the molecule. The charged phosphate and choline moieties form the hydrophilic "head" (19,20).

The fatty acid chains, depending on their length and degree of saturation, can exist in the gel phase, in which the lipids are rigid, impermeable and easily aggregated

upon storage, or in the more fluid liquid-crystalline phase. The temperature at which the gel phase converts to the liquid-crystalline phase is known as the transition temperature (20).

Cholesterol is frequently added in minute quantities to most liposomal formulations to increase the fluidity of the liposomal gel phase, enhance the retention of hydrophilic particles and to stabilize the bilayer membrane in a manner similar to that of biological membranes (19,20).

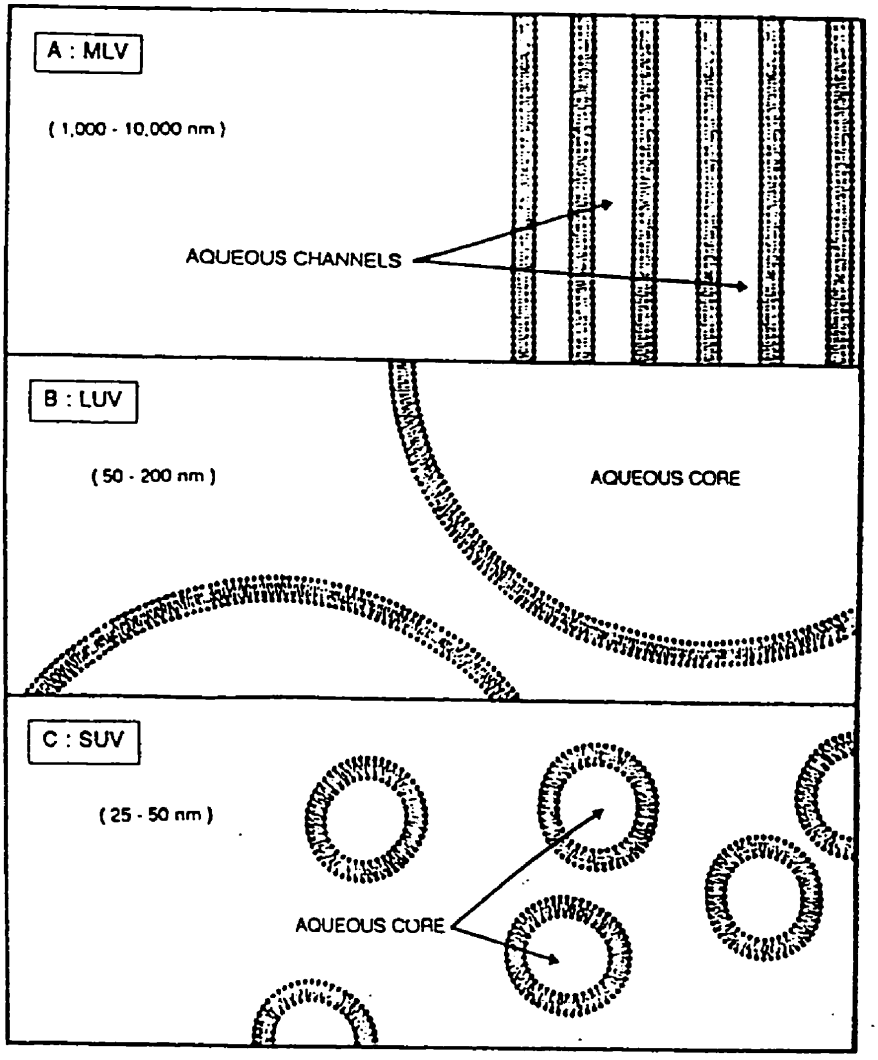


Figure 1. A diagrammatic representation of the three main classes of liposomes. (A) Multilamellar vesicles; (B) Large unilamellar vesicles; (C) Small unilamellar vesicles (Ref 20).

1.3.4. Formulation Methods:

A variety of methods have been developed for preparing liposomes with more than 200 patents and patent applications dealing with liposome technology currently available (12). A pre-requisite to the successful development of liposome products is the capability to scale-up production methods at acceptable costs using processes that provide a high degree of reproducibility required for the finished products.

One of the most frequently used methods, originally described by Bangham (21), is thin film hydration. Here a mixture of lipids dissolved in a volatile organic solvent is deposited as a thin film on the surface of a round bottom flask as the solvent is removed under reduced pressure by rotary evaporation. The MLVs will form spontaneously when an excess volume of aqueous buffer is added to the dry lipid, and the flask is hand shaken vigorously. The drug to be entrapped may be included in aqueous buffer, in case of hydrophilic drugs, or in the lipid film, in case of lipophilic drugs.

An alternative method to prepare MLVs is "hydration in the presence of solvent method", where a two-phase system consisting of equal volumes of petroleum ether, containing lipids, and an aqueous phase are emulsified by vortexing. The ether layer is removed by passing a stream of an inert gas such as nitrogen over the emulsion. As the solvent is removed, MLVs will form in the aqueous phase (22).

Small unilamellar vesicles can be obtained after probe or bath-type sonication of a mixture of MLVs previously produced by conventional methods. Also dispersions of MLVs can be converted into SUVs after extrusion through a french pressure cell where the liposome mixtures are extruded 4-5 times at about 20,000 psi at 4°C. The resulting SUVs will range in size from 30-50 nm (21).

Injection of a water-immiscible solvent such as ether, containing a mixture of bilayer-forming lipids, into an aqueous medium at 55-65°C or under reduced pressure will form single layer vesicles with diameters ranging from 50-200 nm upon evaporation of the ether.

An alternative solvent used to avoid the hazards of diethyl ether is Freon 21, an excellent vehicle for the lipid mixture, which boils at 9°C (760 mm Hg). When this solvent mixture is injected into an aqueous medium at 37°c the lipids are left behind to hydrate and form liposomes. However due to its hazardous effect on the ozone layer the use of Freon 21 has been phased out (22).

A safe ecologically friendly solvent injection method that does not require elevated pressures is the injection of a water-miscible solvent like ethanol. The procedure is simple, rapid and non-destructive to both lipids and the drug to be encapsulated. However the method is restricted to the production of dilute suspensions of SUVs since the final volume of ethanol can not exceed 10-20% by volume. Removal of ethanol by vacuum distillation presents a problem, but ultrafiltration provides a good solution for both removing ethanol and concentrating the suspension (23).

Large unilamellar vesicles can be formed using a detergent removal method in which dialysis, or column chromatography is used to remove the detergent molecules from the aqueous dispersion of phospholipid/detergent mixed micelles. As the detergent is removed, the micelles coalesce to form single-bilayer vesicles (22,24).

The reverse phase evaporation technique is another method designed to form LUVs. A water-in-oil emulsion of phospholipids and buffer in an excess of organic phase is sonicated, followed by removal of the organic phase under vacuum. Removal of

the last traces of solvent transforms the emulsion from the gel state into large unilamellar vesicles (22).

LUVs can be produced from a mixture of MLVs using high pressure extrusion. After repeated extrusion through polycarbonate membrane with uniform pores of selected sizes (80-400 nm), under high pressure (up to 800 psi), liposomes with the desired average diameter will be formed (22).

1.4. Hypothesis, Goals and Objectives:

Drug targeting is one of the most commonly studied areas of pharmaceutical research. The aim is to deliver the drug to the target organ, minimize the drug distribution to other non-target organs and reduce systemic adverse effects (12).

In dermal drug delivery the approach is to apply the drug topically to the skin, to achieve a higher local drug concentration in different skin layers and to minimize drug clearance by lymph or blood vessels, thus reducing undesirable systemic adverse effects. Since the 1980's, liposomes have attracted considerable attention and generated many speculative claims concerning their potential role both as a drug carrier and a storage reservoir for controlled release of drugs within various layers of skin.

Using conventional vehicles like ointments or creams presents a problem since the topical application of some drugs like methotrexate, in cases of psoriasis treatment, failed to achieve penetratation through the *stratum corneum* and reach effective concentrations in the viable epidermis. Other drugs, like corticosteroids, were absorbed through the skin in quantities sufficient to cause suppressive effects upon the adrenal-pituitary axis, but achieved only a short duration of local activity (25).

Liposomes may offer special advantages as topical delivery systems because they are non-toxic, biodegradable, can trap both water-soluble drugs in their aqueous phase, and lipophilic drugs in their bilayer membrane, and can readily penetrate the skin. Their disposition in the *stratum corneum* has been shown to create a drug reservoir, which could result in a decrease in systemic absorption and prolonged activity. These advantages suggest that liposomes have the potential to act as a drug carrier for topical administration of H_1–receptor antagonists. The liposome system for topical administration for the treatment of allergic skin disorders would be considered as a localizer rather than a transporter (26).

Hydroxyzine is one of the most potent first-generation antihistamines. After an oral dose, hydroxyzine distributes into the skin efficiently and sustains higher concentrations than in serum, making it a potential treatment of skin disorders in which histamine is the mediator. Unfortunately hydroxyzine has considerable CNS adverse effects, and sedation presents a problem for most of the patients, limiting its use (27).

In this study we hypothesized that hydroxyzine administration by topical liposomal formulations would yield greater and more persistent effects on the suppression of histamine-induced wheal formation over a 24 hour period, and that liposome formulations would result in lower serum concentrations after topical application compared to traditional non-liposome cream formulations. This method of administration of hydroxyzine should result in a better treatment of allergic skin diseases and also avoid the central nervous system side effects, particularly sedation. Liposomes may also prevent topically applied hydroxyzine from coming into contact with the

dendritive cells in the skin and reduce or remove the possibility od inducing an allergic contact dermatitis (9).

Liposomes consisting mainly of phospholipid products are useful hydrating agents, having hygroscopic (water attracting) properties which enable them to draw water into the *stratum corneum* and hydrate the skin. This property alone could play an important adjunct role in the treatment of dermatitis by softening the skin and counteracting the signs and symptoms of dry skin.

Hydroxyzine was selected as the test H_1–receptor antagonist because of its superior peripheral H_1-receptor antagonistic potency.

The following specific goals and objectives were defined.

The first goal was to choose from the currently available methods for the production of liposomes two different techniques, one method which should produce relatively large MLV liposomes (2-7 μm), and a second method which should yield relatively smaller vesicles (in the range of 100 nm). Both formulation methods should yield liposomes at a relatively low operating cost with acceptable reproducibility, and should have the ability for scale-up production in the future.

The second goal was to evaluate both formulations *in vitro* with regard to the encapsulation efficiency, which should be high enough to avoid the waste of materials; size distribution, which should be relatively consistent from batch to batch; and stability, which should retain the size distribution over time.

The third goal was to measure hydroxyzine serum concentrations in the two selected optimal liposome formulations *in vivo*, to determine both the peripheral H_1-receptor antagonist efficacy after topical administration compared to a typical non-

liposomal cream formulation and to assess the extent of percutaneous systemic H_1-receptor antagonist absorption.

CHAPTER II. METHODOLOGY

2.1. Chemicals, Supplies and Equipment

2.1.1. Chemicals

1. Hydroxyzine dihydrochloride: Sigma, St. Louis, MO 63178, USA.
2. Antazoline hydrochloride: Ciba, Dorval, Quebec.
3. Potassium hydroxide: Fisher Scientific company, Fair Lawn, New Jersey 07410, USA
4. Ethyl ether: Fisher Scientific Company, Fair Lawn, New Jersey 07410, USA.
5. Acetonitrile: Fisher Scientific Company, Fair Lawn, New Jersey 07410, USA.
6. Acetone: Fisher Scientific Company, Fair Lawn, New Jersey 07410, USA.
7. Phosphoric acid: Fisher Scientific Company, Fair Lawn, New Jersey 07410, USA.
8. Nitrogen: Praxair Canada Inc., Mississauga, Ontario L5B 1M2.
9. Ammonium phosphate monobasic: Mallinckrodt, Paris, Kentucky 40361, USA.
10. Evans blue: Fisher Scientific Company, Fair Lawn, New Jersey 07410, USA.
11. Histamine phosphate: Bioniche Inc., London, Ontario N6E 2V6, Canada.

12. Egg phosphatidylcholine (95%): Avanti Polar Lipids Inc., Alabaster, Alabama 35007, USA
13. Cholesterol: Fisher Scientific Company, Fair Lawn, New Jersey 07410, USA.
14. Potassium phosphate monobasic: Mallinckrodt, Paris, Kentucky 40361, USA.
15. Sodium hydroxide: Fisher Scientific Company, Fair Lawn, New Jersey, USA.
16. Glaxal Base: Roberts Pharmaceutical Canada Inc., Mississauga, Ontario L5N 6S2.
17. Ethanol (100%): Commercial Alcohol Inc., Montreal, Toronto.
18. Chloroform: Fisher Scientific Company, Fair Lawn, New Jersey 07410, USA.
19. Outdated Human Plasma, Canadian Red Cross, Winnipeg, Manitoba.

2.1.2. Supplies

1. Syringes (1cc, 5 cc and 10 cc): Becton Dickinson and Company, Franklin Lakes, NJ 07417-1884, USA.
2. Disposable test tubes (16x100mm): VWR Scientific Inc.
3. Sure-Sep II serum plasma separators: Organon Teknika Co., P.O. Box 15969 Durham, NC, 22704-0969, USA.
4. Steripads: Johnson and Johnson Inc., Montreal H1V 2E4.
5. Needles (22Gx1½"): Becton Dickinson and Company, Franklin Lakes, NJ 07417-1884, USA.

6. Needles (25Gx 5/8"): Becton Dickinson and Company, Franklin Lakes, NJ 07417-1884, USA.

7. Needles (27Gx ½"): Becton Dickinson and Company, Franklin Lakes, 07417-1884, USA.

8. Neet hair remover: Boyl-Midway, Toronto, Ontario M8Z 5M5.

9. EMLA cream: Astra Pharma Inc., Mississauga, Ontario L4Y 1M4

10. Alcohol swabs: Becton Dickinson and Company, Franklin Lakes, NJ 07417-1884, USA.

11. Ultrafiltration membranes (YM 100 62 mm): Amicon Inc., Beverly, MA, 01915, USA.

12. Syringes (100 µl): Hamilton company, Reno, Nevada 89520-0012, USA

2.1.3. Equipment:

1. Centrifuge (IEC HN-sII): International Equipment Company.
2. pH meter (Zeromatic SS-3 Model): Beckman Instrument Inc., 2500 Harbar BLV., Fullerton, CA, USA.
3. Balance (Mettler AE 160 Model): Mettler Instrument AG.,Switzerland.
4. Vortex mixer (Multi tube vortexer): Baxter Diagnostic Inc., Deerfield, IL 60015-4633, USA.
5. Ultrafiltration apparatus: Amicon Inc., Beverly, MA 01915, and USA.
6. Milli-Q water system: Millipore Ltd., 3688 Nashua Drive, Mississauga, Ontario L4V 1M5.

7. High performance liquid chromatography system: Waters Associates Inc., Millford, Massachusetts, USA.

   A: 6000 A high pressure pump.

   B: U6K injector (Manual).

   C: LC spectrophotometer (Lambda-Max Model 441).

   D: Waters 745 data module.

   E: Reverse phase column (μBondapak C_{18})

8. NICOMP 370 Submicron Particle Sizer: Particle Sizing System, Santa Barbara, California, USA.

2.2. Chromatographic Quantitation of Hydroxyzine Concentration:

2.2.1. Analysis of Hydroxyzine in a Liposome Formulation:

2.2.1.1. Calibration Curves:

The calibration curves were prepared following the analysis of 0.05% phosphoric acid (H_3PO_4) samples to which known quantities of hydroxyzine and antazoline, the internal standard, were added. In brief, an aliquot volume of hydroxyzine stock solution (1μg/ml) was transferred to a 10 ml volumetric flask, so that hydroxyzine concentrations ranged from 20 to 200 μg/ml. One ml of antazoline solution (24 μg/ml) was added to the test tube. 0.05% phosphoric acid (H_3PO_4) was added in quantity sufficient to make 10 ml. From that solution 50 μl was drawn up in a 100 μl syringe and injected directly into the HPLC system.

2.2.1.2. Sample Preparation:

A 0.25-0.75μl volume of filtrate was transferred to a 10 ml volumetric flask. One ml of antazoline solution (24 μg/ml) the internal standard was added to the volumetric flask. Then 0.05% phosphoric acid (H_3PO_4) was added in a quantity sufficient to make 10 ml. From this solution 50 μl was drawn up in a 100 μl syringe and injected directly into the HPLC system.

2.2.1.3. HPLC Parameters:

The HPLC system (Waters Associates Inc.) was comprised of an M-6000 A high pressure pump, a U6K injector and a Lambda-Max Model 441 liquid chromatography (LC) ultra violet (U.V.) spectrophotometeric detector with a fixed wavelength of 229 nm,

a Waters model 745 data module (integrator) connected with the detector. The mobile phase for hydroxyzine was acetonitrile-phosphate buffer (0.075 M $NH_4H_2PO_4$, pH 2.5) (35:65 v/v). The flow rate was set at 1.5 ml/min. The effluent from the reverse phase column (μBondapak C_{18}) was monitored by UV absorption at 229 nm with 0.2 to 0.005 a.u.f.s. sensitivity settings. The chart speed for the data module was 1.0 cm/min. The peak height ratio of hydroxyzine to antazoline was used for the quantitation based on the calibration curves established during the study period. The retention times were 5.0 and 11.0 minutes for antazoline and hydroxyzine respectively. The calibration curves were prepared from the results of assays of H_3PO_4 samples to which known quantities of hydroxyzine and antazoline were added. All chromatographic separations were carried out at ambient temperature.

2.2.2. Analysis of Hydroxyzine in Serum (Plasma) Samples

2.2.2.1. Calibration Curve

The calibration curves were prepared from the results of assays of blank human plasma samples to which known quantities of hydroxyzine and antazoline, the internal standard, were added. In brief, aliquot volumes of hydroxyzine stock solution (1μg/ml) were transferred to test tubes containing 0.5 ml of blank human plasma so that quantities of hydroxyzine added ranged from 2 to 50 ng. Fifteen microliters of antazoline solution (1μg/ml) were added. The extraction procedure was similar to the one described under the following extraction procedure for hydroxyzine

2.2.2.2 Extraction Procedure for Hydroxyzine

The method used for extracting hydroxyzine from serum or plasma was a modification of the method of Simons et al (28,29).

Fifteen microliters of antazoline solution (1 μg/ml), the internal standard were added to 0.5 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 were added into the test tube containing the transferred ether, followed by mixing on a vortex mixer for 30 seconds and centrifuging for 5 minutes at 2000 rpm. The aqueous layer was again frozen in a dry ice/acetone bath and the ether layer was discarded. The aqueous portion was exposed to a stream of dry nitrogen to remove remaining traces of ether. The test tube was left at room temperature for 24 hours. Of the remaining aqueous solution 100 μl was then taken up in a syringe and injected directly into the HPLC system.

2.2.2.3. HPLC Parameters

The HPLC system (Waters Associates Inc.) was comprised of a M-6000 A high pressure pump, a U6K injector and a Lambda-Max Model 441 LC U.V. spectrophotometeric detector with a fixed wavelength of 229 nm, a 745 data module (integrator) connected with the detector. The mobile phase for hydroxyzine was acetonitrile-phosphate buffer (0.075 M $NH_4H_2PO_4$, pH 2.5) (37:63 v/v). The flow rate was set at 1.5 ml/min. The effluent from the reverse phase column (μBondapak C_{18}) was

monitored by UV absorption at 229 nm 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 ratio of hydroxyzine to antazoline was used for the quantization based on the calibration curves established during the study period. The retention times were 5.5 and 8.6 minutes for antazoline and hydroxyzine respectively. The calibration curves were prepared from the results of assays on blank plasma samples to which known quantities of hydroxyzine and antazoline were added. All chromatographic separations were carried out at ambient temperature. The serum concentration of hydroxyzine was expressed in terms of the free base form.

2.3. Formulation of Ethanol Injection Method (EIM) for Liposomes

2.3.1. Extraction Procedure for Hydroxyzine Free Base

Four ml of stock solution of hydroxyzine dihydrochloride (10 mg/ml) were used to provide the exact amount of drug required for study. This was converted to the free base form of hydroxyzine by adding 1 ml 10% KOH. The precipitated base was extracted twice with 5 ml of freshly distilled ether. Extraction was achieved by mixing the solution on a vortex mixer for 30 seconds. The ether layer was transferred by Pasteur pipet into a 30 ml vial. Evaporation of the ether layer under a nitrogen stream resulted in the formation of the oily free base of hydroxyzine.

2.3.2. Recovery of Hydroxyzine Free Base

The extracted free base was dissolved in 10 ml of ethanol and injected rapidly into 110 ml of KCl solution, then filtered immediately using a >100,000 M.W. cut-off

membrane. The ultafiltration apparatus was washed two times with fresh KCl solution. The recovery of hydroxyzine in the KCl solution was used to evaluate the efficiency of the extraction of hydroxyzine as free base. Analysis of the sum of filtrates revealed 96% recovery of the drug as a free base resulting from the initial dihydrochloride salt of hydroxyzine.

2.3.3. Development of Ethanol Injection Method for the Liposome Formulation

The method used to prepare liposomes using the ethanol injection method was a modification of Batzri et al (23).

2.3.3.1. Potassium Chloride Solution as the Aqueous Phase

Free hydroxyzine base extracted as above was used as the model H_1-receptor antagonist for evaluation after encapsulation into liposomes.

Egg phosphatidylcholine over the concentration range of 21 to 74 μmol/ml and cholesterol over the concentration range of 12 to 70 μmol/ml were dissolved in a sufficient quantity of ethanol together with extracted hydroxyzine base to yield a total volume of 8 ml of ethanol mixture. This solution was rapidly injected, using a 22 G needle, into a rapidly stirred 65 ml volume of 0.16 M KCl solution, then stirred continuously for 3 minutes. The equilibrated liposomal suspension was transferred to an Amicon ultafiltration apparatus where it was concentrated to a 5 ml volume over 150-180 minutes through an ultafilteration membrane with >100,000 M.W. cut-off. This was accomplished with rapid stirring under a nitrogen pressure of < 10 psi.

The concentrated suspension was washed and filtered twice with 100 ml of freshly prepared KCl solution. The sum of the filtrates was used to quantitate the encapsulation efficiency of hydroxyzine by liposomes, using the HPLC method described previously.

2.3.3.2. Phosphate Buffer as the Aqueous Phase

An ethanol solution containing 406 mg egg phosphatidylcholine and 61.5 mg cholesterol was rapidly injected using a 22-G needle into a rapidly stirred 110 ml volume of phosphate buffer at various pH values from 6.6 to 8.0. Exactly 80 mg of hydroxyzine dihydrochloride was added to the liposome suspension and the mixture was stirred continuously for 3 minutes. The equilibrated liposomal suspension was transferred to an Amicon ultrafiltration device where it was concentrated to 5 ml over 150-180 minutes through an ultrafiltration membrane with >100,000 M.W. cut-off. This was accomplished with rapid stirring under a nitrogen pressure of < 10 psi.

2.3.3.3. Method of Assessing Encapsulation Efficiency

Encapsulation efficiency was calculated according to equation 1.

$$\text{Encapsulation efficiency} = (A_1 - A_2)*100/A_1 \quad \text{(Equation 1)}$$

where A_1= Amount of hydroxyzine added initially

and A_2= Amount of hydroxyzine determined in the filtrate by HPLC

(A_1-A_2) represents the amount of hydroxyzine trapped in the liposome formulation. This should equal the total amount added initially, minus the amount determined in the filtrate.

2.3.3.4. Slow Versus Rapid Injection

The same method as above was used except that ethanol mixture is injected slowly using a syringe pump calibrated to deliver the volume over time for periods ranging from 5 to 30 minutes.

2.3.4. Final Production of Ethanol Injection Method Liposome

An ethanol solution containing 406 mg of egg phosphatidylcholine and 61.5 mg of cholesterol was injected slowly over 5 minutes through a syringe pump using a 22 gauge needle into a rapidly stirred 110 ml volume of 400 mM phosphate buffer (pH 7.0). Exactly 80 mg of hydroxyzine dihydrochloride was added to the liposome suspension and the system was then stirred continuously for 3 minutes. The equilibrated liposomal suspension was transferred to an Amicon ultrafiltration apparatus where it was concentrated to 5 ml in 150-180 minutes through an ultrafiltration membrane with >100,000 M.W. cut-off. This was accomplished with rapid stirring under a nitrogen pressure of < 10 psi. Analysis of the filtrate using to the HPLC method described previously revealed an encapsulation efficiency of 10 mg/ml of hydroxyzine in the liposomes.

2.4. Production of the MLV Liposome Formulation

The lipid phase which contained egg phosphatidylcholine (EPC) 406 mg and cholesterol (chol) 61.5 mg was dissolved in a 5 ml volume of chloroform in a round-bottom flask. The solvent was then removed by rotary evaporation at approximately

30°C under vacuum. This resulted in a thin film being deposited on the wall of the flask. The appropriate aqueous phase, 10 ml of phosphate buffer (pH 7.0) was then added. The flask was hand-shaken vigorously for 40 minutes. Then 10 ml of liposome suspension were removed from the round-bottom flask and transferred to an Amicon ultrafiltration device. One hundred ml of phosphate buffer (pH 7.0) was added followed by exactly 80 mg of hydroxyzine dihydrochloride. The liposome suspension was concentrated to 5 ml in 150-180 minutes through an ultrafiltration membrane with >100,000 M.W. cut-off. This was accomplished with rapid stirring under a nitrogen pressure of <10 psi. Analysis of the filtrate using to the HPLC method described previously revealed a typical encapsulation efficiency of 10 mg/ml of hydroxyzine dihydrochloride in these MLV liposomes.

2.5. Production of Control Cream Formulation

Ten mg of hydroxyzine dihydrochloride was weighed and transferred to an ointment slab. A few drops of water were added to enhance the levigation of the hydroxyzine into 4 g of Glaxal Base with the aid of a plastic spatula.

2.6. Particle Size Analysis

2.6.1. Equipment

The Submicron Particle Sizer consists of two subsystems, the light scattering assembly and the controller/analyzer subsystems. A light scattering signal is obtained from the diluted sample by focusing the beam from a laser diode (5 mW, 632.8 nm) into

a temperature-regulated scattering cell. A fraction of the scattered light at a 90-degree angle is captured and transmitted by optical fiber to the photomultiplier detector in the central controller/analyzer subsystem. The central controller/analyzer subsystem controls the light scattering assembly and performs dedicated continuous analysis of the particle size distribution. An IBM-compatible personal computer operating under DOS is used for data collection from the controller/analyzer subsystem.

2.6.2 Method

The parameters for Submicron Particle Sizer were as follows: the temperature was set at 23 °C. viscosity was set at 0.933 cp and the index of refraction was set at 1.333. The sample to be analyzed was diluted with the appropriate aqueous solution until a count rate of 200-300 kHz was achieved. Analysis of the data was done using volume-weighted Gaussian analysis for unimodal distribution or volume-weighted instrument generated non-Gaussian analysis for multi-modal distribution. The run time was determined by the achievement of having either a fitting error of 1, or a Chi-squared value of <1.

2.7. Study Design of *in vivo* Animal Experiments

This study was approved by the Fort Garry Campus Protocol Management and Review Committee of the University of Manitoba

2.7.1 Animals

Six female New Zealand white rabbits, 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 2-day of antibiotic therapy and a two-week environmental adjustment period. The antibiotic therapy did not affect these studies. They were kept individually in metal cages, fitted with wire floors to reduce coprography. Food and water were supplied *ad libitum*.

2.7.2 Procedure

This crossover study required three treatments: hydroxyzine in the MLV liposome formulation, hydroxyzine in the ethanol injection method (EIM) liposomes and hydroxyzine in a control cream formulation, Glaxal Base® (GB).

Before the administration of the topical hydroxyzine formulation, an area of 125 cm^2 (12.5 cm x 10 cm) on the rabbit back was shaved by an electric shaver, followed by the application of depilatory lotion to the area for 5 to 8 minutes. The rabbit ears also had the hair removed in a similar manner to permit a clear view of the blood vessels in the ears. The lotion was completely removed by carefully washing with warm water. Then 0.1 ml, 100 mg/ ml Evans blue was injected through the ear vein to permit easier identification of the histamine wheal perimeter. Additional doses of Evans blue were administered as required. A hairless area, approximately 125 cm^2 was then ready for an application of the topical formulation. Each rabbit received all three treatments in a random order with at least a two-week interval between any two treatments to ensure recovery from blood collection. A dose of 4 g of control cream formulation or 1 ml of

EIM or MLV liposome formulation, each quantity containing 10 mg of hydroxyzine, was applied and spread uniformly over the 125 cm^2 area.

2.7.3 Blood Sample Collection from the Rabbits

The rabbit ear artery was dilated by topical application of an alcohol swab. Then EMLA cream was applied in a thick layer to the ear, and covered with occlusive dressing for at least 1 hour. The cream was completely removed by carefully washing with warm water. Blood samples were collected by means of a 5 ml syringe fitted with a 25 gauge needle. The needle, with the bevel in the upright position, was inserted at a 25° to 30° angle into the skin beside the artery. The needle was lowered until it was almost flush with the skin and aimed directly into the artery. Blood samples, 2 ml were collected pre-dose and at 1,6,12 and 24 hours after the dose. The blood samples were collected in clean 16 x 100 mm glass test tubes without anticoagulants. The blood was allowed to clot and the serum was separated by placing the tubes in a Sure Sep-II separator and centrifuging for 15 minutes at 2000 rpm. Serum samples were stored frozen at –20 °C until analyzed. A total of less than 15 ml blood was withdrawn from a 3-5 Kg rabbit having about 150-250 ml of blood. So less than 10% of the blood volume was removed in each study. The 2-week recovery period was more than sufficient to permit the blood volume to be restored.

2.7.4. Wheal Area Measurement

Prior to the application of each hydroxyzine formulation, 0.1 ml of Evans blue (100 mg/ml) was injected intravenously. Intradermal tests were performed at

0,1,2,3,4,5,6,8,10,12 and 24 hours with 0.05 ml of a solution containing histamine phosphate, 1.0 mg/ml. A different site on the depilated back of rabbits was used for each test. The cutaneous blue spots were traced 10 minutes after each histamine injection and transferred to a transparent paper using a felt-tipped pen. Wheal areas were scanned with a Hewlett Packard Scanjet 4P and measured using a Sigma-Scan® program from Jandel Scientific, Sausalito, California, USA.

2.8. Data Analysis

2.8.1. Pharmacodynamic Data Analysis

The efficacy was calculated as the percent suppression of wheal using Equation 2

$$E = (A_o - A_t)/A_o \times 100 \qquad \textbf{(Equation 2)}$$

where A_o is the wheal area before drug administration.

and A_t is the wheal area at time t after hydroxyzine administration.

2.8.2. Statistical Analysis

absolute wheal areas at each time were compared to pre-dose values for each hydroxyzine formulation using ANOVA (analysis of variance) and the Tukey and Bonferroni Multiple Range Tests with differences being considered significant at $p < 0.05$. Mean wheal areas of each hydroxyzine formulation were compared with each other at corresponding times using ANOVA and the Tukey and Bonferroni Multiple Range Tests with differences being considered significant at $p < 0.05$. Mean serum hydroxyzine

concentrations of each hydroxyzine formulation were compared with each other at corresponding times using ANOVA and the Tukey and Bonferroni Multiple Range Tests with differences being considered significant at $p < 0.05$.

CHAPTER III

RESULTS AND DISCUSSION

3.1. HPLC Assay

3.1.1. HPLC Assay in Liposome Formulation

In order to assess encapsulation efficiency, the concentration of hydroxyzine was determined by HPLC in aliquot volumes of filtrate. The mobile phase used in this study was acetonitrile: phosphate buffer (0.075 M $NH_4H_2PO_4$, pH 2.5), 35:65 v/v.

At a flow rate of 1.5 ml/minute the retention times of hydroxyzine and the internal standard, antazoline, were 11.0 and 5.0 minutes respectively (Figure 2).

Within the concentration range of 20 ng/ml to 200 ng/ml the calibration curves were linear with a coefficient of variation at all concentrations of less than 15% over an 8 month period (Table 1).

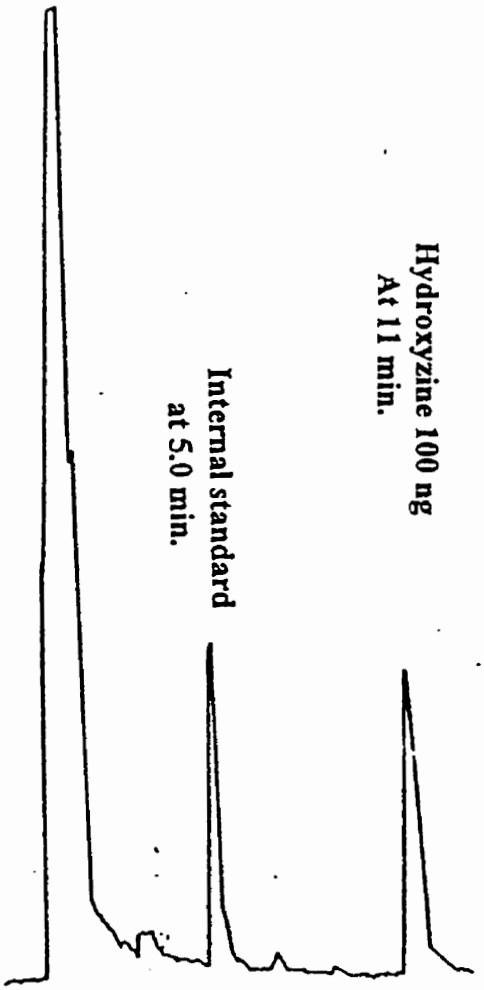


Figure 2. The HPLC chromatograms of hydroxyzine and its internal standard, antazoline in liposome formulation.

CONCENTRATION (NG/ML)	PEAK HEIGHT RATIO (MEAN±SD)	C.V (%)
20	0.106±0.016	14.7
50	0.266±0.029	10.9
70	0.366±0.037	10.1
100	0.546±0.045	8.3
150	0.825± 0.053	6.4
200	1.212±0.038	3.1

Table 1. Variability in HPLC calibration curves for measuring hydroxyzine in liposome formulation filtrate (n=5 over 8 months).

3.1.2. HPLC Assay for Hydroxyzine in Serum

Simons *et al* reported an HPLC method for measuring the serum concentration of hydroxyzine with a sensitivity of 3 ng/ml (28,29). In the present study mobile phases of different compositions were evaluated to optimize the degree of resolution and improve sensitivity. The mobile phase selected for use in the present study was acetonitrile: phosphate buffer (0.075 M $NH_4H_2PO_4$, pH 2.5), 37:63 v/v. The retention times of hydroxyzine and the internal standard were 8.65 and 5.25 minutes respectively (Figure 3).

Within the concentration range of 2.0 ng/ml to 50 ng/ml, the hydroxyzine-antazoline peak height ratios were linear. The coefficient of variation obtained over the study period ranged from 30% at 5 ng/ml to 10% at 50 ng/ml (Table 2).

This assay was considered to be sufficiently sensitive and specific to measure the serum hydroxyzine concentration in rabbits in this study, which was in the range of 2.0 to 50 ng/ml. No difference in the assay results were obtained using either rabbit serum or human plasma.

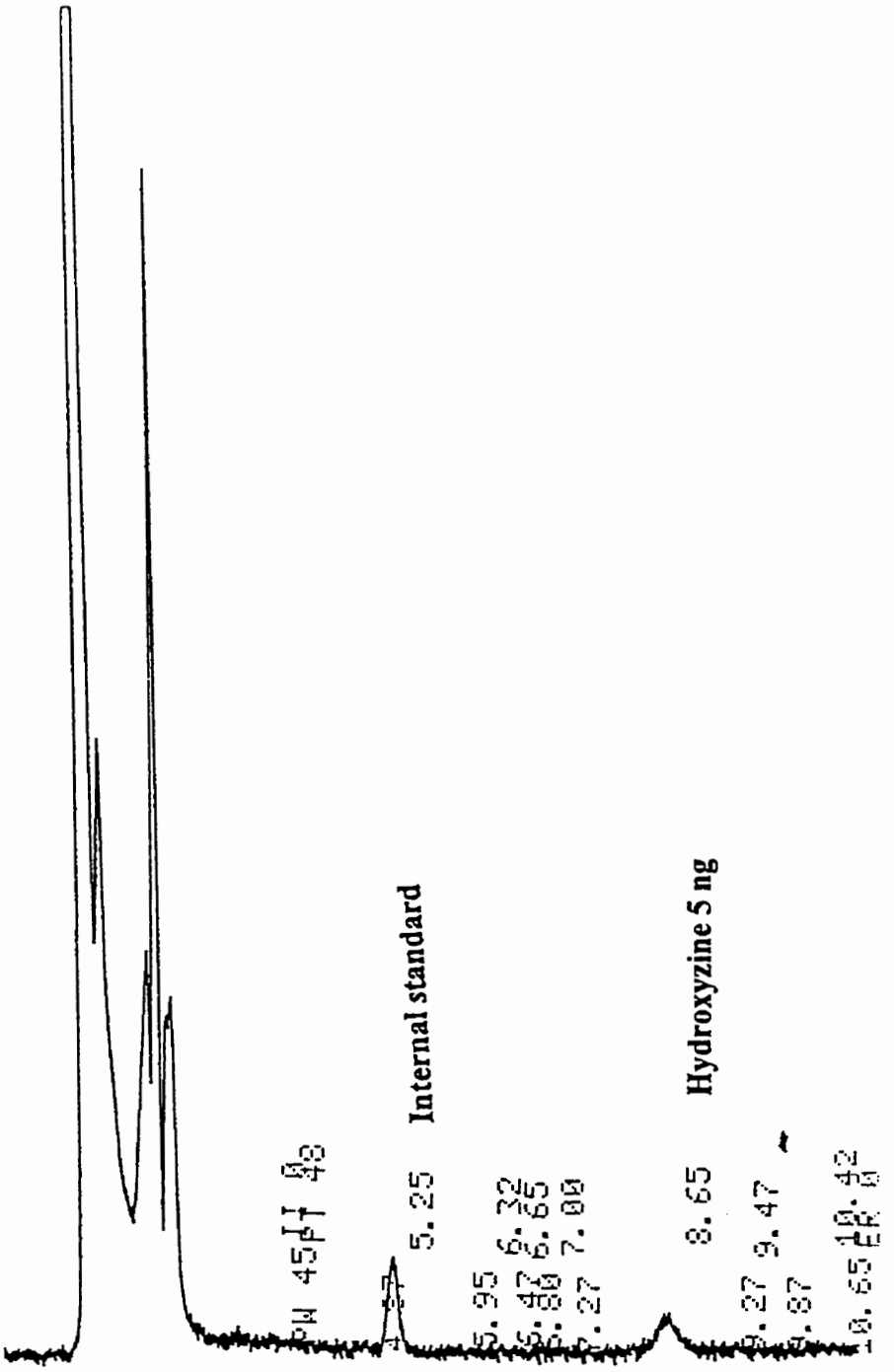


Figure 3. The HPLC chromatograms of hydroxyzine and its internal standard, antazoline in rabbit serum.

CONCENTRATION (NG/ML)	PEAK HEIGHT RATIO (MEAN± SD)	C.V (%)
2.0	0.1123±0.033	30.1
5.0	0.1789±0.045	25.4
10.0	0.2048±0.041	20.1
20.0	0.2312±0.031	13.3
50.0	0.6262±0.065	10.4

Table 2. Variability in HPLC calibration curves for hydroxyzine in plasma (n=5 over 2 months).

3.2. Formulation of Liposomes Using the Ethanol Injection Method

A variety of methods have been developed for preparing liposomes. Recently, a literature survey indicated that there are more than 200 patents and patent applications dealing with liposome technology (12). In order to successfully commercialize a liposome product there is a necessity to find a method capable of commercial scale-up production at acceptable costs.

Batzri and Korn (23) described a method for preparing SUV liposomes that avoids both sonication and exposure to high pressure up to 800 psi in the case of unilamellar vesicles prepared by high-pressure extrusion; the method is called the ethanol injection technique (EIM). Lipids dissolved in ethanol are rapidly injected into an excess of buffer solution and SUVs form spontaneously. The procedure is simple, rapid and non-destructive to both lipids and the drug to be entrapped. One disadvantage of this method is that it results in the production of a dilute suspension of SUVs. Ultrafiltration is an efficient method for both removing ethanol and concentrating the suspension.

In the present study, the EIM has been modified to permit the use of varying proportions of lipids and cholesterol including quantities that exceed the maximum recommended concentration (40 μmoles of egg lecithin/ml) for the production of SUV liposomes. The size of liposomes produced in this study was larger than that reported in the literature, but the number of bilayers resulting from this approach, which should be determined by the use of electron microscopy, was not determined. The liposome vesicles produced in this study could be of either the MLV or LUV type. Micelles are probably also present, but their contribution to the entrapment of hydroxyzine was not included in any calcualations.

3.2.1. Extraction Procedure for Hydroxyzine Free Base

Encapsulation efficiency or loading efficiency in liposomes is usually defined as the percent drug fraction of the total input drug encapsulated in the liposomes at a particular phospholipid concentration and is expressed as %w/w.

In order to achieve optimum efficacy and cost effectiveness for a drug delivery system, it is necessary to have a high encapsulation efficiency. Ideally, the most efficient system would approach 100% encapsulation of the drug, however no system is able to achieve this goal.

For an extremely hydrophobic drug, the optimum quantity of cholesterol additive will yield high encapsulation (>90%) (30). In contrast, for hydrophilic drugs, high encapsulation, although not as high as for hydrophobic drugs, can be attained by preparing LUVs with charged ions on the surface, and also using the optimum quantity of cholesterol.

For drugs of intermediate hydrophobicity, high encapsulation efficiency can be achieved by chemical modification of the drug, for example by increasing the hydrophobic nature by derivatizing it into a fatty acid ester compound.

To convert hydroxyzine hydrochloride into its free base by extracting it into organic solvent was a relatively simple, and efficient process. This will result in the drug being available as the hydrophobic free base and bilayer-compatible so that it can be distributed in the hydrophobic lipid bilayers of the liposomes.

The recovery of hydroxyzine as a free base was as high as 96% provided that freshly distilled ether was used in the extraction process.

3.2.2. The Use of Potassium Chloride as Aqueous Phase

Various proportions of egg phosphatidylcholine (EPC) and cholesterol (Chol) were used to prepare liposomes. The potassium chloride solution was an unbuffered, iso-osmotic aqueous medium with a concentration of 0.16 M KCl. Results of the encapsulation efficiencies of the different formulations are listed in Table 3.

Most phospholipid liposome membranes are permeable and not sufficiently rigid to contain the drug. This allows the encapsulated drug to leak into the external phase. Cholesterol is incorporated into the lipid bilayers to enhance the encapsulation efficiency. From the data in Table 3 it can be seen that when no cholesterol was added to the phospholipid fraction, the encapsulation of hydroxyzine was negligible. The amount of EPC added also had an effect on the encapsulation efficiency of hydroxyzine into liposomes. Lower proportions of EPC (36-39 μmol/ml) yielded encapsulation efficiencies of 30-40% regardless of the amount of cholesterol added. Increasing the amount of EPC up to 73 μmol/ml had a dramatic effect on the encapsulation of hydroxyzine which ranged from 45-73%.

This variation in the encapsulation achieved with high proportions of EPC can be also attributed to the various proportions of cholesterol added. The percentage of cholesterol added had a minimum effect on the encapsulation efficiency up to a 1:1 molar ratio where there was a noticeable decrease in the encapsulation of hydroxyzine compared with lower ratios of Chol to EPC. Encapsulation efficiencies between 25%-40% were achieved at various proportions of EPC to Chol. The maximum effect was seen at 73 μmol EPC: 23 μmol Chol where an encapsulation of 73% was obtained. It

seems that EPC: Chol ratio of 3:1 at 73: 23 μmol/ml is the optimum ratio for maximum encapsulation of hydroxyzine.

Although this technique is designed for the formation of SUV, at the concentration of EPC used, 73 mol/ml which is above the critical micelle concentration (CMC), micelles are possibly also present in the final formulation. The contribution of these micelles, if present, was not considered in the calculation of the degree of entrapment of hydroxyzine. Micelles may be filtered or forced into the liposome structures during filtration. The presence of micelles will need to be considered in future studies.

Ma et al (31,32) observed an increase in the encapsulation of hydrophobic compounds when cholesterol was incorporated into the bilayer. Encapsulation of citicoline increased linearly with the addition of cholesterol up to 1:1 molar ratio to dipalmitoyl phosphatidylcholine (DPPC). This increase was attributed to the reduction in the membrane permeability.

In other studies, the encapsulation of hydrophobic steroids increased slightly as the concentration of cholesterol was increased when the drug input was well below the encapsulation capacity. The opposite effect occurred when the drug input was equal to or higher than the encapsulation capacity of the liposomes (30).

EPC: CHOL (μMOL/ML)	ENCAPSULATION EFFICIENCY(%)
73 : 23 (3:1)	73%
74 : 17.5 (4:1)	51%
71 : 34 (2:1)	45%
36 : 12 (3:1)	40%
55 : 27.5 (2:1)	32%
21 : 13 (1.6:1)	30%
39 : 20 (2:1)	30%
70 : 70 (1:1)	30%
36 : 26 (1.4:1)	30%
36 : 13 (2.75:1)	25%
21 : 0.0 (21:0)	Non Encapsulated

Table 3. The effect of various proportions of EPC and cholesterol on the encapsulation efficiency of hydroxyzine in liposomes.

3.2.3 The Use of Phosphate Buffer as Aqueous Phase

When 0.16 M potassium chloride is used as the aqueous phase, the pH of the filtrate resulting from concentration of the liposome formulation is variable. This solution has an ionic strength of 0.16. Hydroxyzine dihydrochloride has pKa values of 2.6 and 7.0 (33). At pH>7.0, there is a major change in the non-ionized to ionized ratio of hydroxyzine. As the pH is raised, more of the non-ionized form is available than the ionized form, resulting in subsequent changes in the tendency for hydroxyzine to partition more into the lipid layers than to remain in the entrapped aqueous phase. Also as the non-ionized to ionized ratio increases, more hydroxyzine will partition into the lipid phase from the aqueous phase.

These observations suggest that unbuffered 0.16 M potassium chloride is not the optimum aqueous phase to provide the constant pH necessary for consistent hydroxyzine encapsulation. Therefore a change to phosphate buffer was introduced to provide a constant pH medium for the suspended vesicles, and to permit the study of the effect of changing pH on the encapsulation efficiency. The optimum phospholipid to cholesterol ratio was selected from the trials performed during the study of potassium chloride, which was EPC:CHOL (3:1) at 73:23 μmol/ml.

Phosphate buffers at different pH values have been studied with various effects on the encapsulation efficiency of hydroxyzine in liposomes. The results are shown in Table 4.

As seen in Table 4, increasing the phosphate buffer pH from 6.6 to 8 yielded an increase in the encapsulation efficiency of hydroxyzine. A maximum of 77.6% was reached at pH 8, while at pH 6.6 only 50% of the hydroxyzine was encapsulated in the

liposomes. At pH 7.0, 62.5% was encapsulated. The 0.4 M phosphate buffer pH 7.0 selected as the optimal aqueous medium has an ionic strength of 0.44. This ionic strength 0.44 is greater than the ionic strength of potassium chloride solution 0.16. This ionic strength of the phosphate buffer should be sufficient to maintain the integrity of the liposomes. The presence of micelles, and their possible contribution to the entrapment of hydroxyzine, was not considered in these calculations.

Cannon et al (34) found that the encapsulation of tin-mesomorphine is also pH dependent. It was as high as 90% at pH 5 while at pH 7 less than 10% was encapsulated. In the present study pH 7.0 was chosen to be the pH of the buffer in order to match the pH of the skin, and to be consistent with the pH of most topical formulations which are either neutral or slightly acidic.

pH OF AQUEOUS BUFFER	.ENCAPSULATION EFFICIENCY
6.6	50.0%
7.0	62.5%
8.0	77.6%

Table 4. The effect of pH of aqueous buffer on the encapsulation efficiency of hydroxyzine in liposomes.

3.2.3.1. Liposome Characterization

The average diameter of the liposomes and the size distribution of suspended vesicles were determined by submicron particle size analysis. The analyzer was furnished with a 5 mW laser diode at an excitation wavelength of 632.8 nm and a scattering angle of 90°. Devoisselle et al (35) showed that such a scattering angle is adequate because it allows for precise size analysis of both large and small vesicles in the shortest running time.

Volume-weighted Gaussian analysis was used instead of number-weighted Gaussian analysis in order to be consistent with most of the reported literature results. Here, the height of each diameter bin is expressed as percentage of the total volume of all particles counted for the volume-weighted distribution. The average size of 10 different EIM liposome preparations was 103 nm with S.D. of 40% distributed along a bell-shaped unimodal pattern.

3.2.3.2. Effect of Rate of Injection on Liposome Size

Rapid injection of the ethanol/lipid mixture into the aqueous buffer resulted in a trimodal distribution pattern of liposome size as revealed by submicron particle size analysis (Figure 4A). A simple solution to resolve this problem was to inject the ethanol/lipid mixture more slowly using a syringe pump. This resulted in unimodal distribution of vesicles as detected by volume-weighted Gaussian analysis (Figure 4B). Slow injection over a period of 5 minutes or 30 minutes yielded the same size distribution with no effect or improvement on the percentage of drug encapsulated.

The method of ethanol injection developed by Batzri and Korn yielded a homogeneous SUV preparation with an average diameter of 26.5 nm as detected by electron microscopic analysis of unfractionated liposomes (23). Nordlund et al (36) obtained large unilamellar vesicles (LUV) by modifying the ethanol injection method. They used a mixture of lipids in ethanol (10 μmole of phospholipid/ml) that was injected slowly (< 3 ml/hour) up to a maximum concentration of 25% of ethanol in aqueous buffer. Then high-speed centrifugation was implemented to fractionate the liposomes according to size.

The difference between the results obtained currently and the previous ones can be attributed to the fact that in the current preparation a more concentrated solution of lipids was used to prepare liposomes, which exceeded the maximum recommended concentration (40 μmol of egg lecithin/ml of ethanol) necessary for the production of SUV liposomes. As indicated previously, electron microscopy was not used to determine the number of bilayers. There is the possibility that the formulations by this method were comprised of small size MLV vesicles or LUV liposomes.

At these high lipid concentrations, there will probably be a range of structures varying from micelles to liposomes. The micellar structures are very efficient at absorbing hydrophobic molecules. This may partly account for the very high encapsulation efficiencies. These particles would probably be filtered. Light scattering does not detect their presence. Freeze fracture electron microscopy would detect the small micelle size (4-8 nm) and this analysis was not performed in this study.

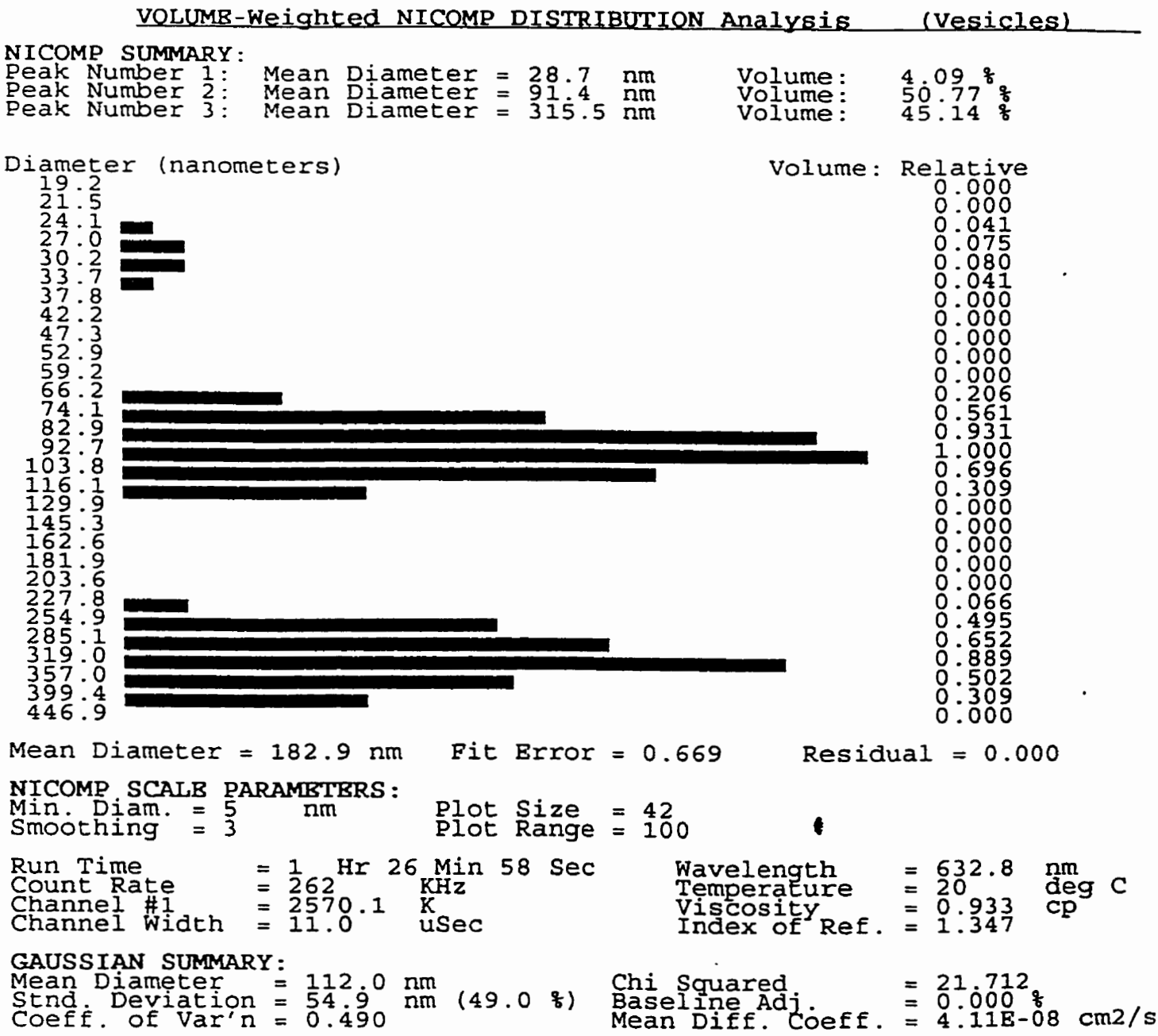
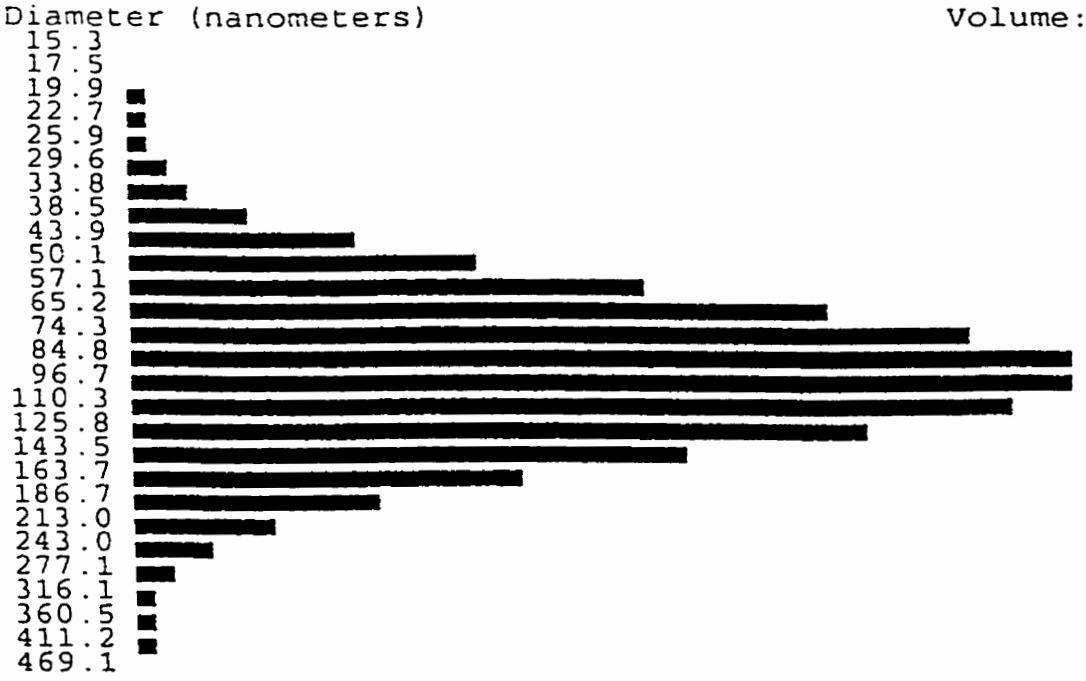


Figure 4A. Particle size distribution of EIM liposomes (Rapid injection).

VOLUME-Weighted GAUSSIAN Analysis (Vesicles)

GAUSSIAN SUMMARY:

Mean Diameter	= 100.4 nm	Chi Squared	= 0.634
Stnd. Deviation	= 42.5 nm (42.3 %)	Baseline Adj.	= 0.000 %
Coeff. of Var'n	= 0.423	Mean Diff. Coeff.	= 4.63E-08 cm2/s



Diameter (nanometers)	Volume: Relative	Percent
15.3	0.000	0.000
17.5	0.000	0.000
19.9	0.001	0.012
22.7	0.004	0.049
25.9	0.011	0.136
29.6	0.028	0.345
33.8	0.061	0.752
38.5	0.122	1.504
43.9	0.220	2.712
50.1	0.361	4.450
57.1	0.537	6.620
65.2	0.725	8.937
74.3	0.889	10.959
84.8	0.989	12.192
96.7	1.000	12.327
110.3	0.917	11.304
125.8	0.764	9.418
143.5	0.577	7.113
163.7	0.396	4.882
186.7	0.247	3.045
213.0	0.139	1.714
243.0	0.071	0.875
277.1	0.033	0.407
316.1	0.014	0.173
360.5	0.005	0.062
411.2	0.001	0.012
469.1	0.000	0.000

Cumulative Results:

25 % of distribution <	64.68 nm
50 % of distribution <	86.01 nm
75 % of distribution <	114.56 nm
90 % of distribution <	148.12 nm
99 % of distribution <	230.61 nm

Run Time	= 0 Hr 35 Min 3 Sec	Wavelength	= 632.8 nm
Count Rate	= 320 KHz	Temperature	= 23 deg C
Channel #1	= 1496.8 K	Viscosity	= 0.933 cp
Channel Width	= 12.0 uSec	Index of Ref.	= 1.333

Figure 4B. Particle size distribution of EIM liposomes (Slow injection).

3.3. Formulation of MLV Liposomes

MLV liposomes were prepared using the solvent evaporation method originally described by Bangham and Standish (37). In this method, lipids were dissolved in an organic solvent in a round-bottom flask. The solvent was removed under vacuum by rotary evaporation, and the lipid residue formed a thin film on the wall of the flask. An aqueous buffer solution was then added to the flask. Agitation of the flask produced large multilamellar vesicles (MLV). This aqueous suspension was further diluted, then hydroxyzine hydrochloride was added in a powder form.

Concentrating the suspension by ultrafiltration was a necessary step for both removing excess non-encapsulated hydroxyzine and producing the desired hydroxyzine concentration (10 mg/ml). This was confirmed by HPLC analysis of the final formulation.

The addition of hydroxyzine after formation of the liposome suspension was possible because in phosphate buffer at pH 7.0 hydroxyzine would have equal proportions of non-ionized and ionized forms. The non-ionized hydroxyzine would be distributed to the maximum in the lipid bilayers. In the aqueous phase, equal proportions of the ionized and the non-ionized hydroxyzine would remain in equilibrium at pH 7.0. The encapsulation efficiency, 63% at an EPC:Chol ratio of 3:1 and 73:23 μmol/ml, was higher than that obtained previously with a buffer at lower pH. This can be attributed to the partition behavior of hydroxyzine between aqueous and lipid compartments.

At these high lipid concentrations, there will probably be a range of structures varying from micelles to liposomes. The micellar structures are very efficient at

absorbing hydrophobic molecules. This may partly account for the very high encapsulation efficiencies. These particles would probably be filtered. Light scattering does not detect their presence. Freeze fracture electron microscopy would detect the small micelle size (4-8 nm) and this analysis was not performed in this study.

Size distribution evaluation showed a bimodal distribution with the majority of vesicles having an average diameter of 7 microns while a smaller population had an average diameter of 2 microns (Figure 5).

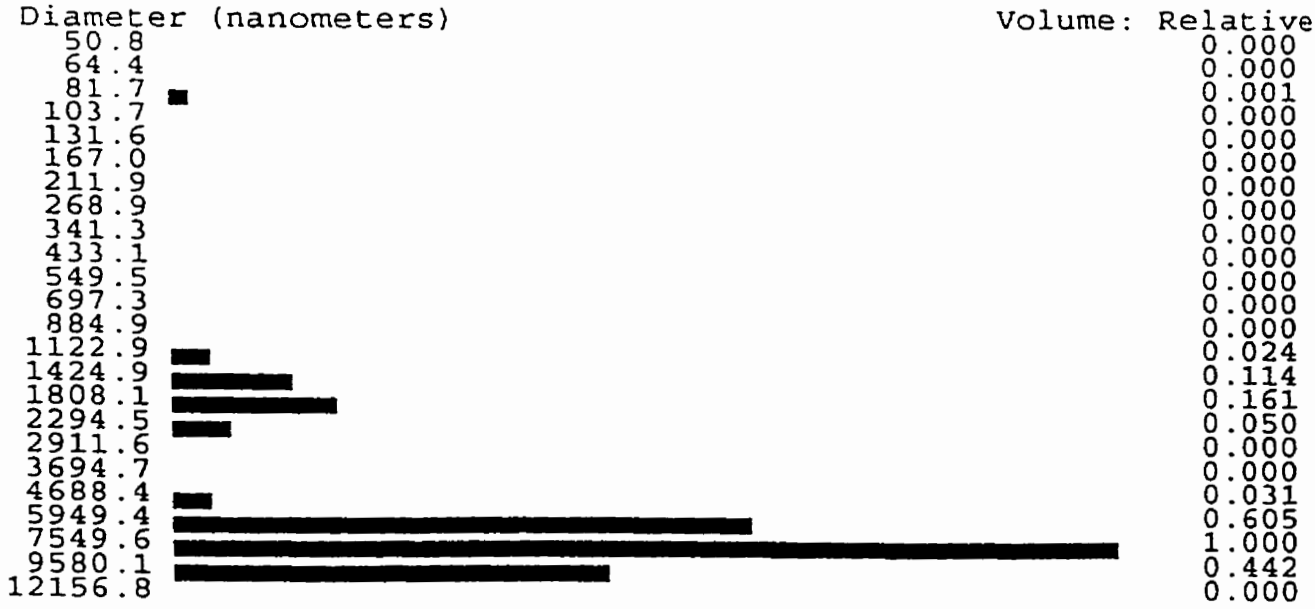
3.4. Selection of Products to be Tested *in vivo*

Three different hydroxyzine formulations were selected for testing in rabbits. These were MLV liposomes of a large size (2-4 microns), ethanol injection method liposomes (EIM) of a relatively small size (100 nm). Both were prepared in phosphate buffer at pH 7.0 and the same phospholipid to cholesterol ratio of 3:1 at 73:23 μmol/ml. The control non-liposome formulation was Glaxal Base, an oil/water cream emulsion base prepared to contain the same concentration of hydroxyzine (10 mg/4g)

The need for the control cream formulation was required in order to confirm our hypothesis that a liposome formulation should demonstrate a higher efficacy and/or lower serum concentration of the active ingredient compared to a conventional vehicle.

VOLUME-Weighted NICOMP DISTRIBUTION Analysis (Vesicles)

NICOMP SUMMARY:
Peak Number 1: Mean Diameter = 80.1 nm Volume: 0.10 %
Peak Number 2: Mean Diameter = 1705.5 nm Volume: 13.85 %
Peak Number 3: Mean Diameter = 7472.6 nm Volume: 86.05 %



Mean Diameter = 6640.9 nm Fit Error = 1.301 Residual = 0.000

NICOMP SCALE PARAMETERS:
Min. Diam. = 40 nm Plot Size = 30
Smoothing = 2 Plot Range = 1000

Run Time = 1 Hr 9 Min 47 Sec Wavelength = 632.8 nm
Count Rate = 378 KHz Temperature = 23 deg C
Channel #1 = 95531.6 K Viscosity = 0.933 cp
Channel Width = 300.0 uSec Index of Ref. = 1.333

GAUSSIAN SUMMARY:
Mean Diameter = 4341.8 nm Chi Squared = 26.380
Stnd. Deviation = 2739.7 nm (63.1 %) Baseline Adj. = 0.006 %
Coeff. of Var'n = 0.631 Mean Diff. Coeff. = 1.07E-09 cm2/s

Figure 5. Particle size distribution of MLV liposomes.

3.5. Serum Concentrations and Pharmacodynamics of Hydroxyzine in Rabbits: The Effect of Different Formulations

The effect of hydroxyzine-induced wheal response on the back of rabbit has been studied previously (43,44). The wheal area suppression can be related directly to the peripheral antihistaminic effect of H_1-receptor antagonists. Hydroxyzine serum concentrations measured by previously established HPLC methods (28,29), were used to evaluate the rate and the extent of absorption of hydroxyzine into the systemic circulation after topical administration.

3.5.1. Evaluation of Hydroxyzine 10 mg in MLV Liposomes

Hydroxyzine, 10 mg, in MLV liposomes was applied to a 125 cm^2 area of the shaved back of each rabbit under the study protocol.

3.5.1.1 Pharmacodynamic Studies of Hydroxyzine in Rabbits After the Topical Administration of 10 mg Hydroxyzine in MLV Liposomes

Results of the efficacy tests calculated as absolute wheal areas induced by the intradermal injection of 0.05 ml of 1mg/ml histamine phosphate before and after the topical application of 10 mg hydroxyzine in MLV liposomes are listed in Table 5 and shown in Figure 6.

Results of the efficacy tests calculated as the percent suppression of wheals induced by the intradermal injection of 0.05 ml of 1 mg/ml histamine phosphate after the topical application of 10 mg hydroxyzine in MLV liposomes are listed in Table 6

and shown in Figure 7. Significant differences (*) in these data were calculated using the absolute wheal areas.

3.5.1.2. Serum Concentrations of Hydroxyzine After the Topical Application of Hydroxyzine in MLV Liposomes

Serum concentrations of hydroxyzine after the topical application of 10 mg hydroxyzine in MLV liposomes on rabbit skin are listed in Table 7. The mean serum concentrations of hydroxyzine after the topical application of 10 mg hydroxyzine in MLV liposomes are plotted against time and shown in Figure 8.

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.629	0.622	0.645	1.163	0.943	0.862	0.811	0.219
0.5	0.0	0.0	0.0	0.0	0.0	0.288	0.048	0.117
1	0.0	0.0	0.0	0.0	0.0	0.156	0.026	0.063
2	0.0	0.0	0.0	0.0	0.0	0.111	0.018	0.045
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.084	0.0	0.0	0.0	0.186	0.0	0.0451	0.077
5	0.083	0.125	0.099	0.078	0.249	0.137	0.128	0.064
6	0.099	0.236	0.222	0.136	0.273	0.391	0.226	0.01
8	0.157	0.285	0.196	0.0	0.099	0.322	0.177	0.119
10	0.0	0.398	0.549	0.242	0.263	0.281	0.289	0.182
12	0.608	0.0	0.532	0.392	0.526	0.338	0.399	0.219
24	N/A	N/A	0.3558	0.9603	N/A	0.5146	0.610	0.313

Table 5. Mean wheal areas (cm^2) induced by histamine phosphate before and after the application of 10 mg hydroxyzine in MLV liposomes.

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	100	100	100	100	100	66.624	94.437	13.62
1	100	100	100	100	100	81.93	96.988	7.377
2	100	100	100	100	100	87.176	97.863	5.235
3	100	100	100	100	100	100	100	0.0
4	86.599	100	100	100	80.241	100	94.473	8.795
5	86.789	79.99	84.849	93.26	73.56	84.136	83.764	6.618
6	84.217	62.086	65.546	88.291	71.047	54.625	70.969	13.05
8	75.039	54.275	69.605	100	89.447	62.62	75.164	17.0
10	100	36.065	15.011	79.187	72.149	67.448	61.643	30.83
12	3.477	100	17.614	66.308	44.172	60.833	48.734	34.99
24	N/A	N/A	44.879	17.443	N/A	40.281	34.201	14.69

Table 6. Percent suppression of histamine induced-wheals after the application of 10 mg hydroxyzine in MLV liposomes.

Figure 6

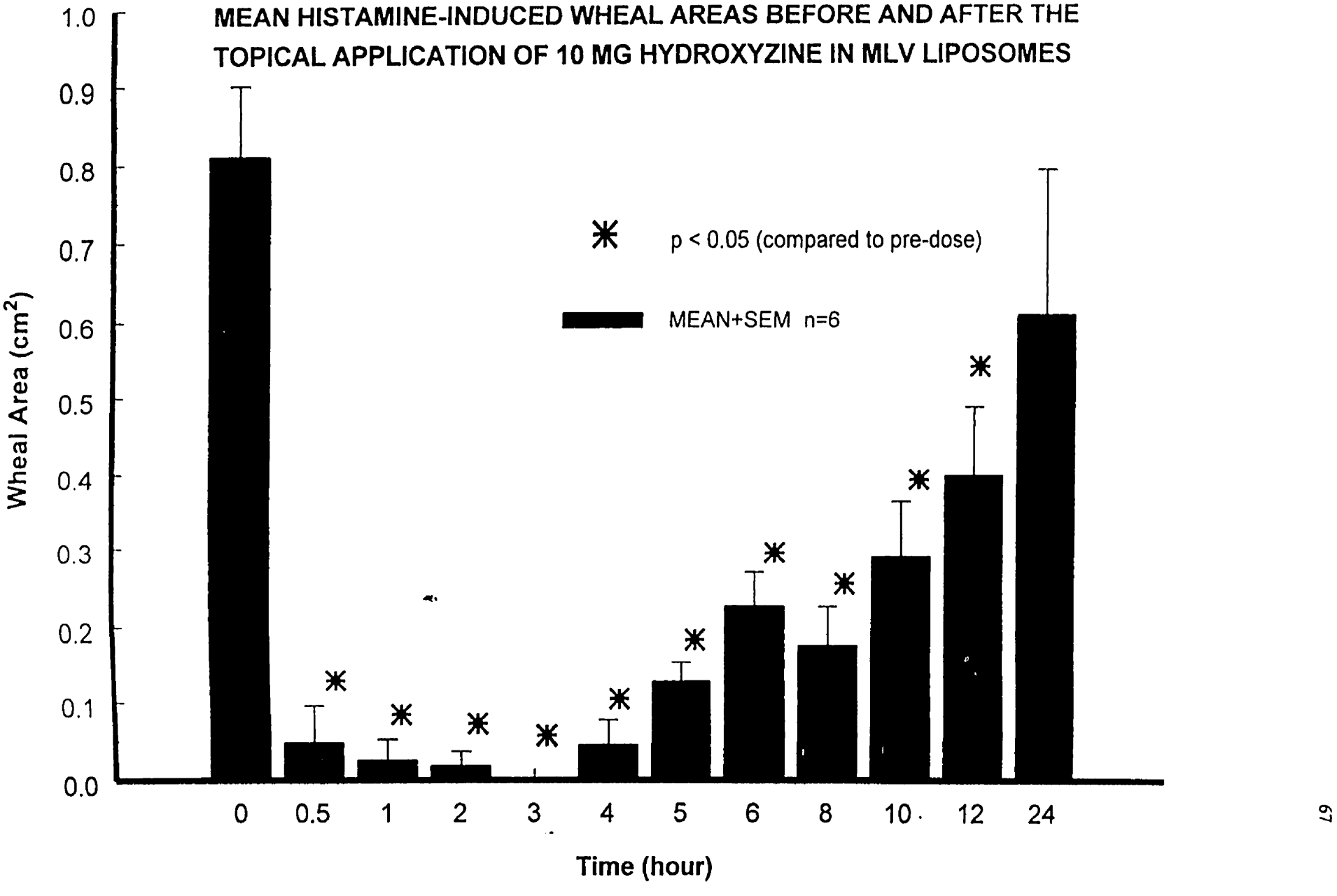
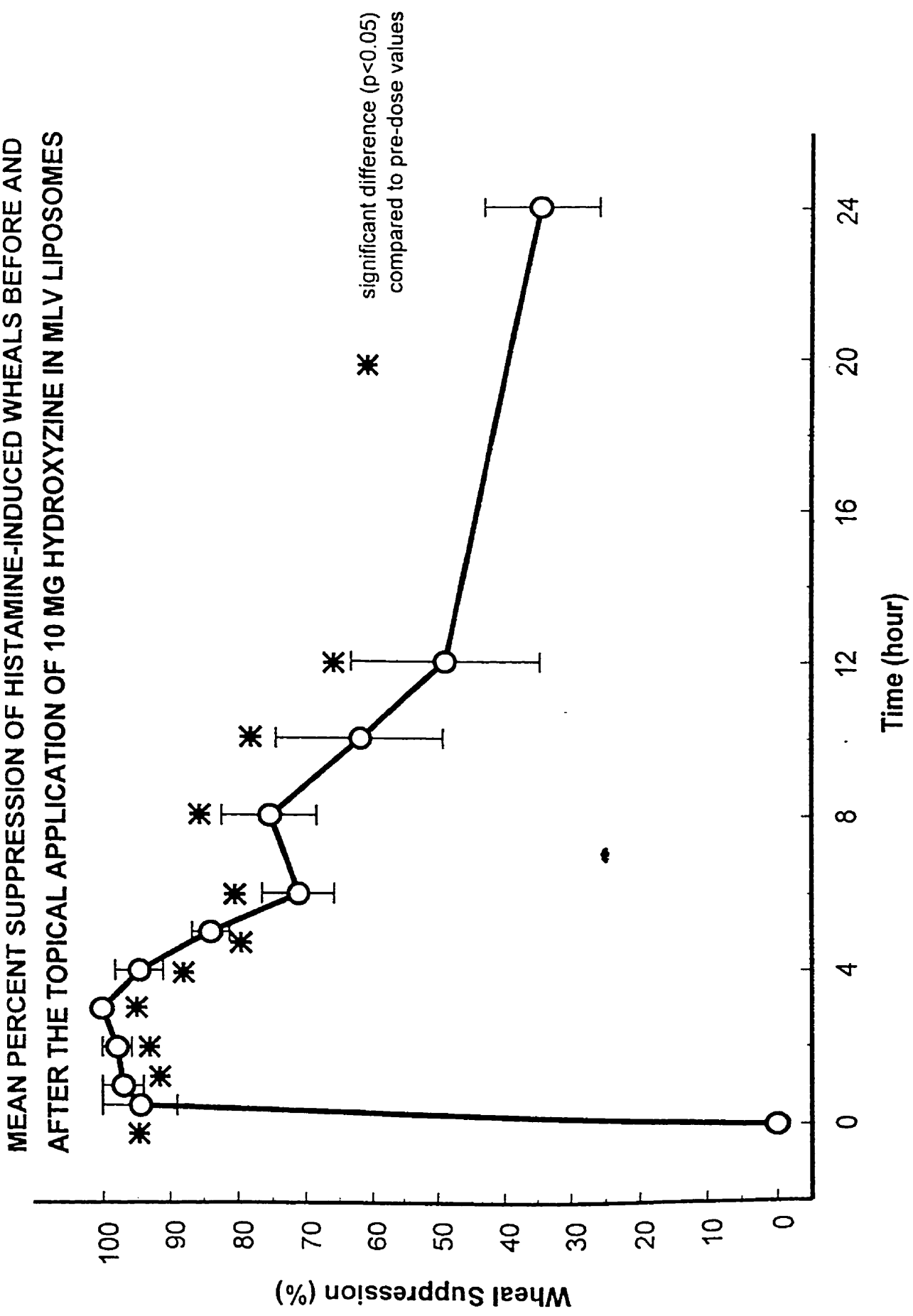

MEAN HISTAMINE-INDUCED WHEAL AREAS BEFORE AND AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN MLV LIPOSOMES
Wheal Area (cm²)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
p < 0.05 (compared to pre-dose)
MEAN+SEM n=6
0
0.5
1
2
3
4
5
6
8
10
12
24
Time (hour)

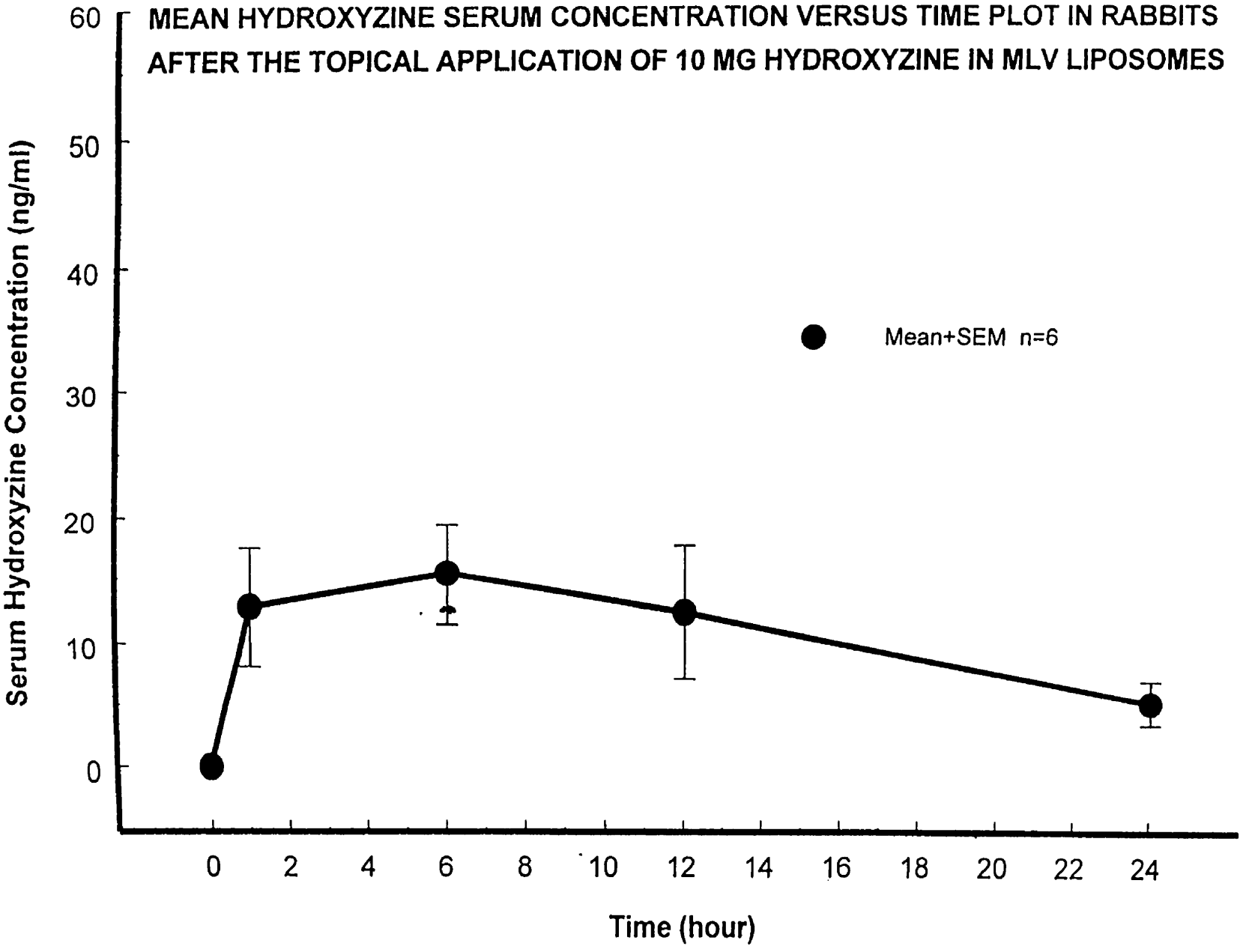
Figure 7


MEAN PERCENT SUPPRESSION OF HISTAMINE-INDUCED WHEALS BEFORE AND AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN MLV LIPOSOMES
significant difference (p<0.05) compared to pre-dose values
Wheal Suppression (%)
Time (hour)
0
10
20
30
40
50
60
70
80
90
100
0
4
8
12
16
20
24

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	29.6	6.3	7.7	24.1	9.3	0.0	12.8	11.4
6	27.3	16.6	7.4	27	7.4	6.9	15.4	9.8
12	N/A	1.9	8.1	29.4	19.7	3.0	12.4	11.8
24	8	4.3	7.9	0.0	10.6	0.0	5.1	4.5

Table 7. Serum concentrations (ng) of hydroxyzine after the application of 10 mg hydroxyzine in MLV liposomes.

Figure 8


MEAN HYDROXYZINE SERUM CONCENTRATION VERSUS TIME PLOT IN RABBITS
AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN MLV LIPOSOMES
Serum Hydroxyzine Concentration (ng/ml)
60
50
40
30
20
10
0
Mean+SEM n=6
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hour)

3.5.2. Evaluation of Hydroxyzine 10 mg in EIM Liposomes

Hydroxyzine. 10 mg, in EIM liposomes was applied to a 125 cm^2 area of the shaved back of each rabbit under the study protocol.

3.5.2.1 Pharmacodynamic Studies of Hydroxyzine in Rabbits After the Topical Administration of 10 mg Hydroxyzine in EIM Liposomes

Results of the efficacy tests calculated as absolute wheal areas induced by the intradermal injection of 0.05 ml of 1mg/ml histamine phosphate before and after the topical application of 10 mg hydroxyzine in EIM liposomes are listed in Table 8 and shown in Figure 9.

Results of the efficacy tests calculated as the percent suppression of wheals induced by the intradermal injection of 0.05 ml of 1 mg/ml histamine phosphate after the topical application of 10 mg hydroxyzine in EIM liposomes are listed in Table 9 and shown in Figure 10.

3.5.2.2. Serum Concentrations of Hydroxyzine After the Topical Application of Hydroxyzine in EIM Liposomes

Serum concentrations of hydroxyzine after the topical application of 10 mg hydroxyzine in EIM liposomes on rabbit skin are listed in Table 10. The mean serum concentrations of hydroxyzine after the topical application of 10 mg hydroxyzine in EIM liposomes are plotted against time and shown in Figure 11.

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.694	0.718	0.751	0.664	0.080	0.806	0.739	0.058
0.5	0.0	0.477	0.195	0.666	0.445	0.430	0.369	0.235
1	0.0	0.0	0.313	0.110	0.0	0.165	0.098	0.126
2	0.0	0.0	0.0	0.084	0.143	0.160	0.065	0.075
3	0.0	0.0	0.0	0.0	0.152	0.180	0.055	0.086
4	0.0	0.0	0.0	0.0	0.578	0.166	0.124	0.232
5	0.0	0.0	0.0	0.075	0.161	0.141	0.063	0.074
6	0.094	0.0	0.0	0.274	0.380	0.316	0.177	0.167
8	0.315	0.179	0.095	0.184	0.0	0.174	0.158	0.105
10	0.229	0.186	0.0	0.408	0.127	0.330	0.213	0.145
12	0.208	0.242	0.0	N/A	0.216	0.227	0.178	0.101
24	0.315	0.427	0.204	N/A	0.628	0.625	0.440	0.187

Table 8. Mean wheal areas (cm^2) induced by histamine phosphate before and after the application of 10 mg hydroxyzine in EIM liposomes.

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	100	33.545	74.012	-0.343	44.457	46.637	49.718	34.41
1	100	100	58.360	83.505	100	79.543	86.901	16.7
2	100	100	100	87.285	82.195	80.115	91.599	9.49
3	100	100	100	100	81.055	77.676	93.122	10.71
4	100	100	100	100	27.920	79.4	84.553	28.94
5	100	100	100	88.66	79.914	82.526	91.85	9.37
6	86.488	100	100	58.763	52.565	60.800	76.436	21.63
8	54.561	75.039	87.386	72.337	100	78.398	77.954	15.25
10	67.062	74.087	100	38.487	84.202	59.084	70.487	21.16
12	70.101	66.296	100	N/A	73.081	71.817	76.259	13.52
24	54.561	40.54	72.796	N/A	21.652	22.461	42.402	21.81

Table 9. Percent suppression of histamine induced-wheals after the application of 10 mg hydroxyzine in EIM liposomes.

Figure 9

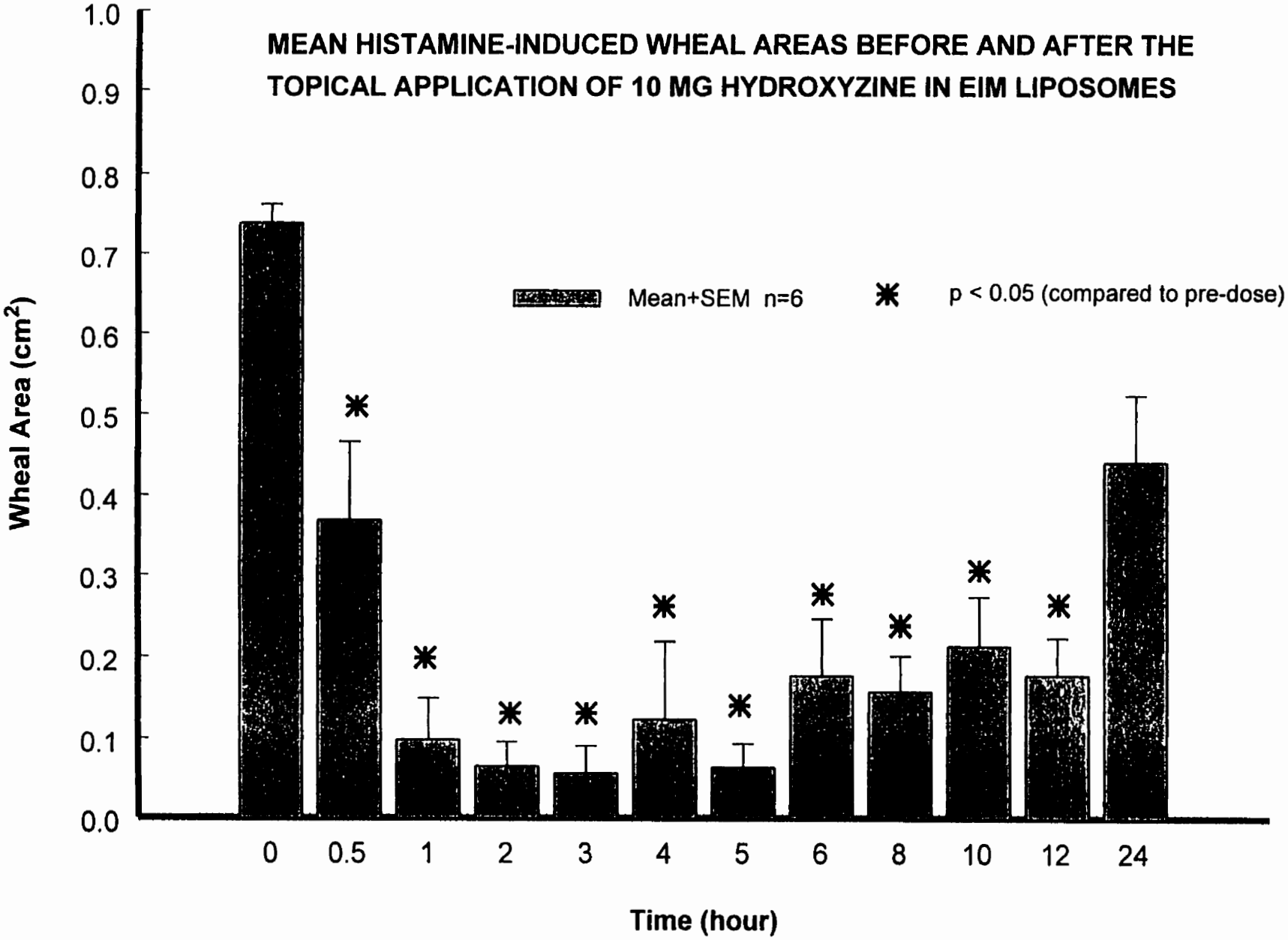
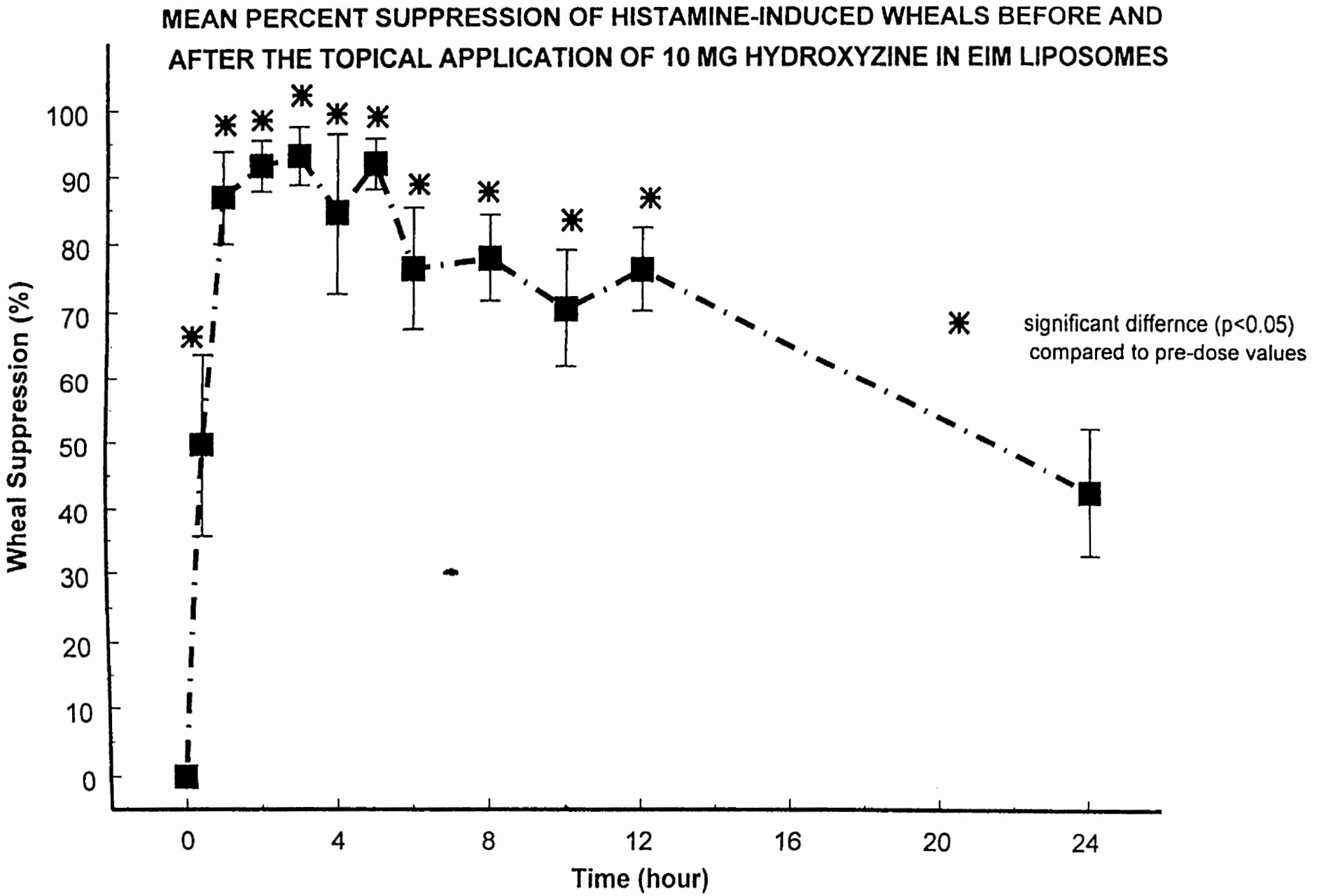

MEAN HISTAMINE-INDUCED WHEAL AREAS BEFORE AND AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN EIM LIPOSOMES
Mean+SEM n=6
* p < 0.05 (compared to pre-dose)
Wheal Area (cm²)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
0.5
1
2
3
4
5
6
8
10
12
24
Time (hour)

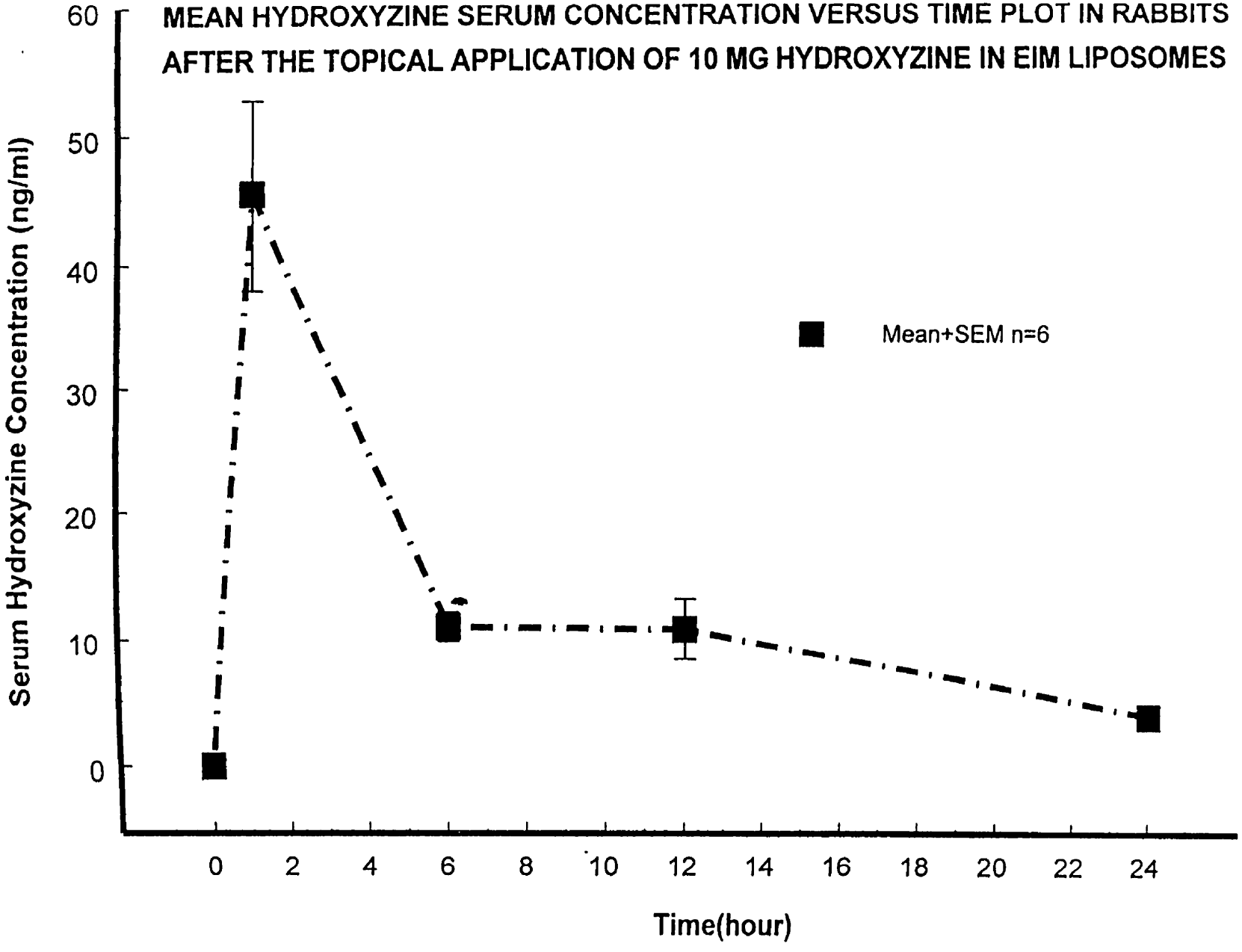
Figure 10


MEAN PERCENT SUPPRESSION OF HISTAMINE-INDUCED WHEALS BEFORE AND AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN EIM LIPOSOMES
Wheal Suppression (%)
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Time (hour)
significant differnce (p<0.05) compared to pre-dose values

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	63.9	37.6	N/A	30.1	50.1	N/A	45.4	14.8
6	12.2	9.8	6.8	10.5	15.1	11.7	11.0	2.8
12	22.5	10.3	8.9	7.9	8.8	6.3	10.8	5.9
24	5.4	3.5	5.6	4.7	3.5	N/S	3.8	2.1

Table 10. Serum concentrations (ng) of hydroxyzine after the application of 10 mg hydroxyzine In EIM liposomes.

Figure 11


MEAN HYDROXYZINE SERUM CONCENTRATION VERSUS TIME PLOT IN RABBITS
AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN EIM LIPOSOMES
Serum Hydroxyzine Concentration (ng/ml)
60
50
40
30
20
10
0
Mean+SEM n=6
0
2
4
6
8
10
12
14
16
18
20
22
24
Time(hour)

3.5.3. Evaluation of Hydroxyzine 10 mg in Glaxal Base

Hydroxyzine, 10 mg, in Glaxal Base was applied to a 125 cm^2 area of the shaved back of each rabbit under the study protocol.

3.5.3.1 Pharmacodynamic Studies of Hydroxyzine in Rabbits After the Topical Administration of 10 mg Hydroxyzine in Glaxal Base

Results of the efficacy tests calculated as absolute wheal suppression induced by the intradermal injection of 0.05 ml of 1mg/ml histamine phosphate before and after the topical application of 10 mg hydroxyzine in Glaxal Base are listed in Table 11 and shown in Figure 12.

Results of the efficacy tests calculated as the percent suppression of wheals induced by the intradermal injection of 0.05 ml of 1 mg/ml histamine phosphate after the topical application of 10 mg hydroxyzine in Glaxal Base are listed in Table 12 and shown in Figure 13.

3.5.3.2. Serum Concentrations of Hydroxyzine After the Topical Application of Hydroxyzine in Glaxal Base

Serum concentrations of hydroxyzine after the topical application of 10 mg hydroxyzine in Glaxal Base on rabbit skin are listed in Table 13. The mean serum concentrations of hydroxyzine after the topical application of 10 mg hydroxyzine in Glaxal Base are plotted against time and shown in Figure 14.

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	1.215	0.543	1.076	0.781	1.019	0.953	0.931	0.238
0.5	0.101	0.0	0.288	0.784	0.015	0.0	0.198	0.307
1	0.0	0.0	0.0	0.304	0.0	0.288	0.099	0.153
2	0.0	0.075	0.0	0.086	0.016	0.404	0.097	0.155
3	0.0	0.272	0.383	0.134	0.322	0.757	0.311	0.259
4	0.0	0.389	0.098	0.392	0.373	0.599	0.308	0.220
5	0.124	0.459	0.255	0.419	0.376	0.602	0.376	0.166
6	0.142	0.605	0.451	0.464	0.423	0.628	0.452	0.174
8	0.0	0.410	0.308	0.357	0.278	0.751	0.351	0.242
10	0.434	0.478	0.414	0.351	0.305	0.479	0.410	0.070
12	0.506	0.8012	0.623	0.4599	0.457	0.570	0.569	0.132
24	0.830	N/A	0.553	N/A	0.607	0.475	0.616	0.153

Table 11. Mean wheal areas (cm^2) induced by histamine phosphate before and after the application of 10 mg hydroxyzine in Glaxal Base.

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	91.699	100	46.162	-0.2957	98.544	100	72.685	41.38
1	100	100	100	61.095	100	69.740	88.473	18.07
2	100	86.136	100	89.053	98.433	57.565	88.531	16.29
3	100	50.0	64.406	82.84	68.421	20.450	64.353	27.43
4	100	28.363	90.889	49.853	63.38	37.116	61.60	28.91
5	89.830	15.526	76.324	46.387	61.157	36.854	54.346	27.07
6	88.321	-11.344	58.051	40.533	58.454	34.042	44.676	33.29
8	100	24.58	71.398	54.291	72.676	21.158	57.351	30.48
10	64.286	11.975	61.547	55.029	70.10	49.764	52.117	20.91
12	58.301	-47.478	41.737	41.123	55.655	40.189	31.588	39.53
24	31.660	N/A	48.622	N/A	40.425	50.118	42.706	8.508

Table 12. Percent suppression of histamine induced-wheals after the application of 10 mg hydroxyzine in Glaxal Base.

Figure 12

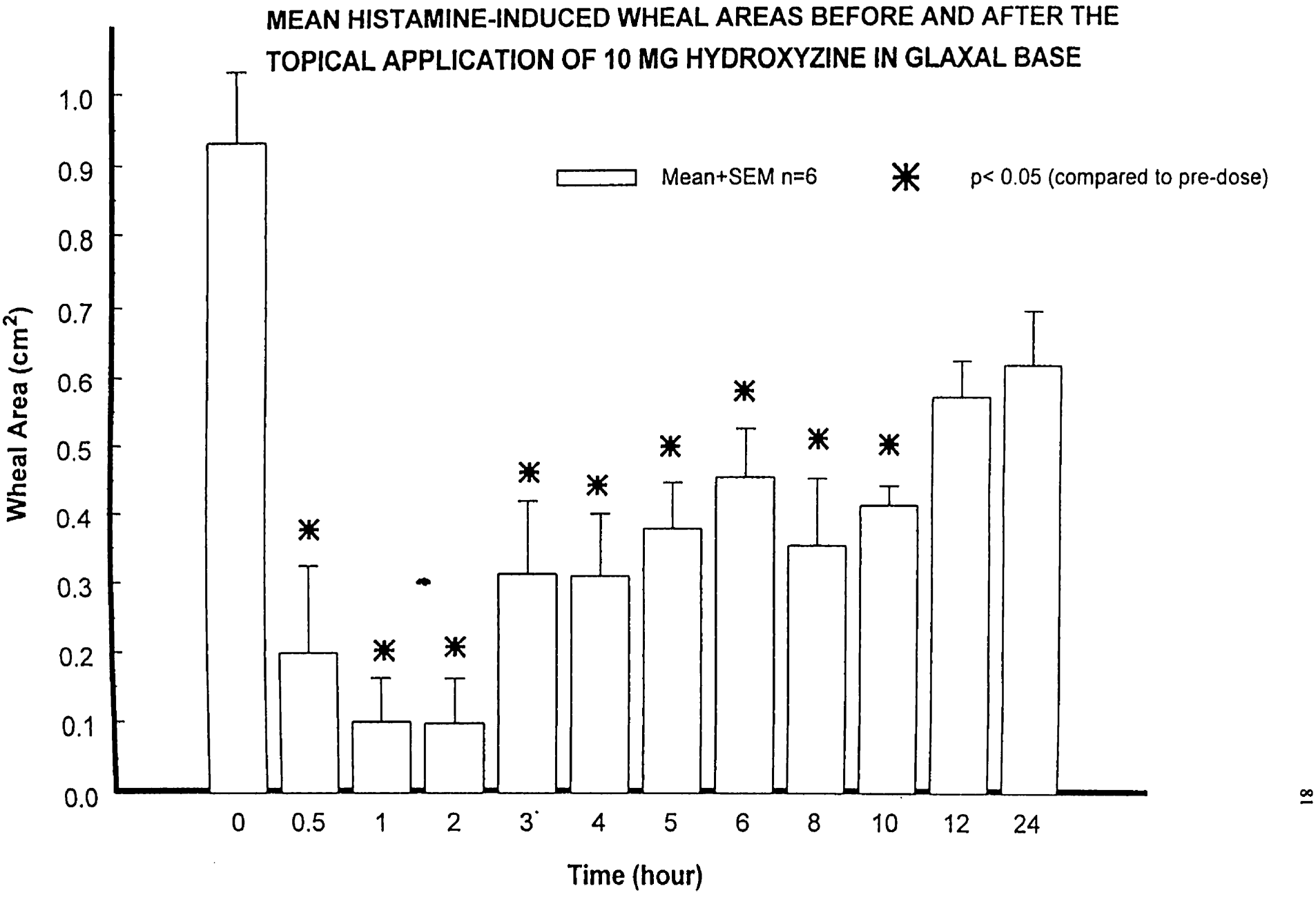
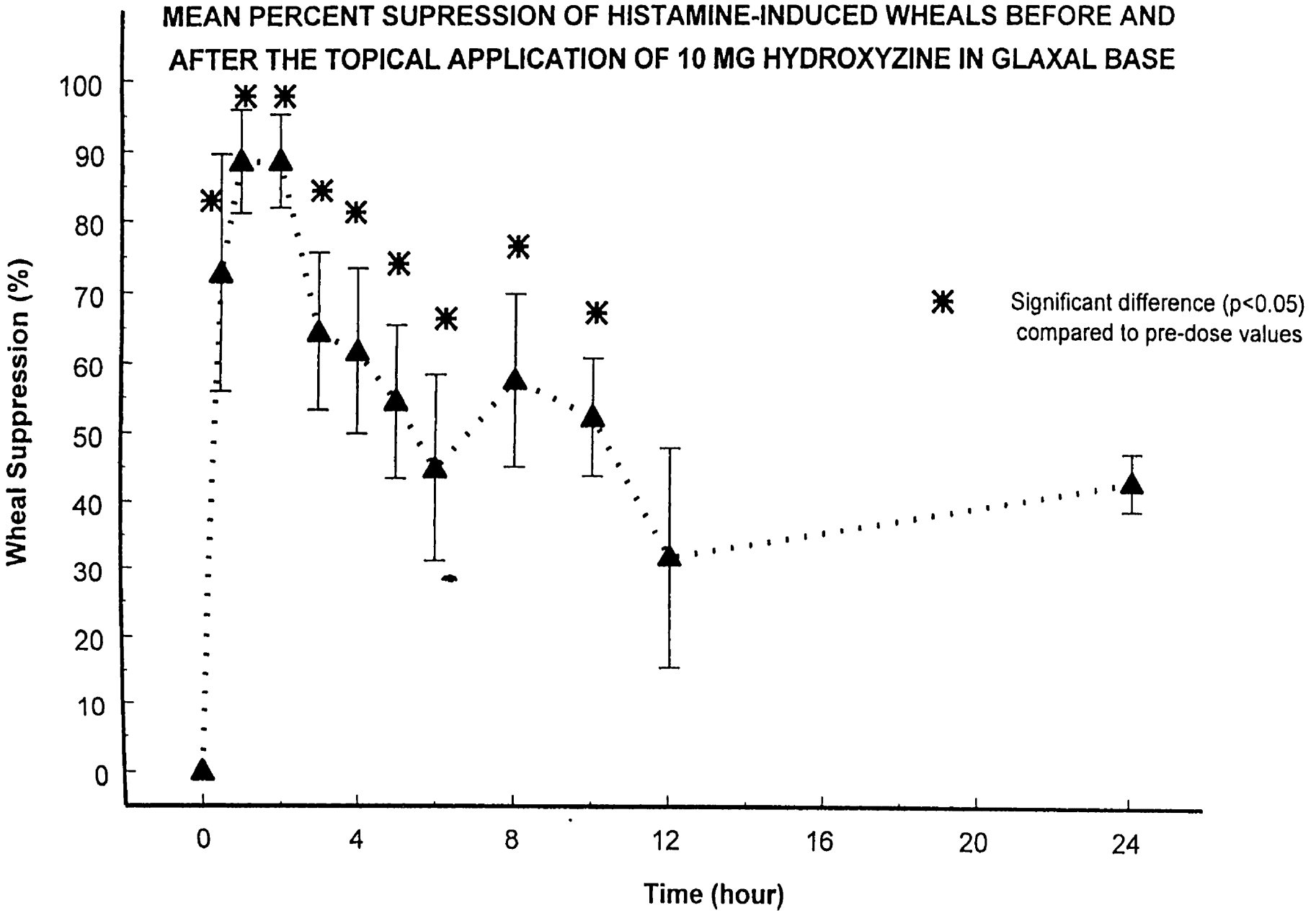

MEAN HISTAMINE-INDUCED WHEAL AREAS BEFORE AND AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN GLAXAL BASE
Mean+SEM n=6
p< 0.05 (compared to pre-dose)
Wheal Area (cm2)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
0.5
1
2
3
4
5
6
8
10
12
24
Time (hour)

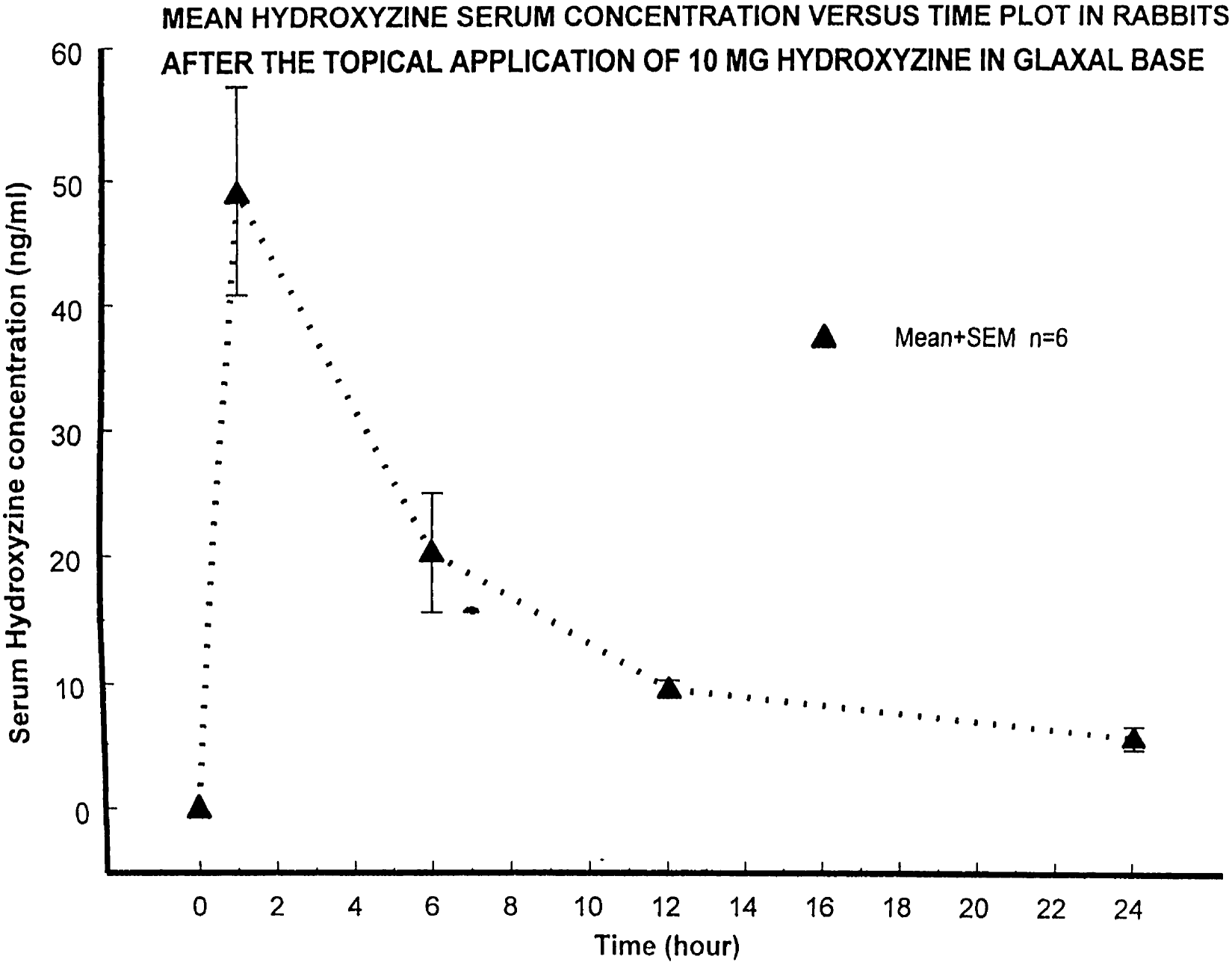
Figure 13


MEAN PERCENT SUPRESSION OF HISTAMINE-INDUCED WHEALS BEFORE AND AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN GLAXAL BASE
Wheal Suppression (%)
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Time (hour)
Significant difference (p<0.05) compared to pre-dose values

SUBJECT	1	2	3	4	5	6	MEAN	S.D.
TIME (hr)								
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	75.3	46.2	20.2	35.7	65.7	50.3	48.9	19.9
6	13.7	28.1	9.5	10.1	38.7	21.1	20.2	11.5
12	10.1	7.1	7.8	9.8	11.2	11.1	9.6	1.7
24	6.5	9.8	4.1	3.7	3.5	5.7	5.6	2.4

Table 13. Serum concentrations (ng) of hydroxyzine after the application of 10 mg hydroxyzine in Glaxal Base.

Figure 14


MEAN HYDROXYZINE SERUM CONCENTRATION VERSUS TIME PLOT IN RABBITS
AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN GLAXAL BASE
Serum Hydroxyzine concentration (ng/ml)
60
50
40
30
20
10
0
Mean+SEM n=6
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hour)

3.5.4. Comparison of Wheal Data

Mean wheal areas at each time were compared to pre-dose values for each hydroxyzine formulation and the mean wheal areas of each hydroxyzine formulation were compared to each other at corresponding times using ANOVA and the Tukey and Bonferroni multiple range tests with differences being considered significant at $p<0.05$. These statistical tests have been used previously and the validity has been proven in several other studies (43.44).

The mean wheal areas before and after the topical application of 10 mg hydroxyzine in MLV liposomes. in EIM liposomes and in Glaxal Base are plotted against time and shown in Figure 15.

The efficiency tests provide some interesting results. Both liposome formulations resulted in a significantly greater suppression of the wheal area on rabbit back skin compared to pre-dose control values from 0.5 hour to 12 hours after a 10 mg hydroxyzine dose in either formulation. Maximum wheal area suppression was 100±0% at 3 hours for MLV liposomes and was 93.1±10.7% at 3 hours for EIM liposomes. In contrast. Glaxal Base yielded significant suppression only up to 10 hours and reached a maximum suppression of 88.5±16.3% at 2 hours (Figure 15).

There were also significant differences at various times among the three formulations (Figure 15). At 3 hours MLV liposomes yielded 100±0% suppression and EIM liposomes yielded 93.1±10.7% suppression which were statistically significantly better than that of Glaxal Base (64.35±27.4% suppression). At 5 hours MLV liposomes had 83.76±6.2% suppression of wheals and EIM liposomes had 91.85±9.37% which were both statistically significantly superior to the 54.3±27.07%

suppression obtained from the Glaxal Base. In addition EIM liposomes resulted in a significant suppression at 6 and 12 hours compared with Glaxal Base. Both liposome formulations were not significantly different from each other at all times.

These results support the hypothesis that both liposome formulations increased the intensity and prolonged the duration of the peripheral H_1-receptor antagonistic effect of hydroxyzine following topical administration when compared with non-liposomal Glaxal Base.

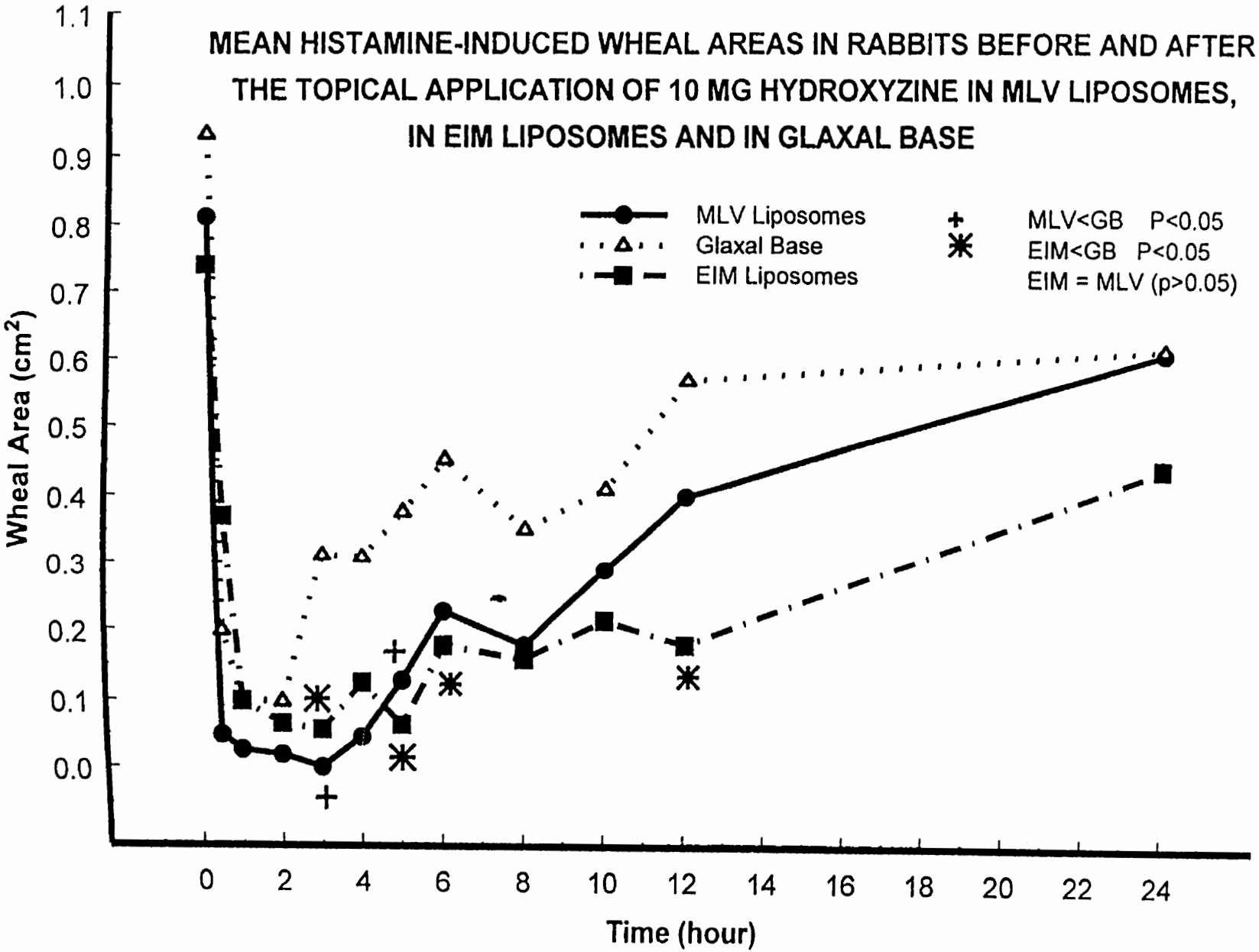
A number of liposomal formulations containing drugs such as econazole, progesterones, local anesthetics and minoxidil have been tested on the skin of animals such as rabbits and guinea pigs in order to determine if the formulations provide a higher concentration in skin layers. The concentration of these drugs achieved in the epidermis and dermis was greater when liposomal formulations were used when compared to conventional topical dosage forms of these drugs.

In one study, Foldvari et al (38) assessed the efficacy of liposome-encapsulated lidocaine in human volunteers compared to a control, lidocaine in Dermabase® cream. The pin-prick test was used to assess pain intensity immediately after the removal of the sample at baseline and at 0.5 hour, 1 hour, 2 hours and 3 hours afterwards. The differences between the liposome dosage form and the cream were statistically significant at every time point except at baseline.

The anaesthetic effect produced by liposome formmulation was significantly longer than that from the cream formulation. Four hours after the removal of the preparations, the effect of liposomal administered lidocaine was still about two fold greater than the conventional dosage form.

In addition, autoradiographic studies demonstrated a higher concentration of ^{14}C-lidocaine localized in the epidermis and the dermis of guinea pigs treated with liposomal lidocaine compared to lidocaine from the Dermabase cream. This study provides evidence for the site of localization and bioavailability of liposomally entrapped drugs. This was also supported by the data from this topical hydroxyzine study, as both liposome formulations yielded a longer duration of wheal suppression and higher efficacy at some test points compared to conventional cream formulation.

Figure 15


MEAN HISTAMINE-INDUCED WHEAL AREAS IN RABBITS BEFORE AND AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN MLV LIPOSOMES, IN EIM LIPOSOMES AND IN GLAXAL BASE
MLV Liposomes
Glaxal Base
EIM Liposomes
MLV<GB P<0.05
EIM<GB P<0.05
EIM = MLV (p>0.05)
Wheal Area (cm2)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hour)

3.5.5 Comparison of Serum Data

The mean hydroxyzine concentration versus time plots after the topical application of 10 mg hydroxyzine in MLV liposomes, in EIM liposomes and in Glaxal Base are shown in Figure 16.

The maximum concentration of hydroxyzine in MLV liposomes at 1.0 hour was 12.8±11.4 ng/ml, significantly lower than EIM liposomes and Glaxal Base, which yielded hydroxyzine serum concentration at 1.0 hour of 45.4±14.8 ng/ml and 48.9±19.9 ng/ml respectively. There was no significant difference between the EIM liposome and Glaxal Base serum hydroxyzine concentrations at this time. At other times, serum hydroxyzine concentrations were not significant from any of the three different formulations. The calculation of routine pharmacokinetic parameters was not possible due to an insufficient number of samples collected during the late elimination phase.

The lower concentration of hydroxyzine detected in the blood after the topical application of MLV liposomes is probably due to the intrinsic action of MLV liposomes acting as a reservoir in skin strata and releasing the drug slowly over time into the skin. EIM liposomes have a much smaller size of liposome vesicles compared to MLV. Hence EIM liposomes might penetrate deeper into the skin layers and come in contact with blood vessels in the dermis resulting in a higher serum concentration.

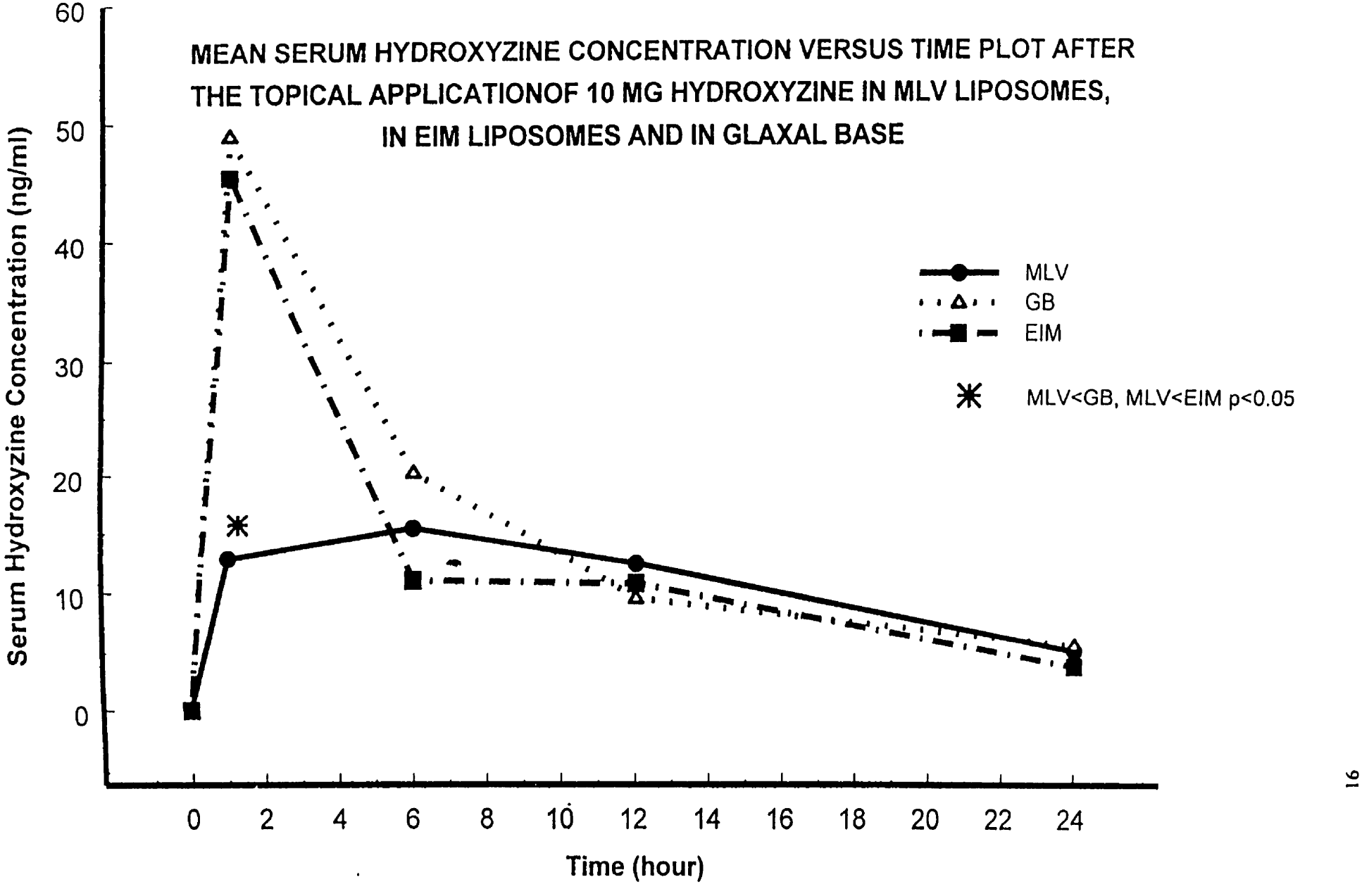
In contrast, with the use of conventional vehicles like Glaxal Base there is no effective way to limit or control percutaneous absorption by the dosage form.

Hydroxyzine in Glaxal Base penetrated the skin readily and was quickly removed by the blood and/or lymph systems, that resulted in a higher serum concentration.

In one study, Kim et al (39) demonstrated that hydrocortisone prepared in a liposome gel and applied to the back of hairless mice with normal skin and *stratum corneum*-removed skin at a dose of 1 mg, resulted in a reduced loss of hydrocortisone from the skin, compared with a conventional ointment formulation. Drug concentrations in the viable skin were maintained at a nearly constant level for over 8 hours by applying a liposome gel formulation. As a result, 5 fold higher viable skin drug concentrations were obtained from liposomes than from a conventional ointment. The plasma level of hydrocortisone at 4 hours from liposome gel was only 25% of that obtained from the ointment, when the drug was applied to the *stratum corneum*-removed skin.

In the present study, similar results were obtained as serum hydroxyzine concentrations were significantly lower at 1 hour after the administration of MLV liposomes compared with either Glaxal Base or EIM liposomes. Both EIM liposomes and Glaxal Base resulted in high serum concentrations at 1 hour. These serum values were similar to the values obtained in human volunteers following an oral dose of 40 mg of hydroxyzine. Serum hydroxyzine concentrations of this magnitude resulted in noticeable sedation and other CNS adverse effects in these subjects (45).

Figure 16


MEAN SERUM HYDROXYZINE CONCENTRATION VERSUS TIME PLOT AFTER THE TOPICAL APPLICATIONOF 10 MG HYDROXYZINE IN MLV LIPOSOMES, IN EIM LIPOSOMES AND IN GLAXAL BASE
Serum Hydroxyzine Concentration (ng/ml)
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hour)
MLV
GB
EIM
MLV<GB, MLV<EIM p<0.05

3.5.6 Correlation Between Wheal Suppression and Serum Concentration

Mean histamine-induced wheal suppression and serum hydroxyzine concentrations in rabbits after the topical application of 10 mg hydroxyzine in MLV liposomes, in EIM liposomes and in Glaxal Base are shown in Figures 17, 18 and 19 respectively.

Both liposomal formulations resulted in significant wheal suppression at 1, 6 and 12 hours compared with pre-dose values while Glaxal Base had significant wheal suppression at 1 and 6 hours only. Corresponding serum concentrations were significantly lower at 1 hour for MLV liposomes compared with Glaxal Base or EIM liposomes. These results indicate that MLV liposome formulation increased and prolonged the duration of the effect of hydroxyzine following topical administration but resulted in the lowest serum concentration of hydroxyzine as detected in the serum by HPLC.

In clinical practice MLV liposomal delivery of hydroxyzine may prove to be of great importance. Topical application provides significant wheal suppression for up to 12 hours and minimal systemic absorption. This should reduce the incidence of systemic adverse effects, particularly sedation.

The fate of topically applied liposomes and the mechanism of penetration into the skin are complex questions which are still under study by several groups. Very few reports about the interaction between lipid vesicles and human skin have been published.

The interaction of liposomes with encapsulated G-gold conjugate in a gel vehicle in the skin has been studied (40). It was found that liposomal size controlled

the extent of penetration of liposomes through the skin. Liposomes up to 600 nm in diameter readily penetrated the *stratum corneum* compared to liposomes > 1000 nm which showed a localization in the *stratum corneum* with the main penetration proceeding along the hair sheath.

In another study, Natsuki et al (41) compared two different liposome samples prepared respectively by sonication and ethanol injection methods containing radiolabeled α-tocopherol. They found that the penetration of the tocopherol into hairless rat back skin was higher from liposomes compared to free tocopherol, and it was higher from sonicated liposomes compared to ethanol injection liposomes. Neither liposomes nor encapsulated tocopherol was detected in the plasma. However, the degree of penetration was dependent on the size of liposomes since sonicated liposomes have a smaller size distribution compared with ethanol injection liposomes.

Dimyristoyl phosphatidyl choline (DMPC) liposomes and their interaction with human *stratum corneum* was investigated by confocal laser scanning microscopy and differential scanning calorimetry (42). It was found that DMPC liposomes do not penetrate intact into the skin and tend to disintegrate at the surface of *stratum corneum*. The lipid molecules then interact with the lipid barrier of the *stratum corneum*. However, to realize the full potential of liposomes in the topical application of drugs it is necessary to investigate the mechanism and the route of penetration of intact liposomes and the several factors that might influence the penetration process and the release of encapsulated drugs.

EAN PERCENT SUPPRESSION OF HISTAMINE-INDUCED WHEALS AND SERUM HYDROXYZINE CONCENTRATIONS IN RABBITS AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN MLV LIPOSOMES

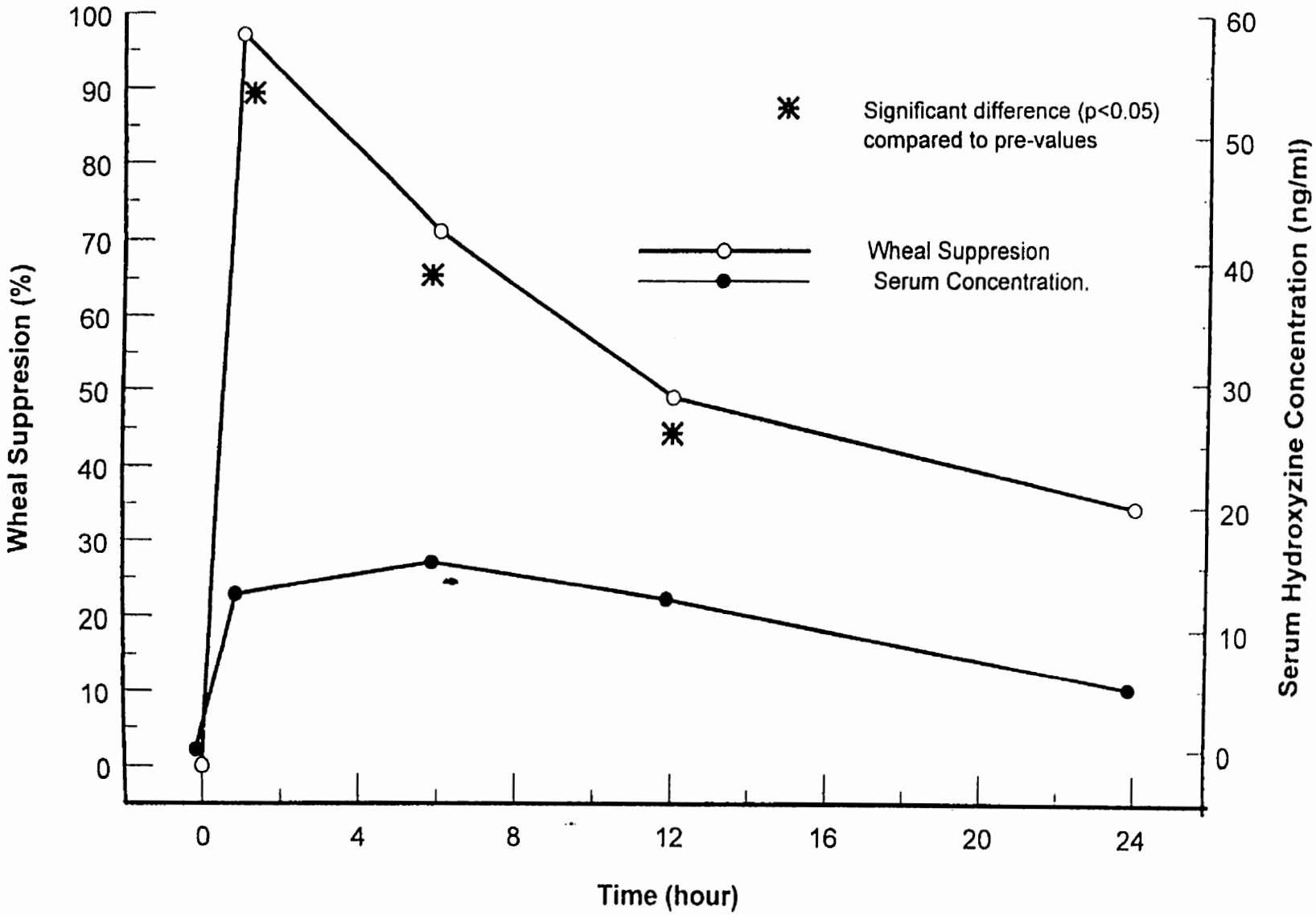


Figure 17

MEAN PERCENT SUPPRESSION OF HISTAMINE-INDUCED WHEALS AND SERUM HYDROXYZINE CONCENTRATION IN RABBITS AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN EIM LIPOSOMES

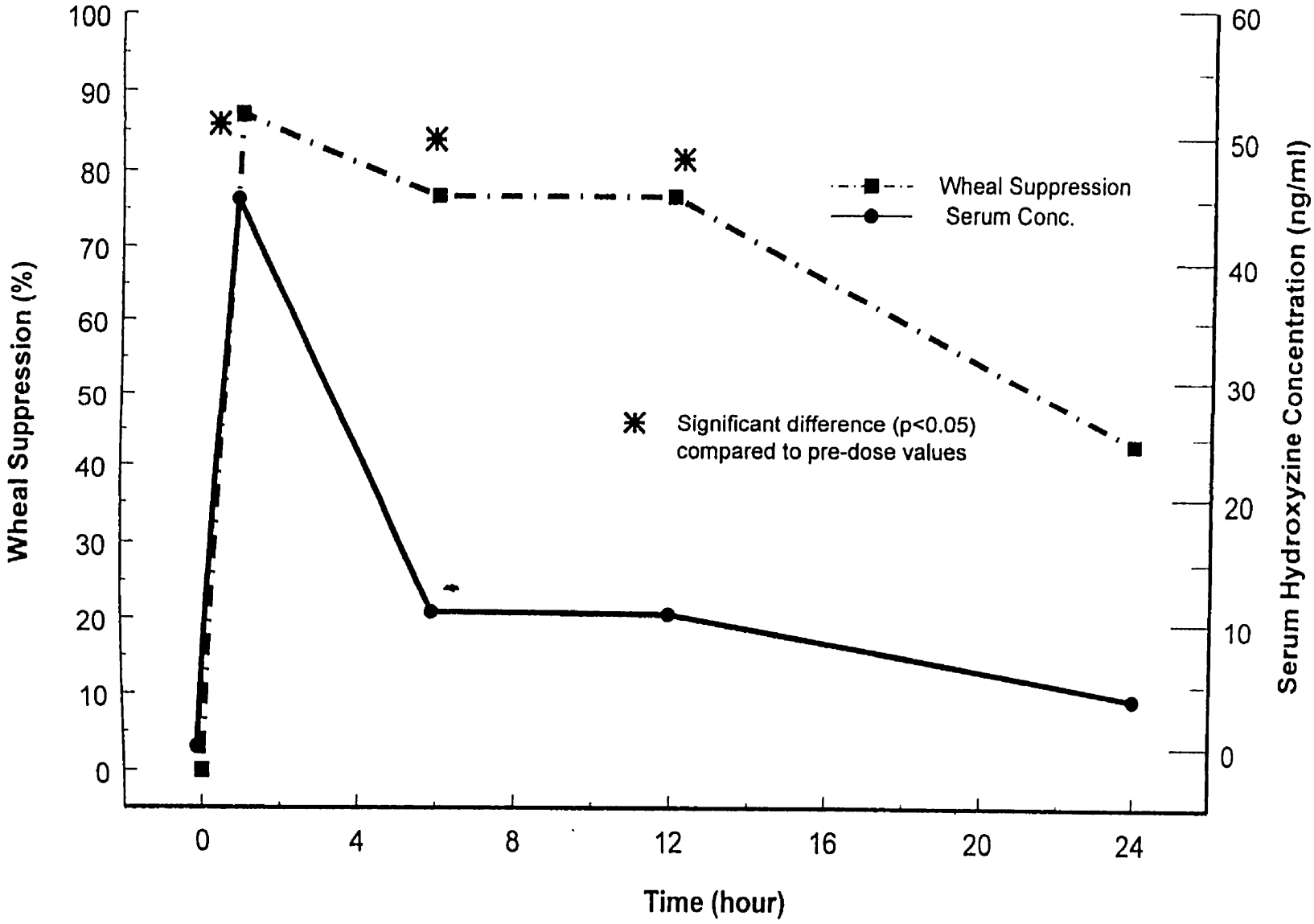


Figure 18

MEAN PERCENT SUPPRESSION OF HISTAMINE-INDUCED WHEALS AND SERUM HYDROXYZINE IN RABBITS AFTER THE TOPICAL APPLICATION OF 10 MG HYDROXYZINE IN GLAXAL BASE

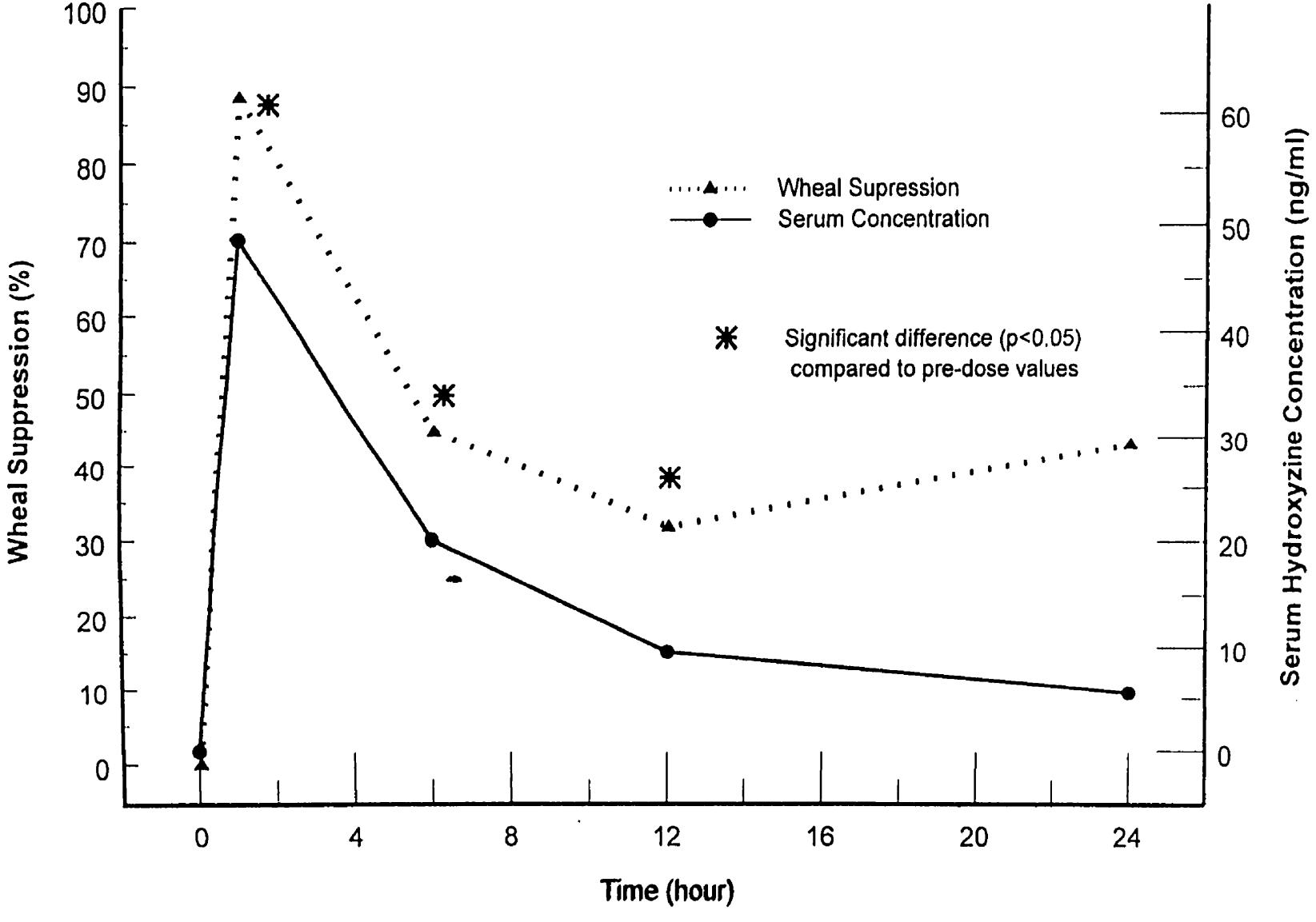


Figure 19

CHAPTER IV

CONCLUSION

The two objectives of this study were, first, to design and formulate different liposome formulations encapsulating hydroxyzine for topical administration; and second, to evaluate the efficacy and the extent of hydroxyzine absorption from two selected liposome formulations compared with a conventional vehicle applied topically to shaved rabbit skin *in vivo*.

The optimum egg phosphatidylcholine to cholesterol ratio was investigated and was found to be 73:23 μmol/ml at a 3:1 molar ratio. Hydroxyzine, one of the most potent antihistamines has two pK_a values at 2.6 and 7. As pH rises more of the non-ionized form is available than the ionized form resulting in an increase in partitioning and trapping in the lipid compartment compared with the aqueous compartment. It was found that 62.5% of hydroxyzine added initially can be encapsulated in the lipid bilayer membrane of liposomes at pH 7.0, with hydroxyzine being added as the dihydrochloride salt after the formation of liposomes by either the ethanol injection method (EIM) or the solvent evaporation method.

Two different liposome formulations were designed, MLV liposomes of a relatively large size (2-7 microns) and EIM liposomes of considerably smaller size (approximately 100 nm). The presence or absence of micelles in these formulations was not studied. These were selected for *in vivo* evaluation in the animal model for comparison with topical hydroxyzine administration from a conventional cream formulation, Glaxal Base.

The second objective of this study was to evaluate the systemic absorption and the peripheral H_1–receptor antagonist efficacy of hydroxyzine after the topical administration on rabbit back skin.

Wheal areas were significantly suppressed from 0.5-12 hours, inclusive, compared to pre-dose values after the topical administration of hydroxyzine in either MLV liposomes or EIM liposomes. In contrast Glaxal Base had a significant suppression from 0.5-10 hours only. Both liposome formulations resulted in significantly higher wheal suppression at 3 and 5 hours compared with Glaxal Base. Furthermore, EIM liposomes suppressed wheal areas significantly better at 6 and 12 hours compared with Glaxal Base.

Serum concentrations were significantly lower at 1 hour after the administration of MLV liposomes compared to either Glaxal Base or EIM liposomes. Both EIM liposomes and Glaxal Base formulations resulted in relatively high serum concentration at 1 hour after either administration.

In conclusion, liposome formulations of hydroxyzine had a better suppression of wheal areas than that of Glaxal Base, particularly at 3.5 and 12 hours. After the topical administration of MLV liposomes, extremely low hydroxyzine serum concentrations were achieved which could reduce the possibility of systemic adverse effects, especially the CNS effects such as sedation and reduced alertness.

The results of this study in rabbits suggest that MLV liposomes applied twice a day may provide excellent suppression of the histaminic inflammatory symptoms with the least possible systemic absorption. Adverse CNS effects may also be minimized.

Further studies are needed to evaluate the extent of penetration of MLV and SUV liposome products into the dermal layers and the mechanism of drug release from the formulations. The results reported in this thesis provide some good basic comparisons that may be used to design future studies.

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IMAGE EVALUATION
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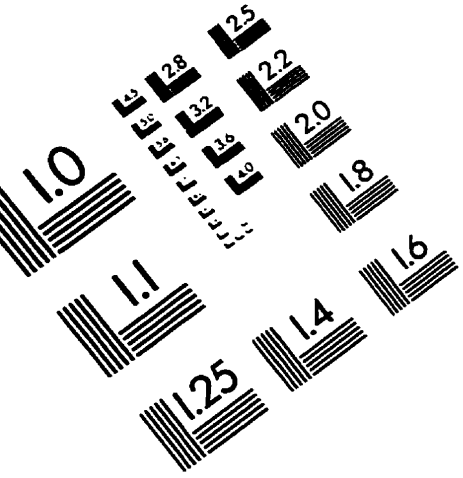

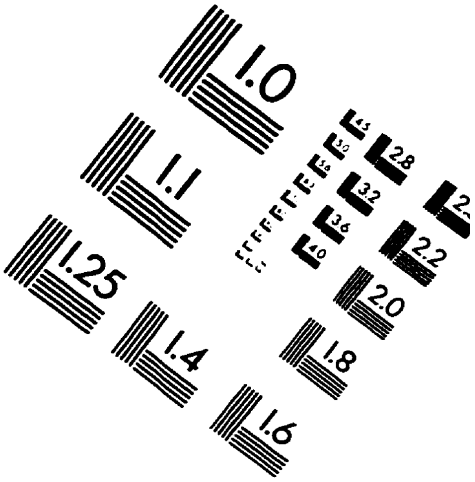

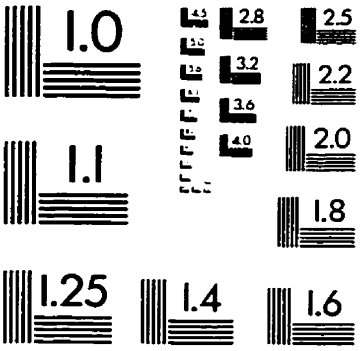

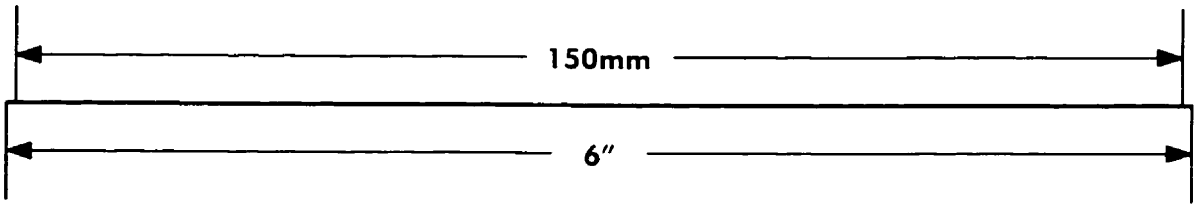

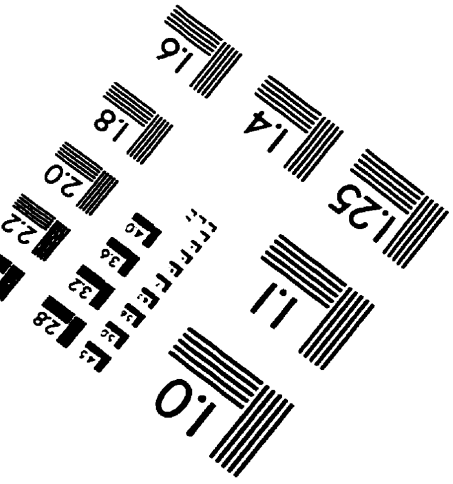

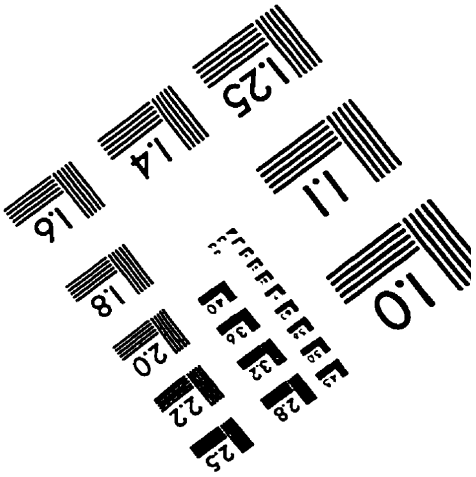

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