



ARI KARPPINEN

Antihistamines in the Treatment of Mosquito-Bite Allergy



ACADEMIC DISSERTATION

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for public discussion in the small auditorium of Building B,
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To my family

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Abbreviations

BID	twice daily
CD	cluster of differentiation
ELISA	enzyme-linked immunosorbent assay
H ₁ receptor	histamine 1 receptor
IgE	immunoglobulin E
IgG	immunoglobulin G
kDa	kilodalton
TID	three times daily

List of original publications

This thesis is based on the following original papers referred to in the text by their Roman numerals I-V.

- I. Reunala T, Brummer-Korvenkontio H, Karppinen A, Coulie P, Palosuo T. Treatment of mosquito bites with cetirizine. *Clin Exp Allergy* 1993;23:72-5.
- II. Karppinen A, Petman L, Jekunen A, Kautiainen H, Vaalasti A, Reunala T. Treatment of mosquito bites with ebastine: a field trial. *Acta Derm Venereol* 1999;79:1-3.
- III. Karppinen A, Kautiainen H, Petman L, Brummer-Korvenkontio H, Reunala T. Loratadine in the treatment of mosquito-bite-sensitive children. *Allergy* 2000;55:668-71.
- IV. Karppinen A, Kautiainen H, Petman L, Burri P, Reunala T. Comparison of cetirizine, ebastine and loratadine in the treatment of immediate mosquito-bite allergy. *Allergy* (in press) .
- V. Karppinen A, Rantala I, Vaalasti A, Palosuo T, Reunala T. Effect of cetirizine on the inflammatory cells in mosquito bites. *Clin Exp Allergy* 1996; 26: 703-709.

A. INTRODUCTION

Mosquitoes are important vectors of many tropical diseases such as malaria and yellow fever. Even in temperate zones mosquitoes may transmit diseases to man. In Finland, examples include Pogosta disease caused by the sindbis virus and bacterial tularaemia (Lundström 1999). In addition to being disease vectors, mosquitoes cause much nuisance by attacking man in dense hordes in many areas in the world. Coastal marshes and lake areas can harbour plenty of *Culex* mosquitoes, whereas forested areas in the northern hemisphere are inhabited mainly by a different *Aedes* species (Peng et al. 1998). When feeding, mosquitoes probe the skin with their mouth parts, inject saliva into the skin and intake blood. In man the skin reacts to the bites by whealing and also with delayed bite papules. The bite reactions are dependent on mosquito saliva which contains biologically active components such as anticoagulants and anaesthetic compounds (McKiel and West 1961b). Whealing was once claimed to be caused by the histamine present in mosquito saliva (Eckert et al. 1951), but Wilson and Clements (1965) showed that histamine cannot be present in the salivary secretions at a sufficiently high concentration to cause whealing. Today it is known that mosquito-bite whealing is an allergic reaction caused by antisaliva IgE antibodies and histamine released from the mast cells into the skin (Brummer-Korvenkontio et al. 1994, Chen et al. 1998, Peng et al. 1996 a, Reunala et al. 1994b). Development of delayed bite papules is also dependent on previous sensitisation. However, the exact pathomechanism of these reactions is not known (Brummer-Korvenkontio et al. 1990, Peng and Simons 1998).

In Finland the mosquito season is short, but during this season people are regularly exposed to variable numbers of bites depending on the place of living and outdoor habits. The bite reactions tend to be more intense at the onset of the mosquito season. About 10% of the people suffer from abnormally large or long-lasting bite reactions (Reunala et al. 1994a, Palosuo et al. 1997). Effective mosquito repellents and a few pruritus-relieving over-the-counter sticks containing e.g. antihistamines are generally available for people suffering from mosquito bites (Fradin 1998). However, no efficient drug therapies have earlier been known for people seeking medical advice for their disturbing mosquito-bite reactions.

Several second-generation antihistamines, including cetirizine, ebastine and loratadine, have been approved for drug use during recent years. These H1 blockers are commonly used in the treatment of perennial and seasonal rhinitis, allergic conjunctivitis and urticaria (Simons and Simons 1993). Two preliminary studies suggested that cetirizine may also affect mosquito-bite whealing and associated pruritus (Coulie et al. 1989, Reunala et al.1991). The present study was, therefore, carried out to extend the observations made on the effect of cetirizine on the immediate, histamine-mediated mosquito-bite reactions. A second goal was to study whether cetirizine could have a clinical effect also on the harmful delayed bite papules and whether eosinophils could be the target inflammatory cells in these reactions (Charlesworth et al. 1989). Thirdly, studies were performed also with ebastine and loratadine antihistamines and, finally, with all the three antihistamines together in order to find the best treatment for mosquito-bite-sensitive subjects.

B. REVIEW OF THE LITERATURE

1 Antihistamines

1.1 Classification and pharmacokinetics

Antihistamines are histamine H₁-receptor antagonists that have been used in various allergic disorders for over 50 years (Simons and Simons 1993; Fig. 1a-d). Guanine nucleotide-binding (G), protein-linked H₁ receptors are expressed especially on the vascular endothelium in the skin and mucosal surfaces, in the gastro-intestinal and bronchial smooth muscles and in the brain. The H₁-receptor genome has been cloned, and the receptor protein deduced from the nucleotide sequence of the gene is composed of 487 amino acid residues. First-generation antihistamines, e.g. difenhydramine and hydroxyzine, have in addition to the H₁-antagonistic also anticholinergic, alpha-adrenergic and anti-5-hydroxytryptaminic effects (Baroody and Naclerio 2000). Second-generation antihistamines are more specific H₁ antagonists that have been in clinical use for over 10 years. Structurally they bear some resemblance to histamine as both contain an ethylamine group (Fig. 1a-d). They penetrate poorly the blood-brain-barrier and therefore have less central nervous system effects than first-generation antihistamines. Terfenadine and astemizole were the first to be approved for drug use in Finland, and cetirizine, acrivastine, loratadine and ebastine were approved later (Fig. 1a-d). Except for acrivastine, these second-generation antihistamines have a long elimination half-life, and therefore they can be administered once a day (Table 1). A group, not yet firmly established, comprise the third-generation antihistamines that are active metabolites or isomers of second-generation antihistamines. These antihistamines include desloratadine, levocetirizine, carebastine and fexofenadine which generally have greater affinity to H₁ receptors than the parent compound and possibly a lower potential for adverse effects.

Histamine H₂ receptors are located on the gastric parietal, cardiac atrial and uterine smooth-muscle cells. H₂-blocking drugs, e.g. ranitidine, are used to control gastric acid secretion in diseases such as reflux oesophagitis or peptic ulcer (McGuigan 1985). Histamine H₃ receptors are involved in the control of histamine synthesis and release and in the inhibition of sympathetic neurotransmission in perivascular nerves (Simons and Simons 1993). They occur both in the nervous system and on

peripheral neurons of the gastrointestinal and bronchial tracts (Malinowska et al. 1998). They may play a role in the pathophysiology of headache and cardiac ischemia. Recently, three functional H₃-receptor isoforms (H₃A, H₃B and H₃C) have been identified in rats (Drutel et al 2001). H₄ receptors occur in the bone marrow and on eosinophils but today no antagonists are in clinical use (Liu 2001).

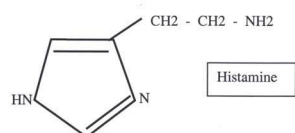


Fig. 1a. Chemical structure of histamine.

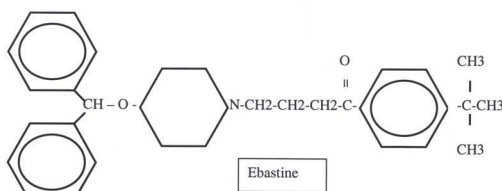


Fig. 1c. Chemical structure of ebastine.

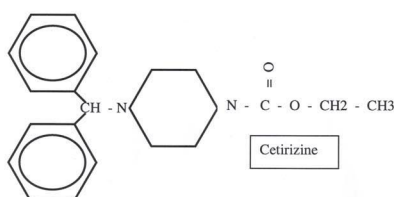


Fig. 1b. Chemical structure of cetirizine.

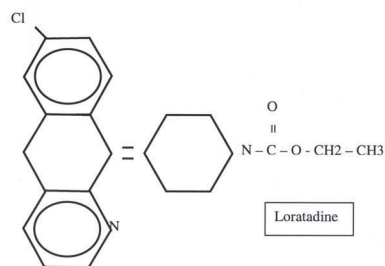


Fig. 1d. Chemical structure of loratadine.

Table 1.

The pharmacokinetic characteristics of second- and third-generation antihistamines.

Drug	t _{1/2} *	Metabolism or elimination	Daily dose
<u>Second generation</u>			
Acrivastine	1.4-2.1	Unchanged, urine	8 mg TID
Cetirizine	7-10	Unchanged, urine	10 mg
Ebastine	13-16	CYP3A4	10 (20) mg
Loratadine	7.8-11	CYP3A4, CYP2A6	10 mg
Mizolastine	8-13	CYP3A4 (first-pass)	10 mg
Terfenadine☼	16-23	CYP3A4	60 (120)mg; 60 mg BID
<u>Third generation</u>			
Desloratadine	17.3-24	Urine	5 mg
Fexofenadine	11-15	P-glycoprotein excretion in the gut (80%)	120 mg

*t_{1/2}=elimination half-life in hours

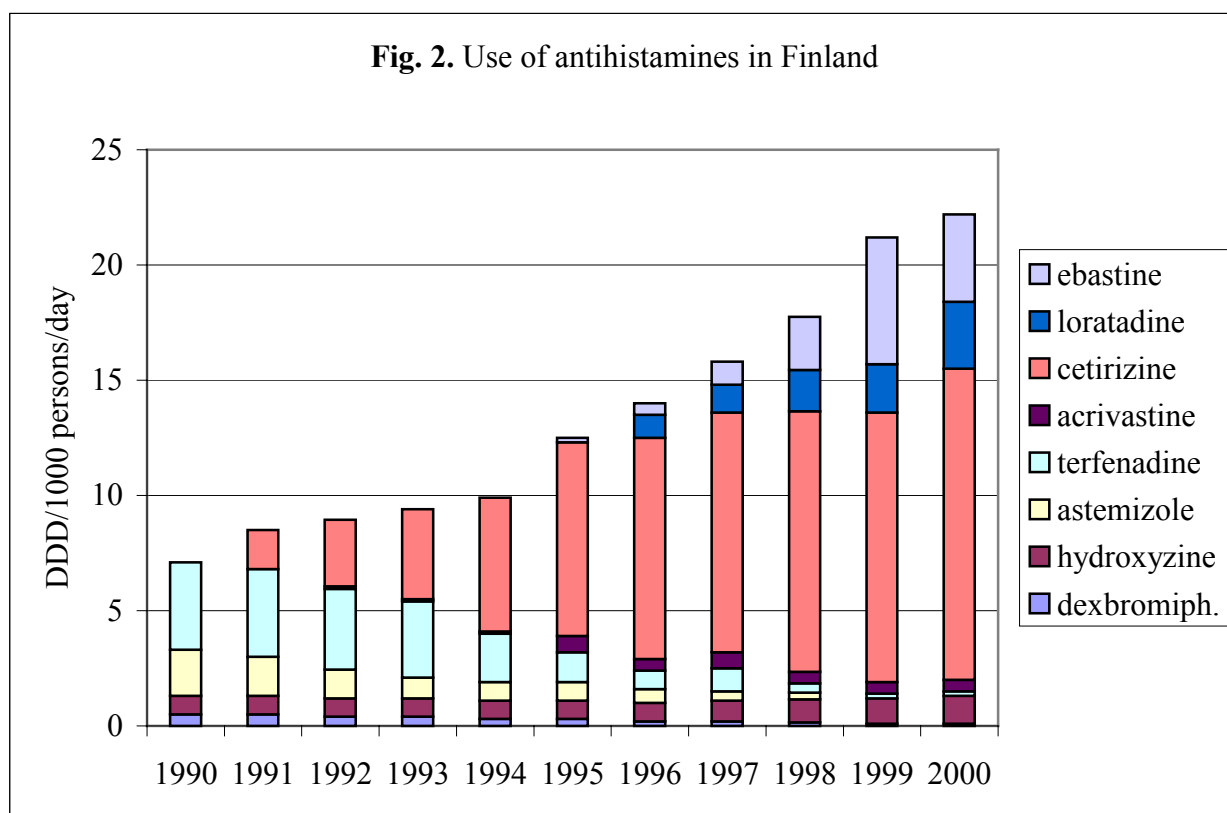
☼active metabolite carboxyterfenadine

CYP= cytochrome P-450

1.2 Clinical use

In IgE-mediated allergic reactions the effects of histamine are rapidly seen as wheal-and-flare reactions in the skin, mucosal swelling in the nose and eyes and wheezing in the lungs. Oral antihistamines are, therefore, widely used in the treatment of allergic disorders such as seasonal and perennial rhinitis, conjunctivitis and urticaria. In Finland there has been a threefold increase in the use of antihistamines in the 1990s (Paakkari 2001; Fig. 2).

In perennial or seasonal rhinitis, orally administered second-generation antihistamines are not as potent as intranasal steroids, but they relieve the majority of nasal symptoms (Foresi 2000, Berlin et al. 2000). Mizolastine and desloratadine have been shown to improve also nasal congestion (Scadding et al. 1999, Bachert 2001). In order to provide relief of nasal congestion, antihistamine preparations with decongestants such as pseudoephedrine are also on the market (Simons and Simons 1993).



From Paakkari (2001)

In various forms of urticaria, oral antihistamines are first-line medication, and a good treatment response is achieved by nearly half of the patients (Simons and Simons 1993, Humhreys and Hunter 1998). Three comparative studies performed with cetirizine 10 mg and astemizole 10 mg, ebastine 10 mg and terfenadine 60 mg BID, and loratadine 10 mg and mizolastine 10 mg showed a similar efficacy (Breneman et al. 1995, Kalis 1996, Leynadier 2000). One urticaria study showed that cetirizine 10 mg was more effective than terfenadine 60 mg BID (Andri et al. 1993). Overall, the treatment responses to different antihistamines in urticaria vary between patients (Grattan et al. 2001). In resistant cases, addition of an H₂ antagonist may also help to control symptoms.

The coexistence of allergic rhinitis and asthma is common. Because antihistamines are often administered in rhinitis, their effects on asthma have also been evaluated. However, in clinical or experimental trials, second-generation antihistamines exhibited only a modest effect on bronchospasm induced by histamine, cold air, exercise or allergens (Larsen 2001). Therefore, second-generation antihistamines do not play a role as a single agent in the treatment of asthma. Interestingly, long-term use of cetirizine in young atopic children in the ETAC study (1998) prevented the development of asthma in half of the children sensitised to aeroallergens. Ketotifen prevented effectively the onset of asthma in genetically predisposed children (Bustos et al. 1995) and decreased the number of days that antiasthma drugs were required by asthmatic children (Kabra et al. 2000).

The first-generation antihistamine hydroxyzine has long been used in the treatment of pruritus in atopic dermatitis (Simons and Simons 1993). The therapeutic effect has been thought to be due to sedation. The effect could also be partly due to its active metabolite cetirizine since the antipruritic effect continues although the serum concentration of hydroxyzine is nearly zero. A study by Hannuksela et al. (1993) in adults with atopic dermatitis showed that cetirizine 20 mg, but not 10 mg, alleviated significantly pruritus, and that cetirizine 40 mg improved also the rash. A similar effect of cetirizine could be shown also in a study in children with atopic dermatitis (LaRosa et al. 1994). The relief of pruritus in higher cetirizine doses could be due to its sedative effect, and the subsequent diminishment of scratching may then have healed the dermatitis (Zuberbier and Henz 1999). It has also been speculated whether the effect of cetirizine in atopic dermatitis could be due to anti-inflammatory mechanisms. In agreement with this view, earlier *in vitro* and *in vivo* studies showed that cetirizine could inhibit eosinophil migration and decrease cutaneous late-phase reactions (Fadel et al. 1987, Charlesworth et al. 1989, Snyman et al. 1994).

In addition to oral administration in rhinoconjunctivitis, antihistamines can be used also topically. Azelastine nasal spray has been shown to be as effective as oral ebastine, loratadine, cetirizine or terfenadine in the treatment of allergic rhinitis (Golden and Graig 1999). Azelastine and levocabastine eye drops are effective in allergic conjunctivitis (Richard et al. 1998, Friedlaender et al. 2000).

1.3 Adverse effects

Terfenadine and astemizole share with old antihistamines the effect on the cardiac conduction tissue by blocking Ether-a-go-go-Related Gene (ERG) potassium channels, causing prolongation of the QTc interval (Mattila and Paakkari 1999). This can lead to serious ventricular arrhythmia, torsades de pointes, particularly in subjects with a pre-existing long QTc interval (e.g. the congenital QT syndromes Jervell & Lange-Nielsen and Romano-Ward). The risk is further increased by liver disease, hypokalaemia, overdose of these antihistamines and CYP3A4 blockers such as erythromycin, ketoconazole and itraconazole. Astemizole is now withdrawn from the market and terfenadine is not an over-the-counter drug anymore in Finland. Ebastine, loratadine and mizolastine, but not acrivastine or cetirizine, have also potential for cardiac arrhythmias, but the risks seem to be low when using recommended daily doses (Hurst 2000, Kosoglou 2000).

Most first-generation antihistamines penetrate the blood-brain barrier and cause sedation that is potentiated by alcohol (Simons and Simons 1993). Newer antihistamines have a low relative lipid solubility that reduces or slows down their penetration through the blood-brain barrier (Mattila and Paakkari 1999). These antihistamines have also a low relative affinity for H₁ receptors in the central nervous system compared with peripheral receptors, which also reduces the incidence of sedation. In practice, however, certain sensitive patients may experience drowsiness or impaired performance while using usual drug dosages. Cetirizine 10 mg may cause sedation, but it has not impaired driving performance or alertness in traffic (O'Hanlon and Raemakers 1995). Higher than normal daily antihistamine dosages are needed to control symptoms in some individuals, and in such instances sedation can appear with several-second generation antihistamines (Mattila and Paakkari 1999). Recently, Mann et al. (2000) showed in the post-marketing surveillance study that the risk of sedation is threefold to fourfold with cetirizine or acrivastine compared with loratadine or fexofenadine.

2 Mosquitoes and mosquito-bite reactions

2.1 Mosquitoes

Mosquitoes are insects belonging to the order diptera in the Culicidae family, and over 3000 species from more than 40 genera have been described (Brummer-Korvenkontio 1996). Although 37 species from five genera are found in Finland, over 80% of man-biting mosquitoes in June-July belong to the *Aedes communis* and *Aedes punctor* species. Female mosquitoes lay their eggs in a moist or wet place where the larvae hatch out from the eggs in two weeks. After one to two weeks the larvae develop into mosquitoes through metamorphosis. Mosquitoes are met around the year in tropical rainforests or salty coastal marshes and in temperate zones, e.g. Nordic countries and the northern part of the USA and Canada, mosquitoes are met mostly during summer time.

Exogenous blood is needed only by female mosquitoes to complete the development of their ovaries (Brummer-Korvenkontio 1996). Temperature, carbon dioxide, smell, moisture, colour and movement attract mosquitoes into the neighbourhood of the victim. Mosquitoes secrete several substances into their saliva, including saliva proteins, which can cause harmful pruritic reactions to sensitised people or animals. Moreover, mosquitoes can transmit a variety of diseases like malaria, yellow fever and arbovirus encephalitis (Lundström 1999).

2.2 Mosquito-bite reactions in man

Reactions to mosquito bites can be classified as immediate or delayed, depending on the time of onset of symptoms (McKiel 1959).

2.2.1 Immediate reaction

An immediate reaction appears in a few minutes after the bite and reaches its maximum in 15-30 minutes in man (McKiel 1959). It consists of a soft, pale wheal usually surrounded by erythema (flare) and accompanied by pruritus (Table 2). Usually it disappears within an hour. Some people experience abnormally large, often even painful local swelling around the bite. Generalized urticaria, asthma or anaphylaxis are rare, but there are, however, some case reports of anaphylactic reactions from mosquito bites (McCormack et al. 1995, Hassoun et al.1999). An immediate cutaneous reaction after a mosquito bite exhibits clinical features of Hymenoptera venom allergy

but is generally much milder (Annala 1999). Both reactions start within minutes and include local subcutaneous swelling accompanied by itch. In contrast to Hymenoptera allergy, large local swelling is not very common after mosquito bites.

Table 2. Clinical types of mosquito-bite reactions

<u>Immediate bite reaction</u>	
● Wheal and flare	Very common
● Large swelling	Uncommon
● Anaphylaxis	Very rare
<u>Delayed bite reactions</u>	
● Pruritic papules	Very common
● Pustular or hemorrhagic lesions	Rather common
● Papular urticaria	Common in children
● Blisters or bullae	Rare
● Erythema multiforme or purpura	Rare

2.2.2 Delayed reaction

A delayed reaction appears several hours after the bite and consists of a rather hard, raised papule (McKiel 1959). Frequently erythema surrounds the papule, the accompanying pruritus may be severe and the reaction tends to last several days (Table 2). There is large interindividual variation, from a few millimetres to many centimetres, in the size of the bite papules. Sometimes the reaction is clinically of the Arthus type with pustular, haemorrhagic or even necrotic lesions possibly accompanied by joint swelling, lymphadenopathy and fever. After repeated bites, especially in children in tropical countries, the older bite sites often flare up, mimicking papular urticaria, also called as strophulus (Reunala et al. 1994b, Reunala 1996). Blisters or large bullae, erythema multiforme and purpura have also been reported (Table 2). It is of interest, that local delayed sting reactions such as swelling or papules may occur also in Hymenoptera allergy (Annala 1999).

3 Mechanism of mosquito-bite reactions

3.1 Immediate bite reactions

There is now convincing evidence that the mosquito-bite wheal is mediated by IgE antisaliva antibodies and histamine in man. Antisaliva IgE antibodies can be demonstrated by immunoblotting or ELISA (Brummer-Korvenkontio et al.1994, Peng et al.1995, Reunala et al. 1994b). In mosquito-bite-sensitive children, IgE antibody levels have been shown to correlate with the size of wheals (Brummer-Korvenkontio et al. 1997). The major allergens in *A. communis* saliva are 36-kDa and 22-kDa proteins (Brummer-Korvenkontio et al. 1997) and in *A. aegypti* saliva a 37-kDa protein (Peng et al. 1998). Confirming *in vivo* reactivity of antisaliva IgE antibodies, the non-reactive recipients develop wheals after passive transfer of antisaliva IgE antibodies (Prausnitz-Küstner test) into their skin (Reunala 1994c). Finally, it has been shown also with the microdialysis technique that histamine is released rapidly into the skin after the bite (Horsmanheimo et al. 1996).

Flare, also called “axon reflex” or “antidromic vasodilatation”, often coincides with the wheal in the immediate mosquito-bite reaction. Unmyelinated sensory nerve fibres are capable of releasing vaso-active neuropeptides, e.g. substance P, from their peripheral terminals when activated by noxious mechanical, chemical or physical stimuli (Chahl 1991). In mosquito-bite reactions, however, the mechanism of flare is probably similar but has not been investigated.

3.2 Delayed bite reactions

The fact that several clinical types of delayed mosquito-bite reactions are expressed suggests that different immune and other mechanisms could be involved in the pathogenesis. A late-phase, type I allergic reaction, depending on IgE antibodies, histamine and leukotriene release from mast cells and subsequent accumulation of neutrophils, eosinophils and T lymphocytes, can be observed 2-4 h after experimental pollen allergen exposure into the skin (Charlesworth et al.1989). Previously, passive transfer experiments with IgE antisaliva antibodies have shown that the recipients develop in addition to 15-min wheals also delayed mosquito-bite reactions lasting up to 6 h (Reunala et al. 1994c) and that also leukotriene C₄ can be released after the bites (Horsmanheimo et al.1996). The cellular reaction involved in this kind of delayed mosquito-bite reaction was not examined. There are, however, a few older studies describing the histological findings in 48-h mosquito-bite reactions in man. Rockwell and Johnson (1952) took biopsies from such lesions and found

infiltrates of macrophages and lymphocytes around the vessels in the dermis and also deep in the subcutaneous fat. They also reported the presence of eosinophils in these lesions, the cells that are regarded as typical histopathological findings in delayed insect bite papules (Melski 1990). The histology of Arthus-type haemorrhagic and necrotic bite lesions showed prominent vasodilatation and necrotizing vasculitis accompanied by neutrophil and eosinophil infiltrations. Moreover, a post-mortem specimen from the kidney of a patient with a severe systemic reaction to mosquito bites showed glomerulonephritis with deposition of C₁q and C₃ (complement component 1q and 3) in the mesangial area of the glomerulus.

There is some *in vitro* data suggesting that type IV cellular immunity could be involved in the delayed mosquito bite reaction. Oka (1989) and Peng et al (1996a) showed with whole-body or salivary-gland mosquito extracts that subjects with delayed bite reactions had increased lymphocyte proliferation responses. Recently, these findings were confirmed by using *A. aegypti* saliva recombinant allergens (Simons and Peng 2001). Exaggerated delayed skin reactions to mosquito bites in association with systemic symptoms including fever are known to occur in patients with various lymphoproliferative disorders such as chronic lymphocytic leukaemia (Weed 1965, Lidén 1977). Recently, similar cases appearing as hydroa vacciniforme-like eruptions with fatal outcome have been described mainly in Japan (Tokura et al. 1990, 1998, Ishihara et al. 1997). Many of these patients had latent or chronic active Epstein-Barr virus infection affecting especially natural killer cells. The reason for the exaggerated mosquito-bite skin lesions in patients developing haematopoietic malignancies often associated with Epstein-Barr virus infection is currently not known.

3.3 Sensitisation and tolerance to mosquito bites

There are many animal and human studies showing that a sensitisation period is necessary prior to the development of skin reactions after mosquito bites. With whole-body extract of the *Anopheles quadrimaculatus* mosquito Dubin et al. (1948) sensitised rabbits to express immediate-type reactions and Hudson et al. (1958) showed that less than 12 *A. aegypti* bites are needed to sensitise rabbits. Allen and West (1962) managed to sensitise guinea pigs with injections of oral secretion of *A. aegypti*, suggesting that the causative factor (or factors) is in the saliva. Brummer-Korvenkontio (1990) demonstrated with *A. aegypti* mosquito that the development of IgG antibodies coincides with the turnup of skin reactions after 40-60 bites.

Mellanby (1946) showed that subjects exposed for the first time to *A. aegypti* mosquitoes were non-reactive. With subsequent bites, delayed skin reactions and, later on, also immediate skin reactions developed. Heilesen (1949) confirmed Mellanby's findings in adults and demonstrated that children never exposed to *A. aegypti* mosquitoes do not react to the first bites, and that delayed skin reactions developed after only four bites in a few days. Later on, Rockwell and Johnson (1952) showed that three of nine newborn infants had no reaction to *A. aegypti* bites and the rest of them had only small delayed papules. Schaffer et al. (1952) tested 30 children for reactions to extracts of mosquitoes, fleas and bedbugs, and concluded that the wheal-and-flare reaction is unusual during the first year of life. Peng et al. (1996a) showed in a cross-sectional study that natural desensitisation developed eventually after long-term exposure to the commonest local mosquito species, *A. vexans*, in Canada. Inverse correlations were found between the size of skin reactions and the number of years lived in Canada. In 1998, Peng et al. showed also in a prospective study with one human volunteer and a rabbit that massive and continuous exposure to mosquito bites eventually leads to desensitisation.

Table 3 summarizes the different stages in the development of mosquito-bite reactions. Non-sensitised, i.e. newborn or others who are not exposed to mosquito-bites, are non-reactive (Stage I). In a few days and after only a few more bites, a delayed reaction appears (Stage II) and in about 1 month and after several dozens of bites also the immediate reaction will develop (Stage III) with subsequent reduction in the latent period preceding the delayed reaction (McKiel 1961b). Continuing exposure to bites, the delayed reaction decreases and disappears (Stage IV) and finally tolerance (Stage V) is achieved along with heavy exposure to bites. Interestingly, these different stages of sensitisation may be valid also in regard to bedbug- or flea-bite reactions (Larrivee 1964).

Table 3.

Development of cutaneous reactions to mosquito bites.

Immunological state	Immediate reaction (15 min)	Delayed reaction (24 h)
Stage I (non-sensitised)	-	-
Stage II	-	+
Stage III	+	+
Stage IV	+	-
Stage V (tolerance)	-	-

From Mellanby (1946), Heilesen (1949) and McKiel (1961b)

4 Treatment of mosquito-bite reactions

4.1 Topical treatment

A variety of topically applied formulations, including calamine lotion, aqueous carbolic acid an alcoholic solution of menthol or topical antihistamines, have been tried for mosquito-bite symptoms, but there are few controlled studies. McKiel et al. (1954) tested antihistamine pyribenzamine (2%) and placebo creams in 146 volunteers. It had, however, no significant effect on the size of wheals, delayed bite papules or even on accompanying pruritus. Previously, difenhydramine cream was on the market as an over-the-counter drug for mosquito bites and other pruritic skin conditions. An antipruritic mosquito-bite-stick containing tripelenamine antihistamine (Etono) is on the market in Finland, but no controlled data exists for this preparation. Recently, Zhai et al. (1998) studied the effect of 3.6% ammonium solution with mink oil in 25 volunteers with a history of immediate mosquito bite symptoms. Compared with placebo, itching was significantly decreased but no effect was found on the size of the immediate bite reaction. Hydrocortisone and other corticosteroid creams are generally recommended and widely used but, to our knowledge, no detailed studies exist on their efficacy in mosquito-bite reactions.

4.2 Systemic treatment

Various non-specific systemic treatments were previously claimed to diminish the symptoms of mosquito bites (McKiel 1961b). These include intravenous procaine hydrochloride, subcutaneous or oral thiamine chloride, local injections of epinephrine or oral magnesium chloride. The results could not, however, be verified e.g. in the case of thiamine.

There are a few previous data on the effects of systemic antihistamines on mosquito-bite symptoms in animals and man. McKiel (1961b) reported that subcutaneous injections of high doses of an antihistamine (Benadryl) suppressed wheals in bite-sensitive rabbits. Recently, Coulie et al. (1989) performed a double-blind, placebo-controlled study with cetirizine 10 mg BID on volunteers exposed to *A. aegypti* laboratory mosquitoes. Cetirizine reduced significantly immediate pruritus but had no effect on the size of the bite lesions. Reunala et al. (1991) performed a similar trial in 23 mosquito-bite-sensitive subjects in the field and showed that cetirizine 10 mg decreased significantly the size of wheals and the intensity of accompanying pruritus.

C. AIMS OF THE STUDY

The aims of the present study were:

- 1 To study the clinical efficacy of cetirizine and ebastine in mosquito-bite sensitive adults.
- 2 To study the clinical efficacy of loratadine in mosquito-bite sensitive children.
- 3 To compare the clinical effect of cetirizine, ebastine and loratadine in mosquito-bite sensitive adults.
- 4 To examine the inflammatory cells in the delayed mosquito-bite reaction and whether cetirizine antihistamine has an inhibitory effect on the cellular influx.

D. MATERIALS AND METHODS

1 Subjects

The subjects participating in the studies were either mosquito-bite sensitive adults (I, II, IV, V) or children (III; Table 4). Study I included 23, Study III 29 and Study V 24 mosquito-bite sensitive patients admitted to the Department of Dermatology, Tampere University Hospital or to the Department of Dermatology and later on to the Skin and Allergy Hospital, Helsinki University Central Hospital. The remaining subjects volunteering in Studies I, II, IV and V were mosquito-bite sensitive employees of the respective hospitals.

Table 4. Number of subjects participating in different studies.

Study	No. of subjects (evaluable)	Females (males)	Mean age, years (range)	Size of the bite-reaction for inclusion
I	28 (18) adults*	15 (3)	42 (18-60)	Large/pruritic reactions
II	28 (28) adults*	24 (4)	40 (18-58)	5 mm or bigger
III	28(25) children#	11 (14)	7 (2-11)	5 mm or bigger
IV	29 (27) adults☼	24 (3)	39 (26-61)	5 mm or bigger
V	26 (26) adults*	24 (2)	41 (19-65)	3 mm or bigger

*patients and employees

☼only employees

#only patients

The patients and the volunteers did not suffer from any clinically relevant disease, e.g. cardiovascular, hepatic, renal, neurological or gastrointestinal disorder, which could interfere with the study. No systemic corticosteroid or H₁-receptor antagonists were allowed within one week (astemizole 12 weeks) prior to the study.

2 Antihistamine administration

In the studies with a single antihistamine and placebo the daily doses were cetirizine 10 mg (one tablet; I) and 20 mg (two tablets; V), ebastine 20 mg (two capsules; II) and loratadine 0.3 mg/kg (mixture; III) as shown in Table 5. The comparative study (IV) included a single daily dose of cetirizine 10 mg, ebastine 10 mg and loratadine 10 mg in identical capsules. Each study (I-V) included a visually indistinguishable placebo product. The drugs were randomised by and obtained from UCB-Pharma (I, V), Rhone-Poulenc-Rorer (II), Schering-Plough (III), and randomised and purchased from Pharmia (IV).

The study protocols were double-blind and cross-over (I-IV), or parallel (V) type. The drugs were taken daily at 8 am for 7 days (I) or for 4 days (II-V). In the 4-day protocols there was a 3-day drug-free period between the cross-over periods.

Table 5. Antihistamines, mosquitoes and measurement of bite reactions in the different studies.

Study	Drug	Setting	Mosquito	Place and time ^o of challenge	Measurements		
					Time	Bite lesion	Pruritus (VAS)
I	Cetirizine 10 mg	Placebo/ Cross-over	<i>A. comm.</i>	Field 8-12 h	15 min, 1,12,24 h	+*	+
II	Ebastine 20 mg	Placebo/ Cross-over	<i>A. comm.</i>	Field 4-7 h	15 min, 2,6,24 h	+☼	+
III	Loratadine 0.3 mg/kg	Placebo/ Cross-over	<i>A. aeg.</i>	Lab. 4-7 h	15 min, 2,6,24 h	+☼	+☺
IV	Cetirizine 10 mg Ebastine 10 mg Loratadine 10 mg	Placebo/ Cross-over	<i>A. aeg.</i>	Lab. 4-7 h	15 min, 2,6, 24 h	+☼	+
V	Cetirizine 20 mg	Placebo/ Parallel	<i>A. aeg.</i>	Lab. 5-8 h	15 min, 2,6,24h #	+*	-

* measurement of diameter in mm

☼ measurement of area in mm²

☺ measured in 12 children older than 7 years

biopsies taken at 2, 6 and 24 h

^oafter drug administration

VAS= visual analogue scale

3 Mosquito-bite challenge and measurement of bite reactions

3.1 Mosquito-bite challenge

In Study I, mosquito-bite exposure took place in the field with the subjects alone. They tried to get on one occasion at least five bites on the forearm between 4 and 8 pm during both trial weeks. In Study II, challenge took place in the forest near the respective hospitals where the majority of the mosquitoes belong to *A. communis* species (Utrio 1978). In this field study the investigator supervised the challenge which took place between noon and 3 pm on day 3. In the laboratory studies III-V the exposures took place in the afternoon on day 3 with *A. aegypti* mosquitoes which bite more eagerly than *A. communis* mosquitoes in a cage. These two *Aedes* species have a common 36 kD antigen in their saliva (Brummer-Korvenkontio et al. 1996). The mosquitoes were obtained from the Laboratory of Protozoology, Institute of Tropical Medicine, Antwerp, Belgium. One female mosquito was put in a special cage and was allowed to bite on the forearm (III, IV). In the biopsy study (V), three separate mosquito bites were given clearly apart from each other on the forearm. In each study the mosquitoes were allowed to feed blood to repletion to ensure delivery of maximal amount of mosquito saliva into the skin.

3.2 Measurement of bite reactions and pruritus

In Study I the size of the largest bite reaction was measured and pruritus evaluated by the patients at 15 min and at 1, 2 and 24 h after the bite challenge (Table 5). Pruritus was scored on an 80-mm visual analogue scale (VAS) ranging from 0 (no pruritus) to 100 (very intense pruritus). In Studies II-IV the size of the bite reaction was measured by the investigator at 15 min and 24 h, and by the subjects or parents (III) at 2 and 6 h. Pruritus was scored on a 100-mm visual analogue scale. Twelve children, aged 7 years and older, evaluated pruritus in Study III.

The size of the bite lesion was measured in Studies I and V as the product of two perpendicular diameters in millimetres and reported as a mean of these measurements (Table 5). In Studies II-IV the corresponding diameters were multiplied by each other and this numerical value was used to represent the area of the bite lesion in square millimetres.

3.3 Sedation and other adverse effects

In Study I the subjects made every day notes of any sedation or other adverse effects that they had experienced. In Studies II-V, adverse effects were evaluated on every visit to the investigator by filling in an adverse effect questionnaire. A brief description, type and severity, duration, date of onset and resolution of the event were asked and written down as was also any change in study drug administration or action taken. Events were classified as minor or major, the latter causing the cessation of the study medication. The relationship of the event to the study drug or study participation was evaluated.

4 Inflammatory cells in bite lesions

In Study V, punch biopsies were taken at 2, 6 and 24 h after the mosquito bites and each biopsy specimen was divided into two pieces; one was fixed with formalin and embedded in paraffin and the other was snap-frozen in liquid nitrogen, embedded in mounting medium and stored in -70°C.

Eosinophils, neutrophils and mononuclear cells were counted from paraffin sections stained with haematoxylin and eosin, and mast cells were counted from toluidine blue-stained paraffin sections. T lymphocyte subsets (CD4⁺ and CD8⁺ cells) were identified in acetone- and chloroform-fixed 5µm cryostat sections using monoclonal antibodies, anti-Leu-3a+3b (CD4) and anti-Leu-2a (CD8). Bound primary antibodies were visualized with the streptavidin-biotin peroxidase technique. Diaminobenzidine was used as chromogen and endogenous peroxidase activity was blocked after the secondary antibody with 0.3% H₂O₂ (hydrogen peroxide) in methanol.

Slides were counted blind with a light microscope using an eyepiece graticule and 600 times magnification. The counting was performed from at least three 0.027-mm² fields on the cell infiltrates in the papillary and mid-dermis.

5 Statistics

Analysis of variance (I) and Wilcoxon's signed rank test, either with exact p-value (II, III) or with Hommel's adjusted p-value (IV), were used for detecting significant differences in the size of the lesions and pruritus between antihistamine and placebo treatments. The size (I-III) and pruritus (I, II) at all four time points and the sum of these measurements were analysed. In Study III, pruritus

was analysed only at 15 min and in Study IV, both size and pruritus were analysed only at 15 min. Correlations were estimated by the Spearman correlation coefficient method, and the McNemar test with binomial distribution and exact p-value was used to analyse side-effects (II). Comparison between antihistamines was performed with Friedman's two-way analysis of variance by ranks with an exact p-value, and the size of the 15-min reaction in the inclusion phase was compared with that in the placebo treatment period by determining the concordance correlation coefficient for agreement (IV). Mann-Witney's U-test was used to analyse the influence of cetirizine treatment on the number of inflammatory cells at various time points. The overall effect of the treatment on the number of cells and on the size of the bite lesion was analysed with the same test, but Kruskal-Wallis test was used to analyse the overall time effect (V). A p-value ≤ 0.05 was considered significant (I-V).

6 Ethics

Informed consent was obtained from all adult subjects participating in Studies I-III and V and from the parents of all 28 children in Study IV. All studies were approved by the Ethics Committees of the Tampere University Hospital and Helsinki University Central Hospital.

E. RESULTS

1 Effect of antihistamines on mosquito-bite symptoms

In the first two trials performed in the field the effects of cetirizine and ebastine on *A. communis* mosquito bite reactions were studied in bite-sensitive adults (I,II). In the third study, bite-sensitive children were exposed to *A.aegypti* laboratory mosquitoes and the effect of loratadine on bite reactions was assessed (III). In the fourth study performed with *A.aegypti* laboratory mosquitoes the effects of all three antihistamines mentioned above were compared with each other and with placebo in bite-sensitive adults (IV). In Studies I and V the size was expressed as a means of the diameter (mm) of the bite lesion. In Studies II-IV the size was expressed as medians of the area (mm²) of the bite. Means were substituted by medians because of the great variation in the size of the lesions.

1.1 Cetirizine and ebastine in mosquito-bite-sensitive adults (I,II)

In Study I the mean diameter of the immediate 15-min wheal decreased by 42% from 10.1 (range 0-40) mm with the placebo to 5.9 (range 0-20) mm with cetirizine 10 mg (analysis of variance; $p<0.05$). The mean diameter of the delayed 24-h reaction decreased by 41% from 12.6 (range 0-90) mm to 7.4 (range 0-70) , respectively (Fig. 3a). At 15 min the mean score of pruritus decreased by 69% from 36.0 with placebo to 11.2 with cetirizine ($p<0.001$) and for 24-h pruritus the decrease was 65% from 18.9 to 6.6 ($p<0.01$), respectively (Fig. 3b).

In Study II, ebastine 20 mg decreased significantly (Wilcoxon's signed rank test; $p<0.001$) the immediate 15-min bite reaction and pruritus. The median size of the wheal was 49 mm² with placebo and 16 mm² with ebastine treatment (Fig. 4a). The median score of pruritus at 15 min decreased from 75 to 20 and from 5 to zero at 24 h, respectively ($p=0.011$, Fig. 4b).

In Study I, one subject dropped out due to eczematous rash during placebo treatment. Two subjects reported mild sedation (5 days total) during placebo and 5 subjects (10 days) during cetirizine treatment (non-significant difference). Further adverse effects, not considered as drug-related, were headache, emesis or arthralgia in four subjects during placebo and in three subjects during cetirizine

treatment. In Study II, five subjects reported mild to severe sedation during ebastine but nobody during placebo treatment.

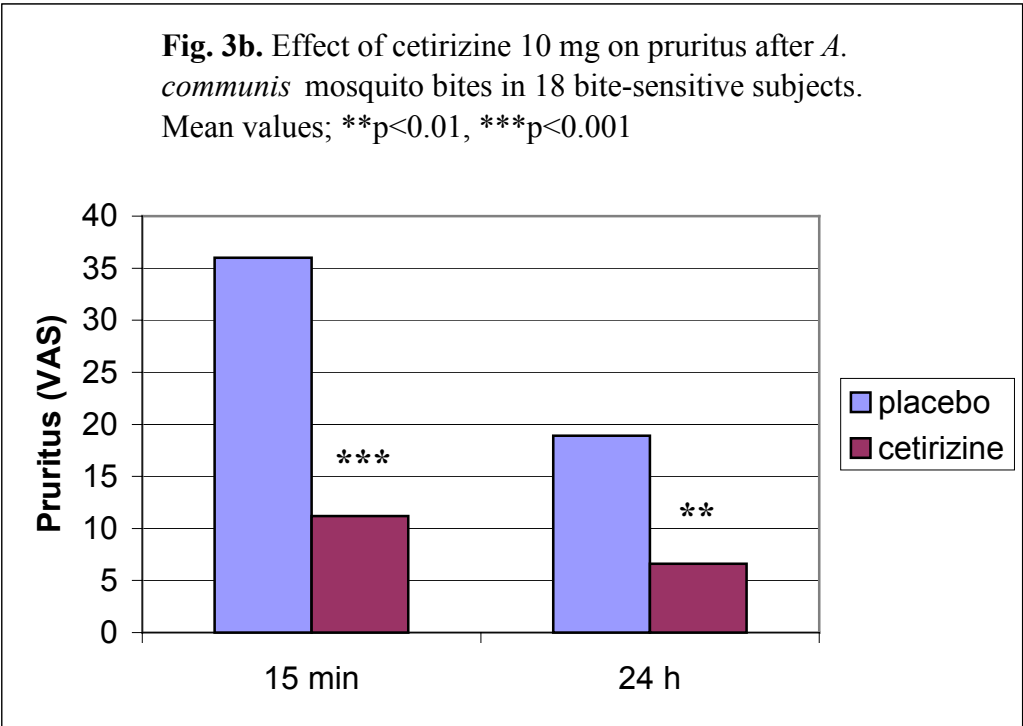
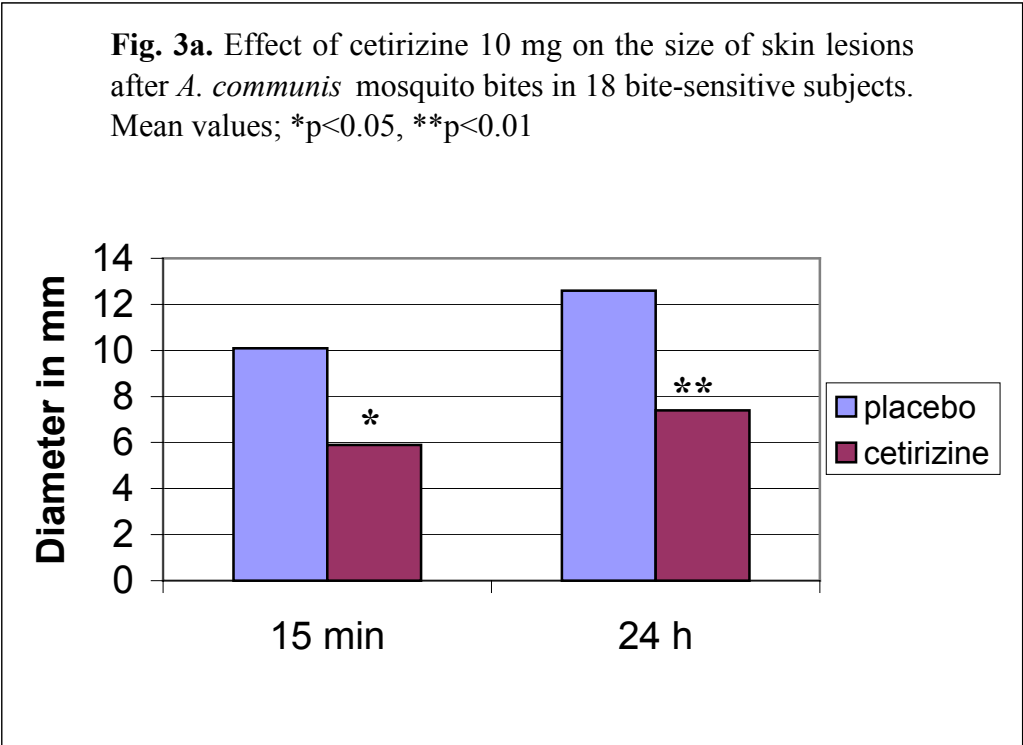


Fig. 4a. Effect of ebastine 20 mg on the size of skin lesions after *A. communis* mosquito bites in 28 bite-sensitive subjects. Median values; ***p<0.001

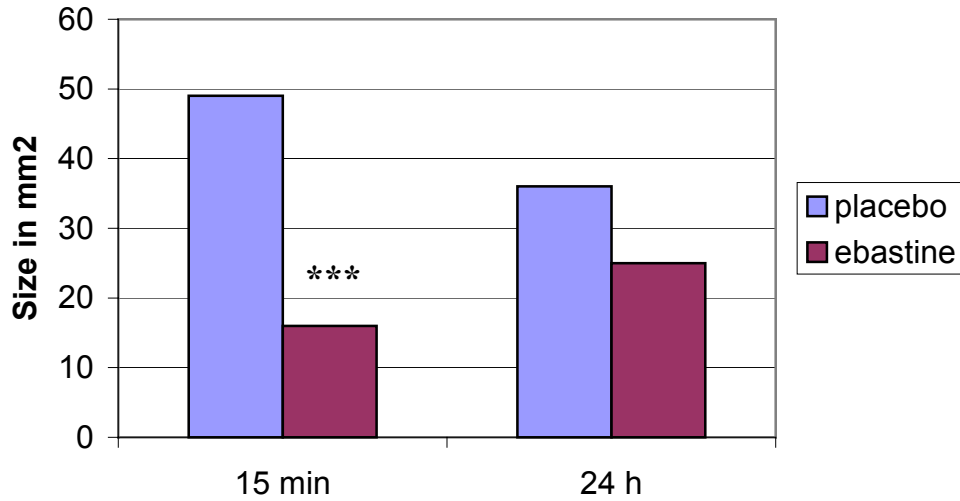
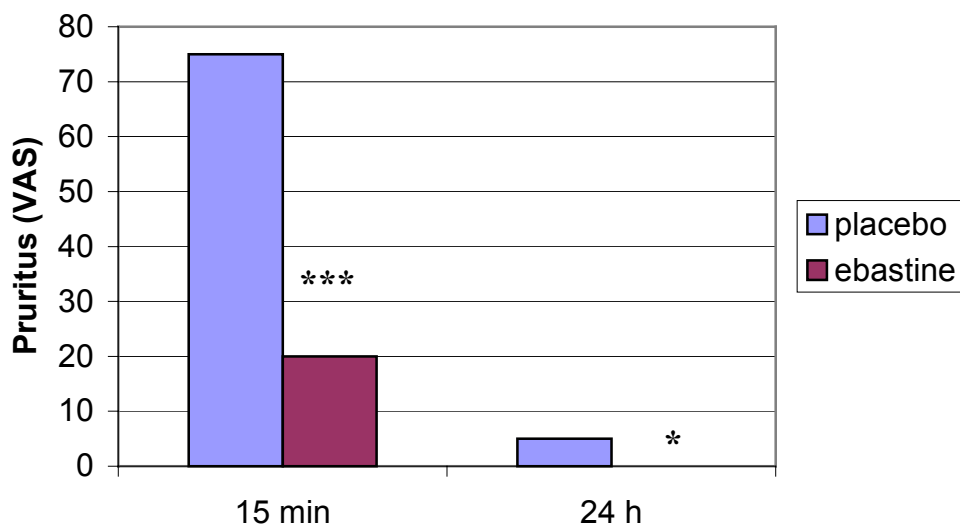


Fig. 4b. Effect of ebastine 20 mg on pruritus after *A. communis* mosquito bites in 28 bite-sensitive subjects. Median values; *p<0.05, ***p<0.001



1.2 Loratadine in mosquito-bite-sensitive children (III)

Loratadine was found effective both in immediate and delayed reactions in children (Fig. 5a). The median size of the 15-min wheal decreased by 45% (55% counted from mean values) from 64 mm² with placebo to 35 mm² with loratadine 0.3 mg/kg (Wilcoxon's signed rank test; $p < 0.001$). The median size of the 24-h bite lesion decreased by 27% (52% counted from mean values) from 49 to 36 mm² ($p = 0.004$). Pruritus was measured in twelve children, and statistical comparison could be made only at 15 min, because at other time points pruritus was very weak (Fig. 5b). The median score of pruritus decreased by 78% (47% counted from mean values) from 45 on placebo to 10 on loratadine ($p = 0.011$).

One child experienced mild, transient abdominal pain after taking loratadine mixture. This adverse event was considered as drug-related, because it occurred after every drug administration. Another child had moderate diarrhoea and nausea during loratadine treatment lasting for two days. This adverse event was not considered as drug-related, because it resolved before the end of the drug administration.

Fig. 5a. Effect of loratadine 0.3 mg/kg on the size of skin lesions after *A. aegypti* mosquito bites in 25 bite-sensitive children.

Median values; **p<0.01, ***p<0.001

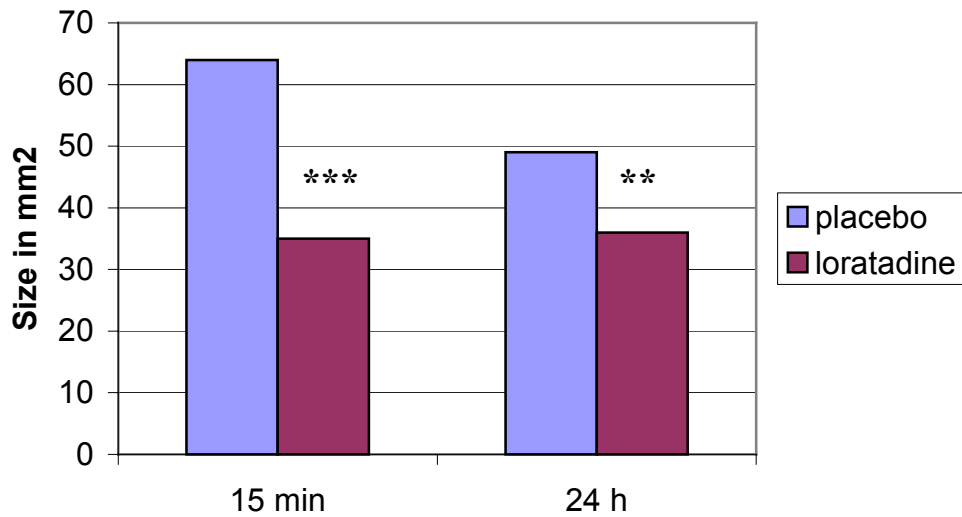
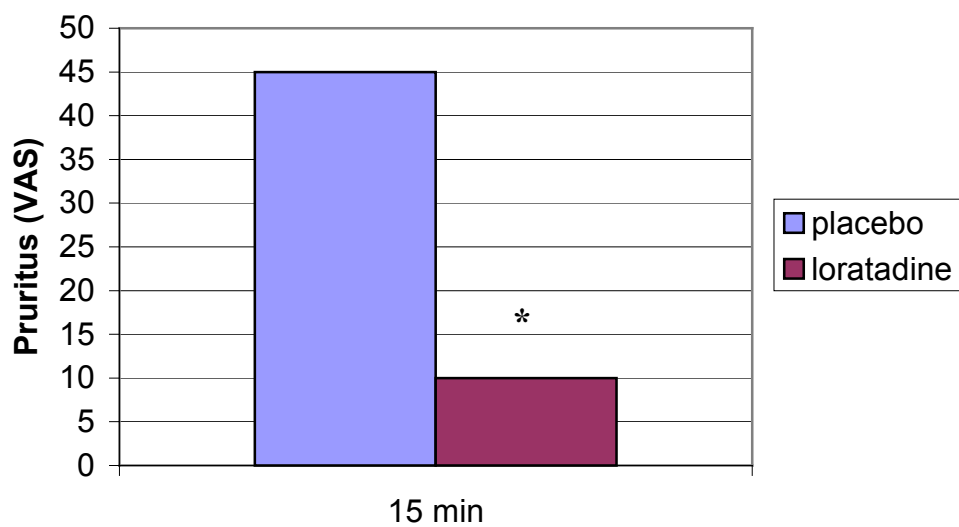


Fig. 5b. Effect of loratadine 0.3 mg/kg on pruritus after *A. aegypti* mosquito bites in 12 bite-sensitive children.

Median values; *p<0.05



1.3 Comparison of cetirizine, ebastine and loratadine in mosquito-bite-sensitive adults (IV)

In Study IV the effects of three antihistamines (cetirizine 10 mg, ebastine 10 mg and loratadine 10 mg) on wheals were compared with each other and with placebo. At 15 min the median size of the wheal decreased by 11% (45% counted from mean values) from 28 mm² with placebo to 25 mm² with cetirizine ($p<0.01$), by 32% (53% counted from mean values) to 16 mm² with ebastine ($p<0.01$) and to 25 mm² with loratadine ($p=0.09$) (Fig. 6a). None of the three antihistamines could be ranked the best in the capacity of decreasing the size of the wheals. At 15 min the median score of pruritus decreased by 100% (64% counted from mean values) from 50 on placebo to 0 on cetirizine, by 80% (61% counted from mean values) to 10 on ebastine and to 30 on loratadine. The effects of cetirizine and ebastine, but not loratadine ($p=0.067$), on pruritus were significant ($p<0.001$). Cetirizine could be ranked better than ebastine and ebastine better than loratadine (Fig. 6b).

A total of 14 subjects reported mild to severe sedation: eight subjects during cetirizine, five during loratadine, four during placebo and two subjects during ebastine treatment (IV Fig 3).

Fig. 6a. Effect of cetirizine 10 mg, ebastine 10 mg and loratadine 10 mg on the size of skin lesions after *A. aegypti* mosquito bites in 27 bite-sensitive adults. Median values; **p<0.01

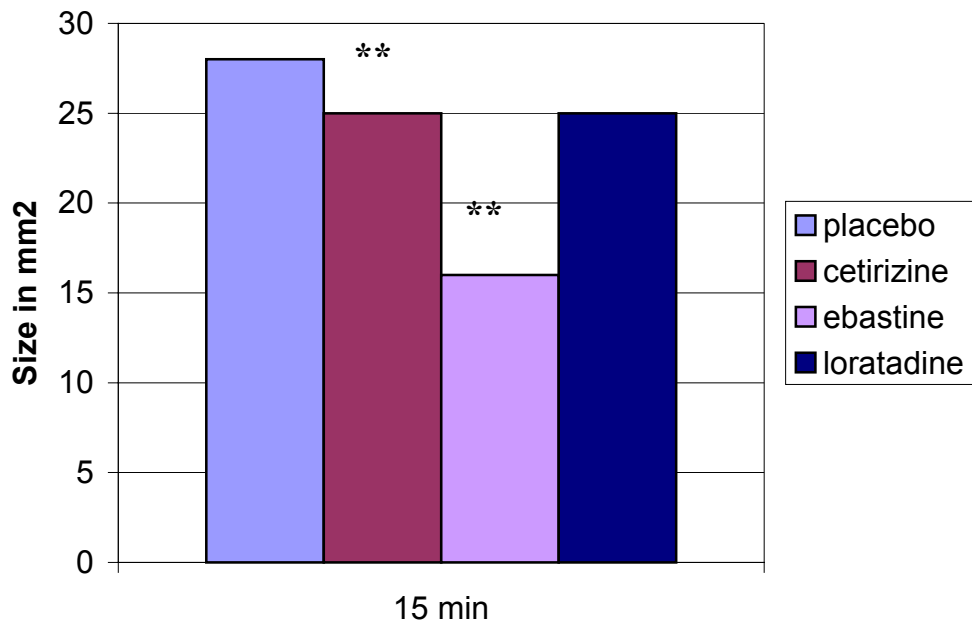
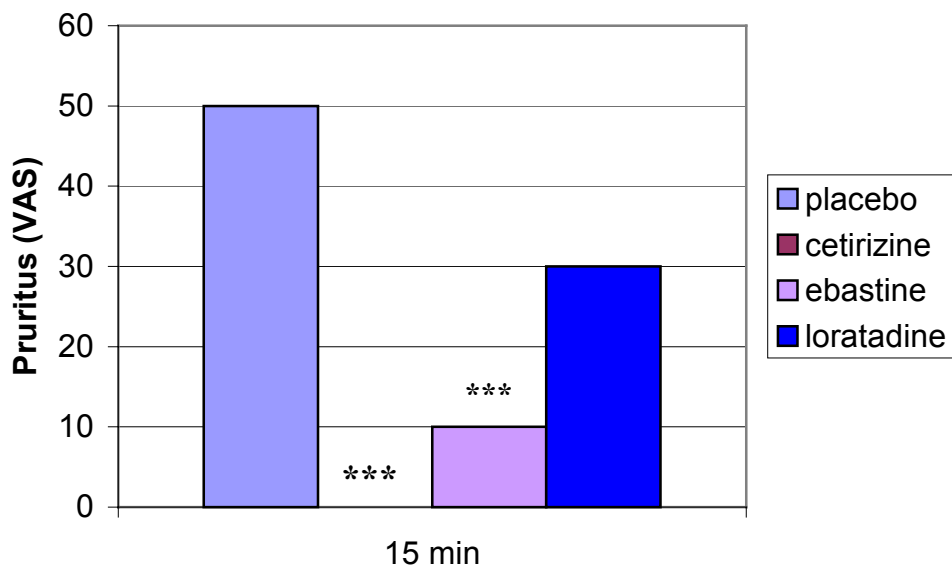


Fig. 6b. Effect of cetirizine 10 mg, ebastine 10 mg and loratadine 10 mg on pruritus after *A. aegypti* mosquito bites in 27 bite-sensitive subjects. Median values; ***p<0.001



2 Effect of cetirizine on inflammatory cells in bite lesions (V)

All 26 subjects (14 on cetirizine and 12 on placebo) completed the study and a total of 73 biopsies (38 on cetirizine and 35 on placebo) were taken at 2, 6 and 24 h.

During placebo treatment eosinophils were detected already in the 2-h biopsy specimens and they located mainly in the upper dermis around the vessels but to a lesser extent also between collagen fibres far from vessels. Extra-cellular granules were also observed in many specimens. A few subjects had high eosinophil counts and in five patients (11 specimens out of 34) no eosinophils were detected in at least one of the three serial specimens. The median number of eosinophils was stable over the time period, but there seemed to be less variation at 24 h compared with the 2- and 6-h specimens (V; Fig. 1). The number of neutrophils between individuals varied largely, especially at 2 h. However, the median numbers of neutrophils were similar in the 2-, 6- and 24-h biopsy specimens (V; Fig. 2). The number of mast cells were low and remained constant over the time period with minimal variation (V; Table 1). In contrast to eosinophils, neutrophils and mast cells, the number of mononuclear cells increased with time (V; Fig. 3). The number of CD4⁺ cells, but not CD8⁺ cells, increased significantly with time from the 2- to 24-h specimens. The median numbers of CD8⁺ cells were four times lower than those of CD4⁺ cells in the 24-h specimens (V; Table 1).

Cetirizine increased the number of eosinophils in the 6-h specimens, but did not contribute to the number of neutrophils or mast cells (Fig. 7a). The number of CD4⁺ and CD8⁺ cells was significantly higher in the cetirizine-treated than in the placebo-treated subjects in the 24-h specimens and also at 6 h for CD4⁺ cells (Fig. 7b,c). Overall, the median number of mononuclear cells was significantly higher in the cetirizine-treated than in the placebo-treated subjects (V; Fig. 3).

Fig. 7a. Effect of cetirizine 20 mg on the number of eosinophils in the 2-, 6- and 24-h lesions after *A. aegypti* mosquito bites in 26 bite-sensitive adults. Median values; * $p < 0.05$

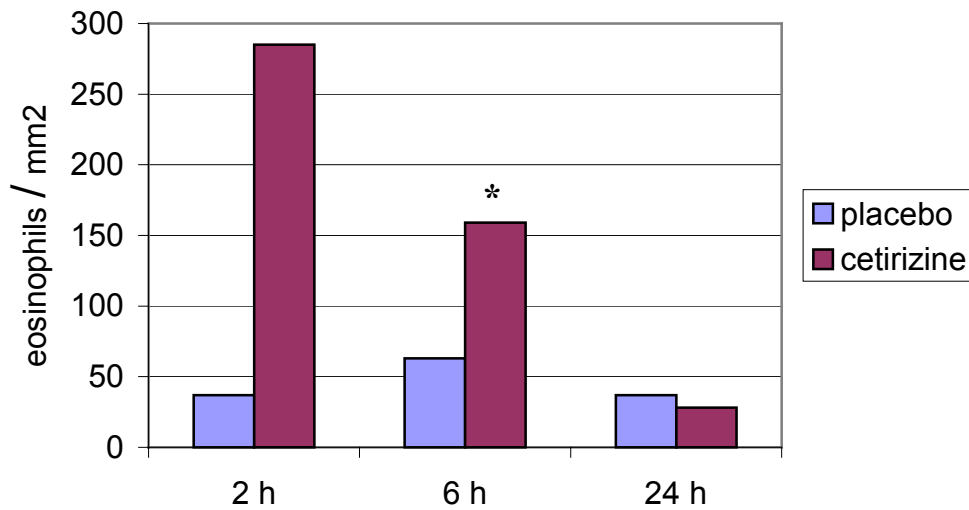


Fig. 7b. Effect of cetirizine 20 mg on the number of CD4+ cells in 2-, 6- and 24-h *A. aegypti* mosquito bite lesions in 26 bite-sensitive adults. Median values; * $p < 0.05$

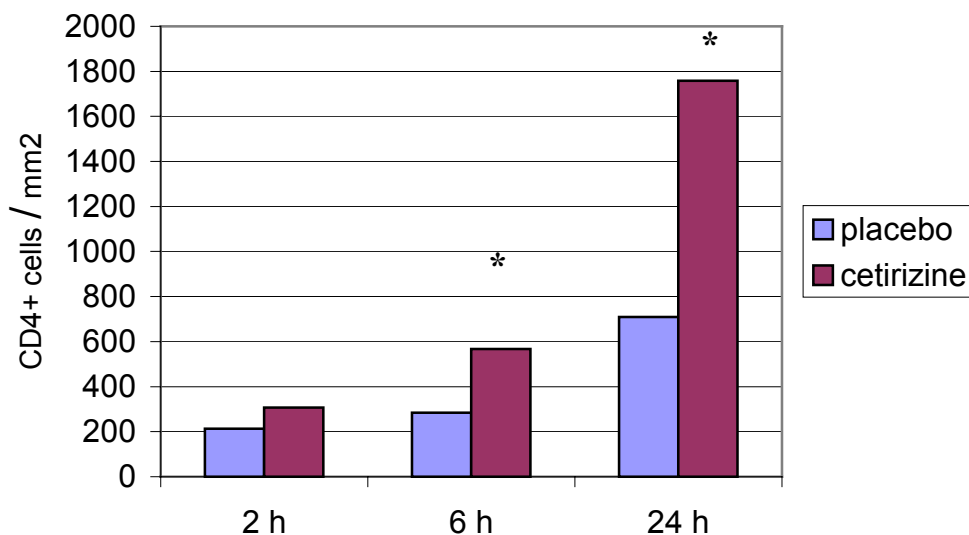
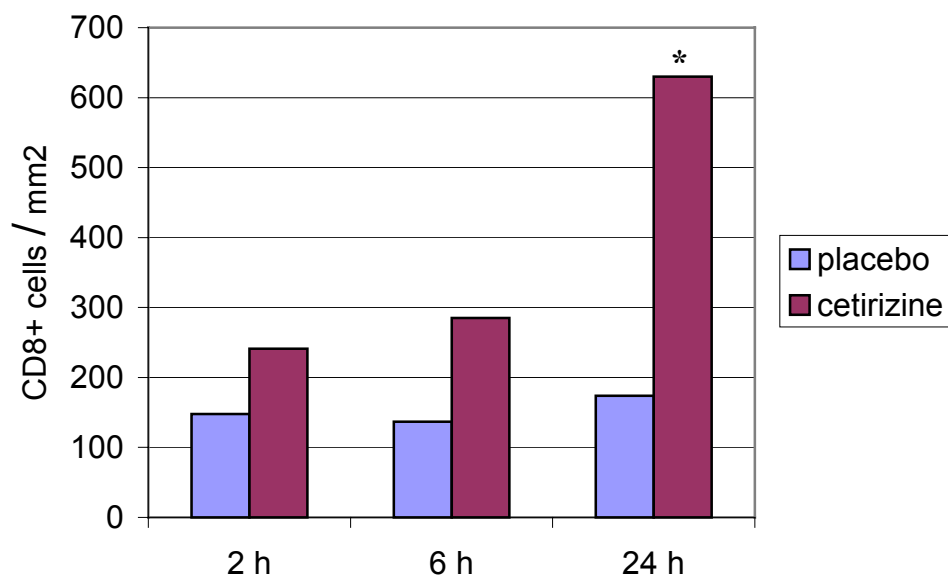


Fig. 7c. Effect of cetirizine 20 mg on the number of CD8+ cells in the 2-, 6- and 24-h lesions after *A. aegypti* mosquito bites in 26 bite-sensitive adults. Median values; *p<0.05



F. DISCUSSION

1 Clinical effects of antihistamines on mosquito-bite reactions

1.1 Cetirizine and ebastine in mosquito-bite-sensitive adults

The two placebo-controlled, cross-over studies were performed in the field by exposing the subjects to the bites of *A. communis* mosquitoes. The first study showed that cetirizine 10 mg decreased significantly the size of wheals and accompanying pruritus. This finding is in a good agreement with a previous parallel-group study performed in the field by Reunala et al. (1991). In contrast, Coulie et al. (1989) did not find in their study performed with *A. aegypti* mosquitoes in the laboratory any effect of cetirizine 10 mg BID on the size of wheals but showed that it alleviated the accompanying pruritus. The reason for the discrepant results on wheals seems to be subject selection and also the rather small reactions from *A. aegypti* bites. The second field study performed with ebastine 20 mg showed a decrease in the size of wheals and accompanying pruritus similar to that found with cetirizine 10 mg. This finding agrees also well with the results of a previous study with ebastine 10 and 20 mg and *A. aegypti* mosquitoes performed in the laboratory (Reunala et al. 1997). In general, the effects of cetirizine and ebastine on mosquito-bite wheals and accompanying pruritus are not unexpected. Cetirizine and ebastine are potent H₁ blockers and mosquito-bite whealing is an antisaliva IgE antibody-mediated allergic reaction where histamine is liberated into the dermis (Brummer-Korvenkontio et al. 1994, Horsmanheimo et al. 1996).

An important and clinically significant finding was that cetirizine 10 mg reduced in addition to the immediate bite symptoms also the size of the 24-h bite lesion and accompanying pruritus. Ebastine 20 mg relieved similarly pruritus at 24 h but did not show any significant effect on the size of the bite lesion. The partially discordant results should be interpreted with caution due to differences in the subject material and protocols. In the cetirizine study the subjects exposed themselves to several mosquito bites and the largest bite reaction was included in the analysis, whereas in the ebastine study the subjects were exposed only to one bite. Moreover, the size of the 24-h bite reactions during placebo was about 30% larger in the cetirizine study than in the ebastine study obviously due to subject selection. On the other hand, the effects of cetirizine and ebastine on the delayed bite

symptoms detected in the present field studies, but not in the previous laboratory studies (Coulie et al. 1989, Reunala et al. 1997), seem to be due to the fact that *A. communis* bites cause as a rule larger wheals and delayed reactions compared with *A. aegypti* bites. Overall, the findings that cetirizine and ebastine could alleviate also the delayed mosquito bite symptoms are of clinical importance and focus attention also to the pathophysiology of the delayed mosquito-bite reactions.

1.2 Loratadine on mosquito-bite reactions in children (III)

The loratadine study was a placebo-controlled, cross-over study with *A. aegypti* laboratory mosquitoes in 25 mosquito-bite-sensitive children. The results showed that loratadine 0.3 mg/kg decreased significantly the size of wheals and also the size of the delayed bite papules. Pruritus could be evaluated only at 15 min in 12 children and it decreased also significantly. Since a large proportion of children in Finland and elsewhere have IgE antisaliva antibodies to *Aedes* mosquitoes and also react to the bites by wheals and delayed bite papules (Reunala et al. 1994b, Brummer-Korvenkontio et al. 1997), it is important to find an effective treatment for the bite symptoms. To our knowledge, no other controlled antihistamine studies have been performed previously on mosquito-bite sensitive children. In regard to the studies in adults, one could expect that cetirizine and ebastine could also be effective in mosquito-bite-sensitive children.

Most of the children in the study were admitted for examinations due to their disturbing mosquito-bite allergy. The study itself could be performed in the allergy laboratory of the respective hospitals without any drop-outs. Moreover, loratadine was well tolerated and no drug-related adverse effects were observed. It is hardly possible to perform with children field studies similar to those done previously with cetirizine and ebastine in adults (I, II). The good results found with loratadine in the present mosquito-bite exposure in the laboratory obviously indicate that this drug would also give a significant relief when children are exposed to local mosquitoes in the field.

1.3 Comparison of cetirizine, ebastine and loratadine in mosquito-bite sensitive adults (IV)

This placebo-controlled, cross-over study with three antihistamines and placebo in 27 mosquito-bite-sensitive subjects showed that cetirizine 10 mg and ebastine 10 mg reduced significantly the size of wheals and pruritus caused by *A. aegypti* mosquito bites. This finding is in agreement with previous studies performed with cetirizine or ebastine in the field (I, II) or in the laboratory (Coulie

et al. 1989, Reunala et al. 1997). A finding that cetirizine and ebastine seemed to have a more profound effect on the bigger than the smaller wheals (IV; Fig. 2) could be of clinical importance, i.e. these antihistamines work best on subjects with intense bite reactions. It should be noted that the size of the wheal in mm² was calculated from the diameter and not from the exact area by drawing the margins of the wheal. Due to this the calculated area used in statistical analysis was a square which is about one fourth larger than the real, usually (more often than 95%) round-shaped wheal. However, as the measurement bias in the wheal area concerns similarly the three antihistamines and placebo, the results are statistically comparable. When the effect on pruritus was compared, cetirizine seemed to give even better relief than ebastine. This may also be of clinical importance but should be confirmed in controlled studies in the field.

Contrary to cetirizine and ebastine, loratadine 10 mg did not show any statistically significant effect on the immediate bite symptoms compared with placebo. This finding is contrary to our previous finding in the mosquito-bite-sensitive children (III). In that study the dose of loratadine given to children was 0.3 mg/kg and in the present study in adults it was 10 mg, i.e. 0.14 mg/kg for a 70-kg person. Though loratadine 10 mg is the recommended dose in the treatment of pollen allergy and urticaria in adults, it seems obvious that a higher dose, e.g. 20 mg of loratadine, is needed to control immediate bite symptoms in adults.

None of the three antihistamines studied showed a significant effect on the delayed bite lesions or accompanying pruritus. The obvious reason for this seems to be the subjects' minor delayed reactivity to the *A. aegypti* bites. The size of the 24-h bite reaction was small and the pruritus score low in many subjects on placebo due to which no significant antihistamine effect could be expected. A comparative study in the field with *A. communis* bites would cause bite reactions big enough and thereafter disclose whether any of the three antihistamines would be superior in the treatment of delayed mosquito bite symptoms.

Assessment of sedation caused by antihistamines is difficult even in the proper laboratory conditions (Mattila and Paakkari, 1999). In the present study we evaluated sedation only by questioning the subjects during the trial and analysed the number of days with reported sedation. The results (IV, Fig. 3) suggest that cetirizine 10 mg might be more sedative than ebastine 10 mg or loratadine 10 mg in the treatment of mosquito-bite reactions. Previously a few clinical or post-marketing studies also showed differences in the frequency of sedation between cetirizine and loratadine but not between cetirizine and ebastine (Meltzer et al. 1996, Mann et al. 2000, Frossard et

al. 2000). It should be noted, however, that subjective sedation does not necessarily mean impaired performance and that tolerance against sedation develops usually a few days after starting antihistamine therapy (Mattila and Paakkari, 1999). The marginal findings of sedation found in the present or in our previous studies (I-III) show that the three antihistamines examined are well acceptable in the treatment of mosquito-bite allergy. Many patients need medication for the whole mosquito season, i.e. up to 3 months, and long-term or post-marketing surveillance studies would implicate the real value of these drugs in the treatment of mosquito allergy.

2 Effect of cetirizine on the inflammatory cells in mosquito-bite lesions (V)

In order to investigate the cellular influx and the effect of cetirizine on the delayed bite reactions, serial biopsies were taken from the lesions at 2, 6 and 24 h. Fourteen subjects received cetirizine 20 mg and 12 subjects placebo in a double-blind fashion. The bite challenges were performed with *A. aegypti* mosquitoes. In the placebo-treated subjects eosinophils and neutrophils were detected already in 2-h lesions. No marked change occurred in their numbers thereafter. An important finding was a significant increase of mononuclear cells from the 2- to 24-h bite lesions. Interestingly, CD4⁺ but not CD8⁺ cells showed a parallel significant increase. These results obviously indicate that the inflammatory cell response in mosquito-bite lesions is characterized by an early influx of eosinophils and neutrophils with a subsequent increase in the numbers of infiltrating CD4⁺ cells.

In experimental cutaneous late-phase reactions produced by ragweed allergen and examined in suction blister chambers, eosinophils and neutrophils have been detected from 4 h after the antigen challenge (Charlesworth et al. 1989), i.e. somewhat later than in the present mosquito-bite lesions. Leiferman et al. (1990) observed extensive deposition of eosinophil major basic protein and neutrophil elastase in the dermis as early as 1-3 h after the ragweed challenge, the findings agreeing well with the present findings of an early influx of eosinophils and neutrophils in the delayed mosquito-bite lesions. We could also detect CD4⁺ and CD8⁺ cells already in the 2-h bite lesions. Thereafter, the numbers of CD4⁺ cells increased significantly and were highest in the 24-h bites, whereas CD8⁺ cells showed no such increase. Overall, the delayed inflammatory cell response observed in the present mosquito-bite lesions fits well to the findings observed after intradermal injection of respiratory allergens (Frew et al. 1988, Charlesworth et al. 1989, Leiferman et al. 1990, Zweiman et al. 1997).

An unexpected finding of the present mosquito-bite biopsy study was the effect of cetirizine on the inflammatory cell response. Overall, the median numbers of eosinophils and CD4⁺ cells were significantly increased in the cetirizine-treated subjects compared with the placebo-treated subjects. In eosinophils this effect was most prominent in the 6-h and in the CD4⁺ cells in the 24-h biopsy specimens. We have previously documented in a clinical study (I) that cetirizine 10 mg decreases the size of the 12- and 24-h mosquito-bite lesions and, therefore, we expected to find that in the present study cetirizine would decrease the number of eosinophils and possibly also of CD4⁺ cells in the bite lesions. In early studies, cetirizine was found to inhibit eosinophil migration and to decrease cutaneous late-phase reactions *in vivo* (Fadel et al. 1987, Charlesworth et al. 1989, Snyman et al. 1994). A recent study could, however, not confirm the effect of cetirizine on the cellular inflammatory responses seen in the cutaneous late-phase responses to injected ragweed allergen (Zweiman et al. 1997). One possible explanation for the increased eosinophil and CD4⁺ cell counts could be that cetirizine modulates adhesion molecule expression and, consequently, these cells become inactive 'bystanders' in the skin (Wardlaw et al. 1994, Snyman et al. 1992, 1994). We observed degranulated eosinophils in several biopsy specimens taken from placebo- but also from cetirizine-treated subjects. However, we could not perform exact eosinophil degranulation measurements because of too strong background staining in eosinophil cationic protein specimens. It is tempting to speculate that after the cetirizine-treatments a high number of eosinophils and CD4⁺ cells in the inflammatory infiltrates of mosquito-bite lesions could be inactive 'bystanders' whose survival is prolonged by autocrine excretion of cytokines such as IL-3 (interleukine 3) or IL-5 (Moqbel et al. 1994). Recent *in vitro* studies show that also mast cells enhance eosinophil survival by secreting these cytokines and GM-CSF (granulocyte-macrophage colony-stimulating factor) (Levi-Schaffer et al. 1998). The mast cell numbers remained, however, low in the present biopsy specimens both on placebo and cetirizine, suggesting that the differences observed are not due to mast cell activities. We conclude from the significantly higher overall number of eosinophils and CD4⁺ cells in the cetirizine-treated than in the placebo-treated subjects that the clinical treatment effect on the delayed reaction is not due to inhibition of cellular recruitment. This is in good agreement with the results of a recent *in vivo* study in which cetirizine was without effect on cellular inflammatory responses in late-phase allergic cutaneous responses (Zweiman et al. 1997).

3 General discussion

Mosquitoes are a common nuisance in tropical and temperate areas around the world by causing immediate wheal-and-flare reactions and delayed bite papules to sensitised people (Alexander 1984, Reunala 1994a). In the Nordic countries the majority of people react to the bites with cutaneous lesions which tend to be more intense at the onset of the mosquito season when the anti-saliva IgE antibody levels are high (Palosuo et al. 1997). People are also known to get harmful reactions when exposed to foreign species when travelling. Mosquito-bite whealing is mediated by antisaliva IgE antibodies and histamine similarly to disorders such as allergic rhinitis and urticaria (Brummer-Korvenkontio et al. 1994, 1997, Peng et al. 1995, 1997, Horsmanheimo et al. 1996). It could be expected, therefore, that H₁-blocking antihistamines would be useful in the treatment of immediate mosquito-bite reactions. Preliminary studies with cetirizine gave promising results (Coulie et al. 1989, Reunala et al. 1991) and led to a set of clinical trials in the laboratory or in the field in Finland. The placebo-controlled, cross-over studies with a single antihistamine are presented in this thesis and the results showed the efficacy of cetirizine 10 mg and ebastine 20 mg in mosquito-bite-sensitive adults (I, II) and loratadine in children (III). A further comparative study in mosquito-bite-sensitive adults (IV) confirmed the significant clinical effect of cetirizine 10 mg and ebastine 10 mg, but not loratadine 10 mg, on the mosquito-bite-whealing and accompanying pruritus. Recently, Grant et al. (1999) compared the effect of different antihistamines on the histamine-induced wheal and flare responses. The onset of action of cetirizine 10 mg was shown to be faster than that of ebastine 10 mg or loratadine 10 mg. Overall, cetirizine decreased better wheal and flare responses than ebastine which, in turn, was more effective than loratadine. These findings are in general agreement with the present treatment results with the same antihistamines in the immediate mosquito bite reactions.

In the present trials with cetirizine and ebastine in adults and loratadine in children the size of whealing was reduced by about 40% and the intensity of pruritus as much as 70%. The present protocols included antihistamine intake 4-8 h before the mosquito-bite exposure. We therefore extended our study results to practice by recommending prophylactic cetirizine or ebastine use for those individuals who suffer from intense immediate bite symptoms during the mosquito season. This approach is similar to that used for seasonal rhinitis. Several points remain, however, to be answered. We have not performed systematic studies to find the optimal dosing or timing for each antihistamine studied. Doubling the cetirizine dose to 20 mg could give even better results in subjects with very intense bite reactions. On the other hand, the results with ebastine 10 mg and 20

mg in the two present studies and in a previous one suggest that the differences between the two doses seem to be small (Reunala et al. 1997). One finding common to cetirizine, ebastine and even to loratadine was the marked effect on the large wheals and intense itching, whereas the response was marginal when the bite reactions were rather small and the pruritus less intense. It seems therefore that prophylactic antihistamine treatment is of special value to those subjects who present with severe allergic cutaneous bite reactions. Mosquito-bite-allergic subjects can sometimes get systemic symptoms such as generalized urticaria and even anaphylaxis (McCormack 1995, Hassoun 1999). Whether the prophylactic use of antihistamines is of any value in preventing these reactions is not known.

Most mosquito-bite sensitive subjects develop disturbing delayed bite papules which appear 4-6 h after the bites and may persist several days (Reunala et al. 1994, Peng et al. 1998). The fact that cetirizine (I), possibly also ebastine (II) and loratadine (III), could have an effect on these delayed bite lesions or accompanying pruritus is of clinical importance and theoretical interest. The mosquito-bite-sensitive subjects often suffer much from these long-lasting bite papules, and scratching increases the risk of secondary bacterial infection. Based on the present studies it seems clear that a marked effect on these bite reactions can be expected by cetirizine and possibly also from the other antihistamines when the delayed bite reactions are big enough, i.e. the diameter is over 10 mm. *A. communis* causes rather often such reactions in the field whereas this was not the case with *A. aegypti*, a clearly smaller mosquito species used in the present laboratory experiments. We conclude that the best setting to study the clinical effects of different antihistamines is obviously a trial with mosquitoes in the field. The clinical effects of third-generation antihistamines, e.g. desloratadine, should be examined in future.

The pathophysiology of delayed mosquito-bite reactions is a matter of discussion and further experiments. Whether a part of these reactions represent allergic type I late-phase reactions mediated by eosinophils and T lymphocytes seems possible and are in line with the results of the present biopsy study (V). A part of the 24-h bite lesions may represent classical type IV cell-mediated reactions. Further research should be performed to study *in vitro* lymphocyte responses to mosquito saliva antigens in man and to study whether the lymphocytes involved in these reactions bear the T helper 2 cytokine profile or not (Chen et al. 1998).

Immune therapy has been used for a long time in pollen and hymenoptera allergy (Bosquet et al. 1998). There have also been attempts to develop immune therapy for mosquito allergy. Scott et al.

(1984) produced allergens in mosquito larval cell cultures, but the allergens caused severe systemic reactions. McCormack et al. (1995) applied rush immune therapy in two anaphylactic patients by using whole-body extracts of *A. aegypti* and *C. pipiens* mosquitoes. The first patient received the therapy without problems, but the second got severe serum-sickness-type symptoms. Recent research has focused on the production of recombinant allergens and on developing gene techniques for immune therapy (Chapman et al. 2000). Recently, Wang et al. (1999) showed with recombinant mosquito saliva allergens that they cause significant IgE response in mice. The same group tried also combined oligodeoxynucleotide vaccination, but this failed to down-regulate ongoing IgE response (Peng et al 2001). For the time being, second-generation antihistamines taken in a prophylactic manner remain the best available therapy for the mosquito-bite-allergic patients (Fradin 1998). Third generation antihistamines have been approved for medicinal use, but their effect on mosquito bites has to be documented in future.

G. SUMMARY

Mosquitoes cause harmful bite reactions to many people. Wheals are mediated by antisaliva IgE antibodies and histamine. The pathophysiology of the common delayed cutaneous bite reactions is, however, not known. Moreover, no appropriate treatment has been available for the mosquito-bite-sensitive subjects. The purpose of the present study was to examine whether oral antihistamines are effective in the treatment of mosquito-bite-sensitive children and adults. The second objective was to study inflammatory cells in the delayed mosquito-bite lesions and to examine whether cetirizine antihistamine would modulate the influx of these cells.

The first two clinical placebo-controlled, cross-over studies were performed in 28 adult subjects exposed to *Aedes communis* mosquitoes in the field (I, II). The results showed that cetirizine 10 mg and ebastine 20 mg decreased significantly whealing and accompanying pruritus. Cetirizine, but not ebastine, decreased also the size of the 24-h bite lesion and both alleviated pruritus. In the third study with loratadine (0.3 mg/kg), 28 mosquito-bite-sensitive children were exposed to *Aedes aegypti* mosquitoes in the laboratory (III). Loratadine decreased significantly whealing and accompanying pruritus and also the size of the 24-h bite lesion. Thereafter, a comparative placebo-controlled, cross-over study was performed with cetirizine 10 mg, ebastine 10 mg and loratadine 10 mg in 29 bite-sensitive adults exposed to *A. aegypti* mosquitoes in the laboratory (IV). Cetirizine and ebastine, but not loratadine, decreased significantly whealing and pruritus. Due to the mild 24-h delayed bite reactions, no comparison could be made between the antihistamines. All three antihistamines were well tolerated but cetirizine seemed to be slightly more sedative than ebastine and loratadine.

Cutaneous inflammatory cell response to *A. aegypti* mosquito bites was examined in 14 cetirizine- and 12 placebo-treated bite-sensitive adult subjects. Serial biopsies were taken at 2, 6 and 24 h after the bites, and inflammatory cells were counted from haematoxylin-eosin- and immunohistochemically stained specimens. The results showed early influx of eosinophils and neutrophils with a subsequent increase of CD4⁺ lymphocytes. These findings suggest that delayed mosquito-bite lesions could represent cutaneous type I late-phase allergic reactions. Unexpectedly,

cetirizine treatment increased, not decreased, the number of eosinophils, CD4⁺ and CD8⁺ cells in the bite lesions. This effect of cetirizine could , e.g. be due to prolonged survival and decreased activity of these cells in the skin.

In conclusion, the present study documented that the second-generation antihistamines cetirizine and ebastine are effective in the treatment of mosquito-bite-sensitive adults and loratadine in children. The inflammatory cell response in the delayed mosquito-bite lesions consists mainly of eosinophils, CD4⁺ and CD8⁺ lymphocytes and the number of the cells is increased by cetirizine.

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