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Main Article

Transtympanic versus intramuscular steroid administration in a histamine-induced inflammatory middle-ear model

T S Chimona, J G Panayiotides*, C E Papadakis, E S Helidonis, G A Velegrakis

Abstract

Objectives: Assessment of the histopathologic effect of transtympanic and intramuscular administration of dexamethasone in an *in vivo* experimental animal model of middle-ear mucosal inflammation.

Methods: Fifty healthy rabbits weighting 1500–1800 g were randomly divided in three groups. In 10 animals (control group), 0.5 ml of a 20 mg/ml histamine solution was injected transtympanically. In 20 rabbits (group A), histamine challenge followed a three day intramuscular pretreatment with dexamethasone at 1 mg/kg per day. In 20 rabbits (group B), histamine challenge followed pretreatment with dexamethasone via a transtympanic route (0.3 ml, 1.2 mg dexamethasone). Middle-ear mucosa was obtained for histopathology 30 minutes after histamine administration. The following parameters were assessed: inflammation, acute inflammatory component, presence of eosinophils, inflammatory activity and fibrosis.

Results: Oedema, vascular dilatation and congestion, inflammation, the presence of an acute (polymorphonuclear) inflammatory component, the presence of eosinophils, and inflammatory activity were found to be of a lesser grade in the mucosae of group B. All differences were found to be statistically highly significant ($p < 0.01$) using the Mann–Whitney test.

Conclusion: Our findings validate the transtympanic route of dexamethasone administration in counteracting histamine effects.

Key words: Middle Ear; Mucous Membrane; Inflammation; Steroids; Animal Model

Introduction

Otitis media with effusion (OME) remains a common problem in the Western world, often necessitating surgical intervention. Animal studies allow observation of the effects of manipulation, which would otherwise not be possible or ethical in humans, since OME is a difficult disease to study clinically.[1]

The inflammatory response starts with relaxation of smooth muscle cells, causing vasodilation and an increase in blood flow. As the process continues, alterations in vascular permeability, migration of phagocytic leukocytes and phagocytosis are observed.

Histamine is involved in the production and persistence of the inflammatory reaction observed in OME.[2] It appears to be a local product of the middle-ear lining rather than a transudate from plasma. Animal studies have shown that histamine is released from mast cells located in the subepithelial layers of the tympanic mucoperiosteum.[3] Mast cells were reported to be present in the pars flaccida of the tympanic membrane as well as in the lamina propria of the middle-ear mucosa, and their number seems to increase considerably in certain types of OME.[4]

The absence of any significant difference between the histamine levels in the effusions of allergic and non-allergic patients indicates that allergy is not the sole mechanism involved in histamine release.[3] Findings from previous studies suggest the importance of local histamine challenge when assessing the effects of anti-inflammatory drugs.[5] On the other hand, little has been done to assess the efficacy of corticosteroid or antihistamine therapy as prophylaxis during an upper respiratory infection or in allergic patients with inhalant allergies.[5]

The present study explored the histopathologic effect of two different routes of steroid administration, within an experimental, histamine-induced middle-ear inflammation.

Materials and methods

Fifty healthy rabbits weighting 1500–1800 g were enrolled in the study and housed in the animal

From the Department of Otolaryngology, University of Crete School of Medicine, Crete, and the *Department of Pathology, University of Athens Medical School (Attikon Hospital), Athens, Greece.
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vivarium at the University of Crete School of Medicine. The project was approved by the animal care and use committee of the University of Crete School of Medicine. Food and water were provided *ad libitum* throughout the study. Before intervention, all ears were found to be normal upon otoscopic examination, and the external auditory canals were cleaned with alcohol. Otorrhoea developed in two ears during the experimental procedure, resulting in exclusion of these rabbits from the study.

Ten animals were used as a control group. After baseline otomicroscopy, these animals received 0.5 ml histamine 20 mg/ml, injected transtympanically with a 25-gauge needle into the inferior-anterior quadrant of the tympanic membrane on the right. In the 20 rabbits comprising group A, a three day intramuscular pretreatment with dexamethasone at 1 mg/kg per day was followed by histamine challenge. In the remaining 20 rabbits comprising group B, pretreatment with dexamethasone via a transtympanic route (0.3 ml, 1.2 mg dexamethasone) was followed by histamine challenge. Animals were comparable regarding age, sex and weight in all groups. All otoscopic examinations and transtympanic injections were performed under general anaesthesia using ketamine HCl (35 mg/kg intramuscularly) and xylazine (5 mg/kg intramuscularly), according to the methods recommended by the Hellenic National Bioethics Commission for anaesthesia of common laboratory animals.

The animals were sacrificed 30 minutes after histamine administration, using intravenous sodium pentobarbital (90 mg/kg) through the left marginal ear vein. Middle-ear mucosa was removed by opening the middle-ear bulla with the use of a surgical microscope. Tissue fragments were fixed in a buffered 10 per cent formalin saline solution for 24 hours and then processed and embedded in paraffin blocks. Six consecutive, 3 μm thick sections were cut and stained with a standard haematoxylin and eosin procedure.

The following parameters were blindly assessed by the same pathologist:[6] oedema, vascular dilatation and congestion, inflammation, acute inflammatory component (i.e. polymorphonuclear neutrophils), presence of eosinophils, inflammatory activity (i.e. infiltration of the epithelium by inflammatory cells), and fibrosis. For all parameters, the number of cells or the vessel area was assessed in five optical fields selected according to the random method described by Fleege *et al.*[7] Assessment was performed using a 63 square grid at a ×400 total magnification (i.e. with a ×40 objective and a ×10 eyepiece, and with a standard optical tube length of 160 mm).[8] A semi-quantitative grading of zero to three was used to quantify all parameters.[9] Moreover, epithelial alterations (squamous metaplasia, tumours) and necrosis, as well as the presence of bone, were noted. All sections were reviewed blindly by the same pathologist.

Statistical testing was performed with the non-parametric Mann–Whitney test. A p value of <0.05 was considered significant.

Results and analysis

All ears had normal tympanic membranes before the intervention. Figure 1 shows normal rabbit middle-ear mucosa, with a single layer of squamoid–cuboidal epithelium and scant lymphocytes and histiocytes in the lamina propria. The effect of histamine on middle-ear mucosa was observed in the control group (Figure 2); sections of middle-ear mucosa observed 30 minutes after transtympanic injection of histamine revealed dilated, hyperaemic vessels, many eosinophilic leukocytes and excessive stromal oedema. Table I shows the results reported by the pathologist, using a semi-quantitative grading system, for the seven parameters studied.

The mucosae from group B (transtympanic administration of dexamethasone) (Figure 3) was compared with that from group A (systemic administration of dexamethasone) (Figure 4). Group B muscosae showed lower grades of the following: oedema, vascular dilatation and congestion, inflammation, the presence of an acute (polymorphonuclear) inflammatory component, the presence of eosinophils, and inflammatory activity. All differences were found to be statistically highly significant ($p < 0.01$) using the Mann–Whitney test. Fibrosis was the only parameter for which no statistically significant difference was found between the two groups ($p = 0.560$) (Table II).

An unexpected finding was a squamous cell carcinoma of the middle-ear mucosa in one rabbit of group A.

Discussion

The reported incidence of allergy in children with otitis media with effusion (OME) varies, with the highest reported incidences ranging from 15 to 35 per cent.[10,11] The child with OME is usually (80–90 per cent) atopic, with primed eosinophils, mast cells and neutrophils which, for undetermined reasons, respond in a different manner from those of non-atopic individuals. Like any other allergic disease, OME in an atopic child will resolve only when the patient's atopy is identified and

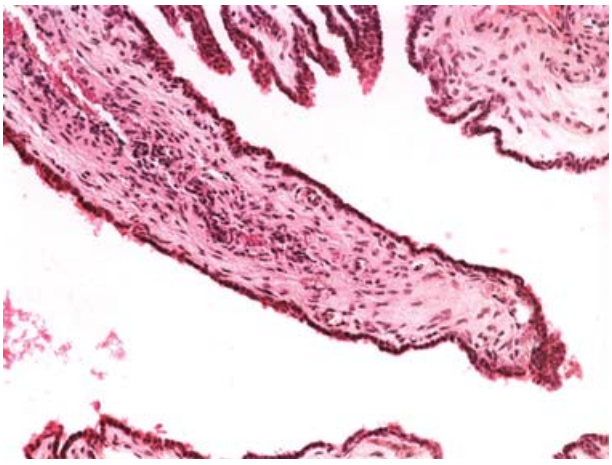

FIG. 1
Normal middle-ear mucosa (H&E; ×400).

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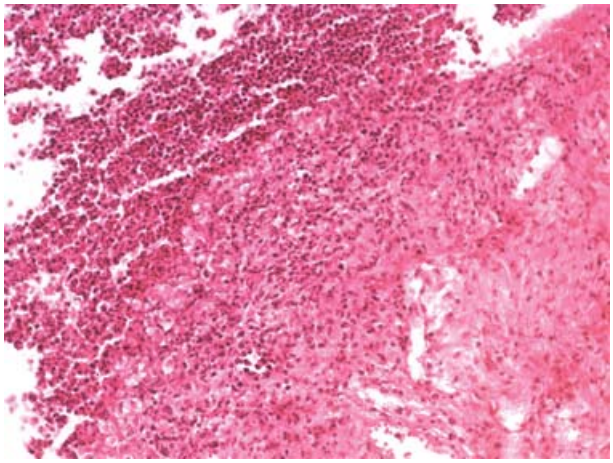

FIG. 2
Middle-ear mucosa after transtympanic administration of histamine, control group (H&E; ×400).

aggressively treated. On the other hand, allergy is not the only mechanism involved in histamine release.[3]

Rabbits are among the most frequently used animals in experimental models, as their maintenance, housing and reproduction are comparatively easy. Cases of human and rabbit OME have been reported commonly to share various features: effusion, goblet cell transformation, mucosal inflammation and airway bacterial colonisation.[12] The present study of middle-ear inflammation was based on *ex vivo* methodology. The inflammation model was designed with transtympanic administration of histamine solution.

Histamine is a hydrophilic molecule comprising an imidazole ring and an amino group connected by two methylene groups. The pharmacologically active form at all histamine receptors is the monocationic Nγ-H tautomer.[13] Histamine is one of the preformed mediators stored in the mast cell. Its release

TABLE I
HISTOLOGICAL EFFECTS OF HISTAMINE ON RABBIT MIDDLE-EAR MUCOSA, GRADED FROM 0 TO 3*

Subject	Oedema	Vascular dilation	Inflammation	Acute inflammatory component	Eosinophils	Inflammatory activity	Fibrosis
Group A†							
1	1	2	2	2	2	2	0
2	2	2	1	2	3	2	0
3	2	3	3	1	2	2	1
4	3	2	2	2	3	2	0
5	1	2	1	2	1	2	3
6	2	0	3	2	2	1	0
7	2	1	2	2	1	3	0
8	2	2	1	2	2	0	1
9	3	0	0	1	2	1	3
10	3	3	2	1	3	0	2
11	2	2	3	2	1	3	0
12	2	1	2	1	2	0	1
13	1	3	2	2	3	0	3
14	1	2	2	2	2	3	2
15	3	1	2	2	2	2	0
16	3	2	2	0	2	2	1
17	2	2	3	1	2	2	0
18	2	1	2	2	3	3	3
19	1	2	2	2	3	3	0
20	3	3	3	0	2	2	1
Group B‡							
21	1	1	0	0	1	0	0
22	0	0	0	0	0	0	0
23	0	1	0	0	0	0	0
24	0	0	1	0	1	1	2
25	1	2	0	1	0	1	2
26	0	0	1	1	0	1	0
27	0	1	0	1	1	2	2
28	1	0	1	0	0	0	0
29	0	1	0	0	0	0	2
30	0	0	0	1	2	0	0
31	0	1	1	0	0	1	1
32	0	0	0	1	2	1	0
33	1	2	1	0	0	0	0
34	2	0	0	1	1	1	0
35	0	2	0	1	0	0	3
36	0	1	0	1	2	1	3
37	1	0	0	0	0	1	0
38	0	1	1	0	1	0	0
39	1	0	1	1	0	2	1
40	0	1	0	1	0	0	1

*0 = normal middle-ear mucosa (no evidence of acute or chronic inflammation), and 3 = most intense effect (for each parameter).
†Group A (20 rabbits) = histamine challenge following 3 day intramuscular dexamethasone pretreatment; ‡group B (20 rabbits) = histamine challenge following transtympanic dexamethasone pretreatment. See text for explanation of parameters.

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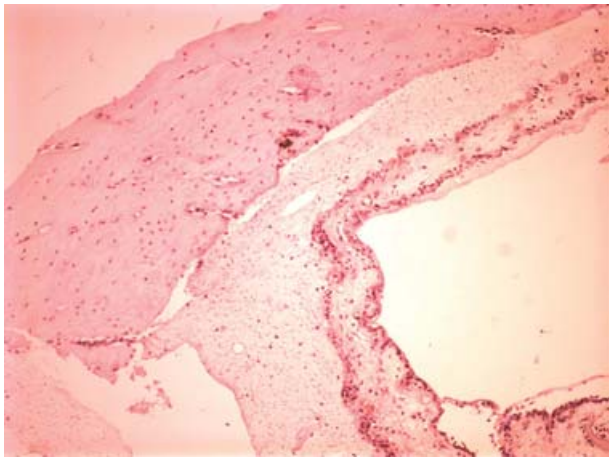

FIG. 3

Middle-ear mucosa after histamine challenge and pretreatment with transtympanic dexamethasone. A fragment of osseus tissue is seen on the left (H&E; ×250).

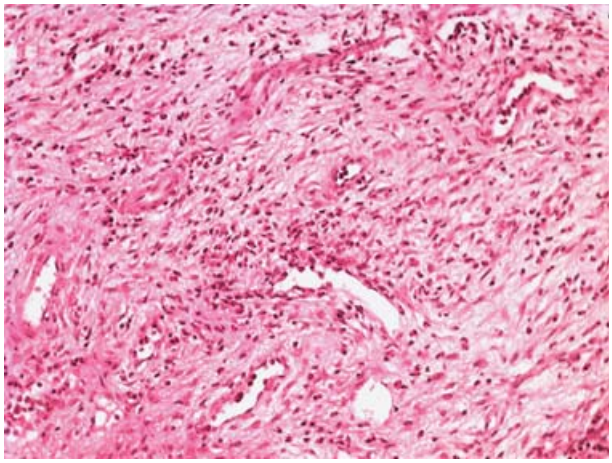

FIG. 4

Middle-ear mucosa after histamine challenge and pretreatment with systemic dexamethasone (H&E; ×400).

(as a result of interaction between antigen and immunoglobulin E antibodies on the mast cell surface) plays a central role in immediate hypersensitivity and allergic responses. Histamine causes dilation of the finer blood vessels and greater capillary permeability. Vasodilation involves both H1 and H2 receptors distributed throughout the resistance vessels in most vascular beds. The H1 receptors have a higher affinity for histamine and mediate a dilator response that is relatively rapid in onset and short-lived. By contrast, activation of H2 receptors causes dilation that develops more slowly and is more sustained.[13]

It has been shown that histamine injection into the guinea pig middle-ear cavity produces dilation and endothelial disjunction in capillaries, leading to striking mucosal oedema and effusion, persisting for at least several hours.[14] Increased capillary permeability results mainly from the actions of histamine on postcapillary venules; H1 receptors clearly are important in this response. Histamine causes the endothelial cells to contract and separate at their boundaries, thus exposing the basement membrane, which becomes freely permeable to plasma protein and fluid.[13]

Normal rabbit middle-ear mucosa is covered by a single layer of cuboidal epithelium, with scant lymphocytes and histiocytes in the lamina propria. Transtympanic administration of histamine caused histopathologic changes in the middle-ear mucosae of the control group (Figure 2). In order to determine the best administration route for anti-inflammatory agents (e.g. dexamethasone), the effect on histamine provocation was assessed.

Dexamethasone (9-fluoro-11b,17,21-trihydroxy-16a-methylpregna-1,4-diene-3,20-dione) is a synthetic corticosteroid. Synthetic corticosteroids are primarily used for their potent anti-inflammatory effects on a variety of organ systems. They are metabolised primarily in the liver and are then excreted by the kidneys. Dexamethasone is a long-acting glucocorticoid with a 36- to 54-hour half-life. It is used therapeutically for endocrine disorders, rheumatic disorders, collagen and skin diseases, allergic states, ophthalmic diseases, respiratory diseases, neoplastic disorders, and oedema.[15] However, the prolonged use of high-dose steroids is not innocuous. Their serious side effects include: diabetes mellitus, psychosis, glaucoma, osteoporosis, aseptic necrosis of femoral and humeral heads, peptic ulcers, menstrual irregularities, and development of a cushingoid state.[15]

Corticosteroids have been administered via the transtympanic route to treat inner-ear disorders (such as sudden idiopathic sensorineural hearing loss, autoimmune hearing loss and Ménière's disease), with the most common risks being pain, momentary vertigo, otitis media and tympanic membrane perforation.[16]

TABLE II

STATISTIC ANALYSIS OF HISTOLOGICAL PARAMETERS

Test	Oedema	Vascular dilatation	Inflammation	Acute inflammatory component	Eosinophils	Inflammatory activity	Fibrosis
Mann–Whitney U	24.500	74.000	24.000	55.000	33.000	81.000	180.000
Wilcoxon W	234.500	284.000	234.000	265.000	243.000	291.000	390.000
Z	−4.932	−3.558	−4.965	−4.166	−4.711	−3.357	−0.583
Asymp sig (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.001	0.560
Exact sig [2(1-tailed sig)]	0.000(a)	0.000(a)	0.000(a)	0.000(a)	0.000(a)	0.001(a)	0.602(a)

Asymp = Asymptotic; sig = Significance

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Theoretically, the transtympanic route of corticosteroid administration has two main advantages.[17] Firstly, there is an immediate onset of action limited to the area of inflammation, thus reducing systemic steroid absorption and toxicity. Secondly, there does not seem to be any toxic effect on the inner ear. Shirwany *et al*. showed that transtympanic injection of dexamethasone increased cochlear blood flow and had no effects on auditory sensitivity or cochlear histology in a guinea pig model.[17]

Anaesthesia and intratympanic injections were performed according to our institutional guidelines regarding animal experimentation. After specimen collection, preparation and standard haematoxylin and eosin staining, sections were assessed by the same pathologist in order to achieve uniformity of assessment.[18] A semi-quantitative, zero to three scale was used to grade each of the inflammatory parameters assessed.[6] Zero represented normal middle-ear mucosa, without any evidence of acute or chronic inflammation; three represented the most intense effect for each parameter.

- **Rabbit middle-ear mucosa can be used as an *ex vivo* model for the study of otitis media with effusion (OME)**
- **Corticosteroids may be used against allergy-mediated OME, either by systemic or transtympanic administration**
- **Transtympanic dexamethasone administration seems to be more effective than systemic administration against histamine-induced OME in an *ex vivo* experimental model**
- **This study demonstrates the possible efficacy of transtympanic corticosteroid administration as a prophylactic treatment in allergic patients with persistent OME or in cases of immune-mediated OME**

Based on Mann–Whitney statistical analysis, group B showed very significant statistical differences ($p < 0.01$) for all inflammatory parameters except fibrosis. Thus, our data validate the transtympanic route of dexamethasone administration in counteracting the inflammatory effects of histamine.

Future study will assess the use of other anti-inflammatory agents (e.g. methylprednisolone, ranitidine, piroxicam and loratadine), singly and in combination. We will aim to determine the optimal route of administration, and the most effective anti-inflammatory factors, for the treatment of allergy or immune-mediated otitis media with effusion.

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Address for correspondence:
Dr T S Chimona,
9 Iosif Vriga St,
GR-73100 Chania,
Crete, Greece.

E-mail: chimonath@yahoo.gr

Dr T S Chimona takes responsibility for the integrity of the content of the paper.
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