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Anti-inflammatory effect of erythromycin on histamine-induced otitis media with effusion in guinea pigs

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Abstract

In this study, the anti-inflammatory effect of erythromycin was investigated in a model of histamine-induced otitis media with effusion (OME). OME was induced in guinea pigs by the transtympanic injection of histamine solution into the middle-ear cavity. Guinea pigs were randomly assigned to one of three groups: control, erythromycin treatment, or methylprednisolone treatment. After histamine injection, the animals were treated with intraperitoneal medication for five days consecutively. Afterwards, the animals were sacrificed and the temporal bones were removed. The samples were examined stereologically.

In the erythromycin-treated group, it was observed that neutrophil infiltration was significantly inhibited when compared to the control group. This result shows that erythromycin may produce a significant anti-inflammatory effect in this model of OME.

Key words: Otitis Media with Effusion; Erythromycin; Guinea Pigs

Introduction

Otitis media with effusion (OME) is a very common disease, especially in childhood and infancy and is characterized by non-purulent fluid in the middle ear and fluctuating conductive hearing loss. OME is an inflammatory response of the middle ear caused by multiple factors such as viral or bacterial infection, eustachian tube dysfunction, or allergy. Inflammatory mediators, such as histamine, kinin, leukotrienes, prostaglandins, platelet-activating factor, lysosomal enzymes, hydrolytic enzymes, or protease and neutrophil chemotactic factors, seem to play a major role in the pathogenesis of OME.[1–4] Current treatment of OME consists of antimicrobial therapy, decongestants, corticosteroids, in certain cases antihistamines or the insertion of ventilation tubes in the eardrum in unresponsive cases. OME is an important problem, making life harder for the patients and leading to economic loss. Therefore, physicians have investigated more effective treatment strategies.

Macrolide antibiotics have been used for the treatment of infectious diseases. Erythromycin is a well-known macrolide antibiotic and it is active against such organisms as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Streptococcus viridans*.[5] Apart from their antibacterial activity macrolide antibiotics, also have anti-inflammatory activity, increase mucociliary transport and decrease goblet cell secretion.[6–9]

There is also increasing evidence that macrolide antibiotics are effective in the treatment of chronic diseases such as OME, sinobronchial syndrome and diffuse panbronchiolitis.[10–12] The clinical effects of macrolide antibiotics may depend on their anti-inflammatory and anti-secretory effects, as well as their antimicrobial properties.

We hypothesized that erythromycin would be as effective as anti-inflammatory agents, including corticosteroids, for the treatment of experimental aseptic OME. Therefore, the objective of the present experiment was to determine the anti-inflammatory effect of erythromycin and methylprednisolone on histamine-induced OME in guinea pigs. Stereological and histopathological findings (neutrophil infiltration and vasodilatation in blood vessels) were considered for evaluation of the degree of severity of experimental OME.

Materials and methods

Animal, management and treatment

All animals received humane care in compliance with the guidelines of Atatürk University Research Council's criteria. Twenty-one albino guinea pigs between 600–650 g obtained from the Research Institute of Animal Diseases, Erzurum, Turkey,

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were used in this study. After two weeks adjustment, histamine solution was injected to the right middle-ear cavity of the animals. Twelve hours after histamine injection, the animals were separated into three groups, receiving erythromycin or methylprednisolone or saline. Guinea pigs were administered intraperitoneal medication twice daily for five days. Erythromycin was given at 25 mg/kg per day and methylprednisolone was given at 2 mg/kg per day. Two hours after ending the administration period, animals were anaesthetized (100 mg/kg ketamine hydrochloride and 3 mg/kg diazepam, intraperitoneal) and were sacrificed with an intracardiac injection of a high dose of sodium pentobarbital.

Induction otitis media with effusion

Absence of middle-ear pathology was established by otomicroscopic examination and tympanometry. Histamine dihydrochloride (Sigma) was added to normal saline solution (1 mg/ml), and the pH of this solution was adjusted to 7.4 with sodium hydroxide. Before the histamine injection, animals were anaesthetized (100 mg/kg ketamine hydrochloride and 3 mg/kg diazepam, intraperitoneal) and 0.1 ml histamine was injected via a 27-gauge needle through the right tympanic membrane to the middle-ear cavity.[1]

Histopathological procedures

Immediately after sacrifice, the temporal bones were carefully dissected. For histopathological examinations, the bullae were fixed with formalin (10 per cent, vol/vol). After fixation, the bullae were decalcified with EDTA (10 per cent, wt/vol) for 10 days at 4°C and the right bulla of each animal was embedded within paraffin.

Microscopy for stereological examination

Embedded sections (4 μm) were cut serially with a Leica RM2155 microtome, and stained with haematoxylin and eosin (H & E).

A detailed description of the dissector method is found in Sterio (1984).[13] Data were obtained from dissector pairs, which consisted of pairs of parallel sections taken at known intervals until the tissue samples were exhausted. Two consecutive sections (a section and its adjacent section) were mounted on each slide. The same field on two consecutive sections on a slide was found and photographed. Thirty dissector pairs were found and photographed in each block to be analysed in this study. The section pairs, consisting of two adjacent sections, on slide were examined together according to the dissector counting method rule. First rule an unbiased counting frame was superimposed on each micrograph that was obtained from the adjacent section on slides to perform the counting. The bottom and left hand edges of the frame were considered to be the forbidden lines together with the extension lines. Other boundaries of the frame and the top-right corner were considered to be inclusion points and any particle which hit these lines or was located inside the frame counted as a dissector particle. The profiles of interesting object (neutrophil leukocyte in our study) were counted in case they were visible in the references section (one of consecutive sections on each slides) but not in the look-up section (other section on each slide).[14]

The mean numerical density of neutrophils (N_v neutrophils) per μm^3 was estimated using the following formula.[13]

$$Nv\text{neutrophils} = \Sigma Q^{-}\text{ neutrophils} / t \times A$$

Where $\Sigma Q^{-}_{(\text{neutrophils})}$ is the total number of counted neutrophils profiles appearing only in the reference sections; t is the section thickness and A is the area of the counting frame.

Rate vascular dilatation with stereological method

In the vascular response of inflammation, stereological assessments are based on the Cavalieri volume estimation method. According to the first important rule of this principle, an unbiased estimate of the volume of the object to be studied must be obtained by sectioning the object with a series of parallel planes separated by a fixed distance (t).[15–17]

The volume fraction of a phase that is estimated within the reference volume is the proportion of each unit volume of the reference space. Volume fraction was estimated by the following formula in our study:

V_v = *Volume of phase interesting area in reference space/Volume of reference space*

We placed a frame with known size on the area of interest, and then a modified point counting grids were placed on first frame with known size to obtain the surface area of interesting profiles (blood vessels in this study). The point density of the point grid was designed to obtain an appropriate coefficient of error (CE) for the serial sections of our study. The coefficient of error and coefficient of variation (CV) were estimated according to Gundersen and Jensen's formula, 1987.[18] All points hitting the object of interest were counted according to a fixed rule. The area (a μm^2) of per test point (k μm) is equal to k × k μm^2. Subsequently, the volumes of the blood vessels in each section are estimated from

$$Volume\ (\mu m^3) = t\ (\mu m) \times a/p\ (\mu m \times \mu m) \times (\Sigma P)$$

Where V is the volume of the object of interest (blood vessels) in one section plane, t the section thickness, a/p interpoint area, and ΣP the number of point hitting the temporal bone in that section. After the same formula was applied to the other sections, total volume that is wanted to be estimated was obtained from

$$Total\ Volume = V_1 + V_2 + \ldots + V_n$$

According to our sampling procedure, an average of 30 pairs of sections for the physical dissector method, 10 sections for the Cavalieri volume

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TABLE I
MEAN ± SD VALUES OF NEUTROPHIL INFILTRATION (NUMBER DENSITY) AND VASODILATATION (%) IN MIDDLE EAR MUCOSA OF GUINEA PIG WITH EXPERIMENTAL OTITIS MEDIA WITH EFFUSION

	Control (n = 7)	Methylprednisolone (n = 7)	Erythromycin (n = 7)
Neutrophil infiltration (number density)	0.11 ± 0.04	0.01 ± 0.005[b]	0.04 ± 0.03[b]
Vasodilatation (%)	5.2 ± 1.8	7.9 ± 2.6[c]	14.4 ± 8.2[a]

a = $p<0.01$, b = $p<0.001$, *vs.* control group, c = $p<0.05$, *vs.* erythromycin.

estimation method were sufficient to a significant coefficient of error of which the highest limit is accepted as five per cent.[18]

Finally, we calculated a ratio of our interesting volume by using Cavalieri method within the reference space.[15–17]

Statistical analysis

For statistical analysis, parameters were analysed by one-way analysis of variance (ANOVA). The least significant difference (LSD) multiple range test was used to compare means (significance = $p<0.05$). Statistical analyses were performed with the Statistical Package for the Social Sciences (version 10.0, SPSS Inc., Chicago, IL). Results are reported as mean ± SD.

Results

All parameters are shown in Table I. As seen from the Table, neutrophil infiltration and vasodilatation were detected across all treatment and control groups. Neutrophil infiltration was the most severe in guinea pigs treated with saline (Figure 1). The degree of severity of neutrophil infiltration was reduced in the animals treated with methylprednisolone or erythromycin when compared with those treated with saline ($p<0.001$). Inhibition of neutrophil infiltration was the most pronounced in animals treated with methylprednisolone (Figure 2), but there was no significant difference between the methylprednisolone group and erythromycin group ($p>0.05$).

Vasodilatation was observed in all the treatment and control groups. Among three groups, vasodilatation was found to be most pronounced in the erythromycin group ($p<0.01$) (Figure 3) and the least pronounced in the saline group. Although there was a significant difference between the erythromycin and methylprednisolone groups ($p<0.05$), no significant difference was found between the methylprednisolone and saline groups ($p>0.05$).

Discussion

Stereological procedures are efficient methods, if you want to estimate quantitative analyses, interesting number of objects in any biological tissue. This method used in our study involves a hierarchy of systematic random sampling combined with the physical dissector.

Although a lot of useful information has been obtained from different types of studies of inflammatory cells such as structural,[19] ultrastructural,[20] quantitative[21] and clinical[22] information up until now there has only been one study using this stereological method in the literature.[23]

It is known that recalcitrant sinusitis may occur in association with a prolonged and excessive state of inflammation rather than a simple bacterial infection.[24] The neutrophil exudation and generation of chemotactic mediators by the exudative cells at the site of inflammation results in sinusitis becoming chronic.[25] The degree of mucosal destruction is correlated to the number of infiltrating inflammatory cells, but not with the number of invading bacteria.[26]

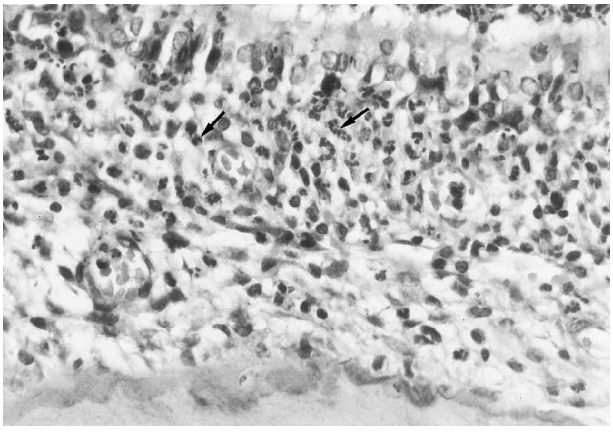

FIG. 1
Control group: most severe neutrophil infiltration (arrow) (H & E; ×200).

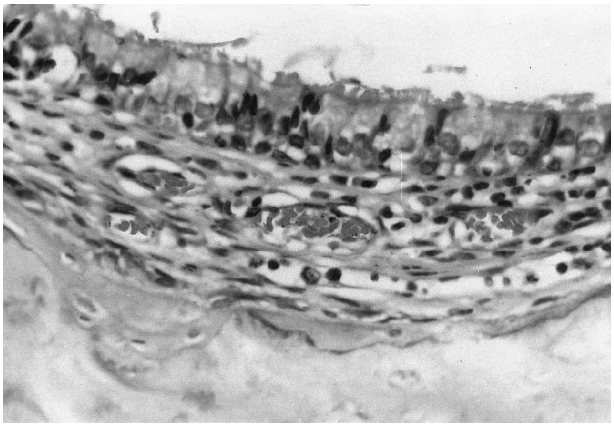

FIG. 2
Methylprednisolone group: minimal neutrophil infiltration and vasodilatation (H & E; ×200).

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FIG. 3
Erythromycin group: minimal neutrophil infiltration and severe vasodilatation (arrow) (H & E; ×200).

This explanation may be valid for OME. It is supported by the fact that some cases of refractory OME may be treated with corticosteroids.

- **The anti-inflammatory effect of erythromycin is investigated in the model of histamine-induced otitis media with effusion (OME) in guinea pigs. They were randomly assigned to three groups: control, erythromycin treatment, or methylprednisolone treatment**
- **In the erythromycin treated group, it was observed that neutrophil infiltration was significantly inhibited as compared to the control group**
- **Erythromycin may produce a significantly anti-inflammatory effect in this model of OME**

In the present study, administration of erythromycin inhibited significantly neutrophil infiltration to the middle ear mucosa when compared to the control group. This result showed that erythromycin had an anti-inflammatory effect in the middle ear. This inhibition may be attributed to the inhibiting effect of erythromycin on the production of cytokines and pro-inflammatory mediators.[9,27] It was observed that there was a significant reduction in neutrophil chemotactic activity after erythromycin treatment in the patients with diffuse panbronchiolitis.[28] Moreover, it was suggested that erythromycin inhibits neutrophil infiltration into the middle-ear cavity, affecting both the first and second stages of leukocyte adhesion by modulating the expression of adhesion molecules such as L-selectin and Mac-1.[8]

Unexpected vasodilatation was the most pronounced in animals treated with erythromycin and there was no meaningful relationship between the degree of neutrophil infiltration and vasodilatation in all the groups. It may be possible that erythromycin may cause vasodilatation. Further studies are necessary to find out the effect of erythromycin on blood vessels.

Because of their side-effects, it is preferable not to use corticosteroids in the treatment of OME. But, in our study, inhibition of neutrophil infiltration was the most pronounced for guinea pigs treated with methylprednisolone in this model of OME. However, there was no statistical difference between the erythromycin group and the methylprednisolone group with regard to neutrophil infiltration. So, due to the fact that erythromycin unlike corticosteroids does not have adverse effects and has considerable anti-inflammatory effects,[9] improves ciliary motion,[10,29] and inhibits mucus secretion,[6] it may be the most effective antibiotic for the treatment of OME. However, further studies will be necessary to find out the effects of other macrolide antibiotics on OME.

References

1 Boisvert P, Wasserman SI, Schiff M, Ryan AF. Histamine-induced middle ear effusion and mucosal histopathology in the guinea pig. *Ann Otol Rhinol Laryngol* 1985;**94**:212–6
2 Skoner DP, Stillwagon PK, Casselbrandt ML, Tanner EP, Doyle WJ, Fireman P. Inflammatory mediators in chronic otitis media with effusion. *Arch Otolaryngol Head Neck Surg* 1988;**114**:1131–3
3 Goldie P, Hellstrom S, Idahl LA. Middle ear effusion induced by various inflammatory mediators and neuropeptides. An experimental study in the rat. *Acta Otolaryngol* 1989;**108**:246–52
4 Rhee CK, Jung TT, Miller S, Weeks D. Experimental otitis media with effusion induced by platelet activating factor. *Ann Otol Rhinol Laryngol* 1993;**102**:600–5
5 Chambers HF. Protein synthesis inhibitors and miscellaneous antibacterial agents. In: Hardman JG, Limbird LE, eds. *The Pharmacological Basis of Therapeutics*. 10 edn. New York: McGraw-Hill, 2001:1239–71
6 Takahashi Y, Shimizu T, Sakakura Y. Effects of indomethacin, dexamethasone and erythromycin on endotoxin-induced intraepithelial mucus production of rat nasal epithelium. *Ann Otol Rhinol Laryngol* 1997;**106**:683–7
7 Sugiura Y, Ohashi Y, Nakai Y. Roxythromycin stimulates the mucociliary activity of the Eustachian tube and modulates neutrophil activity in the healthy guinea pig. *Acta Otolaryngol* (Suppl) 1997;**531**:34–8
8 Enomoto F, Ichikawa G, Nagaoka I, Yamashita T. Effect of erythromycin on otitis media with effusion in experimental rat model. *Acta Oto-Laryngol Suppl* 1998;**539**:57–60
9 Ianaro A, Ialenti A, Maffia P, Sautebin L, Rombola L, Carnuccio R, *et al*. Anti-inflammatory activity of macrolide antibiotics. *J Pharmacol Exp Ther* 2000;**292**:156–63
10 Iino Y, Sugita K, Toriyama M, Kudo K. Erythromycin therapy for otitis media with effusion in sinobronchial syndrome. *Arch Otolaryngol Head Neck Surg* 1993;**119**:648–51
11 Fujii T, Kadota J, Kawakami K, Iida K, Shirai R, Kaseda M, *et al*. Long term effect of erythromycin therapy in patients with chronic pseudomonas aeruginosa infection. *Thorax* 1995;**50**:1246–52
12 Ernstson S, Sundberg L. Erythromycin in the treatment of otitis media with effusion. *J Laryngol Otol* 1984;**98**:767–9
13 Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the dissector. *J Microsc* 1984;**134**:127–36
14 Unal B, Bradley PM, Sahin B, Canan S, Aslan H, Kaplan S. Estimation of numerical density and mean synaptic height in chick hippocampus 24 and 48 hours after passive avoidance training. *Brain Res Dev Brain Res* 2002;**136**:135–44
15 Cruz-Orive LM, Weibel ER. Recent stereological methods for cell biology: a brief survey. *Am J Physiol* 1990;**258**:L148–56

16 Gundersen HJ. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. *J Microsc* 1986;**143**:3–45
17 Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, *et al*. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* 1988;**96**:379–94
18 Gundersen HJG, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. *J Microsc* 1987;**147**:229–63
19 Norlander T, Forsgren K, Kumlien J, Stierna P, Carlsoo B. Cellular regeneration and recovery of the maxillary sinus mucosa. An experimental study in rabbits. *Acta Oto-Laryngol Suppl* 1992;**492**:33–7
20 Polosukhin VV. Ultrastructure of the blood and lymphatic capillaries of the respiratory tissue during inflammation and endobronchial laser therapy. *Ultrastruct Pathol* 2000;**24**:183–9
21 Knipping S, Holzhausen HJ, Mir Salim PA, Berghaus A. Chronic rhinosinusitis in hypogammaglobulinemia. A morphological study. *HNO* 2002;**50**:644–8
22 Engelke W, Schwarzwaller W, Behnsen A, Jacobs HG. Subantroscopic laterobasal sinus floor augmentation (SALSA): an up-to-5-year clinical study. *Int J Oral Maxillofac Implants* 2003;**18**:135–43
23 Fagerland JA, Hagemoser WA, Ireland WP. Ultrastructure and stereology of leukocytes and platelets of normal foxes and a fox with a Chediak-Higashi-like syndrome. *Vet Pathol* 1987;**24**:164–9
24 Norlander T, Westrin KM, Stierna P. The inflammatory response of the sinus and nasal mucosa during sinusitis: implications for research and therapy. *Acta Otolaryngol Suppl* 1994;**515**:38–44
25 Suzuki H, Takahashi Y, Wataya H, Ikeda K, Nakabayashi S, Shimomura A, *et al*. Mechanism of neutrophil recruitment induced by IL-8 in chronic sinusitis. *J Allergy Clin Immunol* 1996;**98**:659–70
26 Lundberg C, Engquist S. Localization of bacteria and the cause of tissue destruction in maxillary sinusitis. *Acta Oto-Laryngol Suppl* 1984;**407**:30–2
27 Suzuki H, Asada Y, Ikeda K, Oshima T, Takasaka T. Inhibitory effect of erythromycin on interleukin-8 secretion from exudative cells in the nasal discharge of patients with chronic sinusitis. *Laryngoscope* 1999;**109**:407–10
28 Kadota J, Sakito O, Kohno S, Sawa H, Mukae H, Oda H, *et al*. A mechanism of erythromycin treatment in patients with diffuse panbronchiolitis. *Am Rev Respir Dis* 1993;**147**:153–9
29 Tamaoki J, Chiyotani A, Sasaki N, Takeyama K, Takizawa K. Effect of erythromycin on ciliary motility in rabbit airway epithelium in vitro. *J Antimicrobial Chemother* 1992;**29**:173–8

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