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The effects of methylprednisolone, montelukast and indomethacine in experimental otitis media with effusion

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ABSTRACT

Objectives: The present study aimed to investigate the effects of indomethacine, montelukast and methylprednisolone in management of experimental otitis media with effusion.
Methods: Forty Wistar albino rats of which the weights ranged between 310 and 370 g were included in this study. Middle ear effusion was created by transtympanic histamine injection. The presence of effusion was confirmed by otomicroscopic examination. Thirty-seven rats with effusion were divided into 4 groups (methylprednisolone, montelukast, indomethacine and saline-control groups). All agents were administered for a period of consecutive 10 days. At the 11th days of administration, the recovery of effusion was confirmed by otomicroscopic examination. Tympanic bullae of the rats were removed and histopathological examinations were carried out. In the histopathological examination, the neutrophil leukocytes accumulated in the middle ear submucosa were counted.
Results: The mean numbers of submucosal neutrophils in the methylprednisolone, montelukast, indomethacine, and saline groups were 24.6 ± 8.1, 54.1 ± 6.2, 52.3 ± 7.3, 55.7 ± 8.3, respectively. The otomicroscopic recovery rates of effusion in the methylprednisolone, montelukast, indomethacine, and saline groups were 18/18 (100%), 8/18 (44%), 2/14 (14%), 2/18 (11%) respectively.
Conclusions: Methylprednisolone and montelukast ameliorate the middle ear effusion. However, only methylprednisolone reduces the submucosal infiltration of the neutrophil leukocytes which are the most evident cell of inflammatory process. Montelukast is effective in the resolution of experimental otitis media with effusion.

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1. Introduction

Otitis media with effusion (OME) is one of the most common diseases among preschool children, and it also occurs in adults as well as in older children [1]. OME is defined as inflammation of the middle ear space accompanied by fluid collection without signs or symptoms of acute infection such as otalgia, fever or a red, bulging tympanic membrane [2]. The mechanism of OME is still not clearly, although was determined inflammatory mediators such as prostaglandins (PG), leukotrienes (LT) and platelet-activating factors (PAF) have been identified in human OME, and in the experimentally induced OME of animals [3–7].

The current treatment options for OME include eliminating the risk factors, watchful waiting without treatment, use of decongestant medication, use of antibiotic medication, maneuvers to open Eustachian tubes, and if medical treatment fails, tympanostomy tube placement [8,9]. The anti-inflammatory treatment of OME by steroids, non-steroid anti-inflammatory drugs (NSAID) and leukotriene antagonists is still controversial.

The purpose of this study was to evaluate the effects of NSAID (indomethacine), leukotriene antagonists (montelukast) and steroids (methylprednisolone) in otitis media with effusion. For this purpose, we investigated the submucosal neutrophil accumulation in the tympanic bullae with experimental OME, and evaluated otomicroscopic examination of the tympanic membranes of rats. To our knowledge, this is the first experimental study examining the comparison of effects of these three agents in OME.

2. Methods

Animals used in the present study were housed, supervised, and handled according to the Suleyman Demirel University Medical

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School Guidelines for Care and Use of Laboratory Animals. Forty male, healthy, Wistar-albino rats, with no middle ear pathology and weighing 310–370 g were enrolled in this study (Fig. 1). The experimental animals were housed in polypropylene cages under laboratory conditions of 28 ± 2 °C temperature with 75% relative humidity and photoperiod of 12 h light/dark cycle. The rats were given standard pellet diet and water ad libitum. The animals were anesthetized with intraperitoneal injections of ketamine (100 mg, Ketalar®, Parke-Davis, Turkey). Histamine dihydrochloride, (Sigma®) was dissolved in normal saline solution (1 mg/ml). Histamine solution (0.1 ml) was administered via 27-gauge needle through the right and left tympanic membranes into the middle ear cavities of all animals. All tympanic membranes and middle ears were examined by otomicroscopic examination 24 h after the histamine injection. The effusion was confirmed in 72 middle ears of 37 rats (35 bilateral, 2 unilateral) (Fig. 2).

The animals with OME were divided into four groups which have 18 middle ears with effusion in each group. Group I was the methylprednisolone group (9 rats with bilateral effusion), Group II was the montelukast group (9 rats with bilateral effusion), Group III was the indomethacine group (9 rats with bilateral effusion) and Group IV was the control group (8 rats with bilateral effusion and 2 rats with unilateral effusion). Methylprednisolone was administered to Group I animals intraperitoneally once in a day as a dosage of 1 mg/kg. Montelukast was administered to Group II animals via orogastric tube once in a day as a dosage of 1 mg/kg. Indomethacine was administered to Group III animals via orogastric tube once in a day as a dosage of 10 mg/kg. Saline was administered to Group IV animals intraperitoneally once in a day as a dosage of 0.1 ml. All these agents were administered consecutively for a period of 10 days.

After the administration of the agents for a period of 10 days, at 11th day, all animals were anesthetized by ketamine 100 mg/kg and sacrificed. All tympanic membranes and middle ears were examined by otomicroscopic examination 11th day just before the scarifications. Bilateral temporal bullae of rats were removed successfully, and fixed with 10% formalin during 24 h for histopathological examination. Thus, the bullae were decalcified with EDTA for 10 days at 4°, and embedded within paraffin. Tissue blocks (5 μm) were sectioned serially by a microtome, and stained with haematoxylin and eosin. The histopathology specimens were than blindly evaluated with light microscopy by the same pathologist. Neutrophil leucocytes were counted in 25 areas of specimens which were prepared from sub-mucosa of each tympanic bulla (100× magnified). The mean counts of submucosal neutrophil leukocyte obtained from 25 areas (MCN) in all groups were compared. Statistical analysis was performed with the non-parametric Mann–Whitney *U* test. The significance level was set at $p < 0.05$.

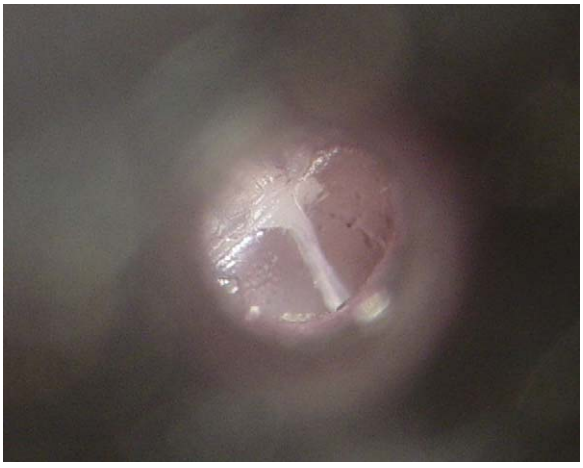

Fig. 1. The otomicroscopic view of healthy middle ear of rats, before administration of histamine.

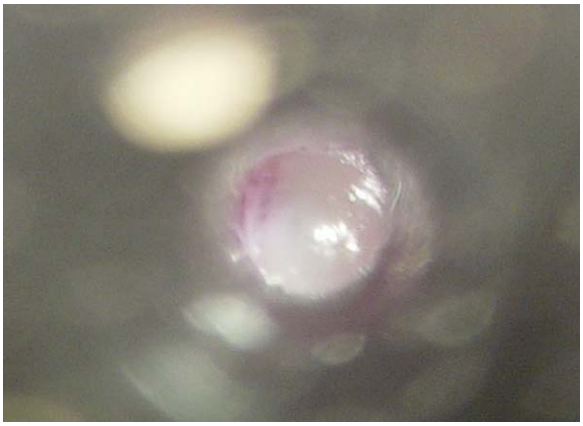

Fig. 2. The otomicroscopic view of formation of middle ear effusion.

3. Results

This study was begun with 37 rats which were confirmed to have OME. Two rats died in 4th day of the treatment in Group III. At the 11th days of histamine administration, the status of effusion in other 35 rats was evaluated by otomicroscopic examination. Then, all animals were sacrificed following otomicroscopic examination. Bilateral temporal bullae of rats were removed successfully. All histopathological specimens were examined with respect to the submucosal neutrophil infiltration.

The mean of MCN was 24.6 ± 8.1 (14–38) in Group I. The mean of MCN in Group I was 24.7 ± 9.7 (14–38) for the right ears and 24.6 ± 6.8 (17–35) for the left ears. At 11th day (otomicroscopic examination) effusions of all middle ears in Group I were recovered completely. The recovery rate of effusion in this group was 18/18 (100%) (Table 1).

The mean of MCN was 54.1 ± 6.2 (44–68) in Group II. These values were 53.6 ± 6.0 (44–63) and 54.7 ± 6.7 (46–68) for the right and left ears respectively. Effusion was recovered in 8 middle ears of Group II. The recovery rate of effusion in this group was 8/18 (44.4%) (Table 1).

The mean of MCN was 52.3 ± 7.3 (46–72) in Group III. These values were 53.1 ± 9.0 (46–72) and 51.4 ± 5.8 (46–63) for the right and left ears respectively. Effusion was recovered in 2 middle ears in Group III. The recovery rate of effusion in this group was 2/14 (14.3%) (Table 1).

The mean of MCN was 55.7 ± 8.3 (46–80) in Group IV. These values were 53.8 ± 5.2 (47–62) and 58.0 ± 11.1 (45–80) for the right and left ears respectively. Effusion was recovered in 2 middle ears in Group IV. The recovery rate of effusion in this group was 2/18 (11.1%) (Table 1).

According to the MCN, there was statistically significant difference between Group I and other groups ($p = 0.00$). There was no significant difference among other treatment groups (Group II and III) and Group IV ($p > 0.005$) (Table 2).

The recovery rate of the effusion in Group I was higher than other treatment groups (Group II and III) and control Group IV. According to the recovery rate of effusion, there was statistically significant difference between Group I and other groups ($p = 0.00$). There was statistically significant difference between recovery rate of Group II and Group IV effusions ($p = 0.03$). There was significant difference neither between Group II and Group III nor between Group III and Group IV ($p > 0.05$) (Table 2).

Table 1
Histopathological and clinical findings: MCN, mean count of submucosal neutrophil leucocytes in an ear; RO, recovery of OME; +, clinical improvement; –, no clinical improvement; RRO: recovery rate of OME.

	Number of rat	MCN/RO		The mean of MCN/RRO		
		Right ear	Left ear	Right ear	Left ear	Both ears
Group I	Methylprednisolone 1	36/+	18/+	24.7 ± 9.7/100%	24.6 ± 6.8/100%	24.6 ± 8.1/100%
	Methylprednisolone 2	34/+	28/+			
	Methylprednisolone 3	38/+	35/+			
	Methylprednisolone 4	18/+	33/+			
	Methylprednisolone 5	28/+	17/+			
	Methylprednisolone 6	14/+	21/+			
	Methylprednisolone 7	23/+	21/+			
	Methylprednisolone 8	13/+	19/+			
	Methylprednisolone 9	18/+	29/+			
Group II	Montelukast 1	52/–	49/+	53.1 ± 9.0/33.3%	54.7 ± 6.7/55.6%	54.1 ± 6.2/44.4%
	Montelukast 2	53/–	61/–			
	Montelukast 3	61/–	46/+			
	Montelukast 4	63/–	52/+			
	Montelukast 5	56/–	51/+			
	Montelukast 6	44/+	68/–			
	Montelukast 7	53/+	53/–			
	Montelukast 8	53/–	58/–			
	Montelukast 9	47/+	54/+			
Group III	Indomethacine 1	48/–	46/–	53.1 ± 9.0/28.6%	51.4 ± 5.8/0%	52.3 ± 7.3/14.3%
	Indomethacine 2	56/–	49/–			
	Indomethacine 3	47/–	63/–			
	Indomethacine 4	52/–	49/–			
	Indomethacine 5	72/–	47/–			
	Indomethacine 6	51/+	54/–			
	Indomethacine 7	46/+	52/–			
Group IV	Saline 1	49/–	80/–	53.8 ± 5.2/20%	58.0 ± 11.1/0%	55.7 ± 8.3/11.1%
	Saline 2	62/–	47/–			
	Saline 3	58/–	60/–			
	Saline 4	49/–	63/–			
	Saline 5	51/+	51/–			
	Saline 6	54/–	57/–			
	Saline 7	60/–	61/–			
	Saline 8	47/+	45/–			
	Saline 9	51/–				
	Saline 10	57/–				

4. Discussion

In inflammation, the predominant effects of the cyclooxygenase and lipoxygenase products are vasodilatation, an enhanced vasopermeability and chemotaxis for polymorphonuclear (NL) leucocytes. LTs and PGs are potent inflammatory mediators which may play a role in the formation of OME. Previous studies have shown that these metabolites are identified in OME such as 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, TXB_2, PGE_2, PGD_2, LTB_4, LTC_4, LTD_4, 5-HETE, 12-HETE, and 15-HETE [3,10].

Indomethacine is an indole derivative. It is a potent nonselective COX inhibitor, reducing neutrophil migration, and decreasing T cell and B cell proliferation [11].

Methylprednisolone is a glucocorticoid which has potent anti-inflammatory effect. This agent dramatically reduces the manifestations of inflammation. This is due to its profound effects on the concentration, distribution, and function of peripheral leukocytes and to their suppressive effects on the inflammatory cytokines and chemokines and on other lipid and glucolipid mediators of inflammation [11,12].

Table 2
Statistical analyses: MCN, mean count of submucosal neutrophil leucocytes in an ear; RRO, recovery rate of OME.

Groups	p^* (MCN)	p^* (RRO)
I–II (methylprednisolone–montelukast)	0.00*	0.00*
I–III (methylprednisolone–indomethacine)	0.00*	0.00*
I–IV (methylprednisolone–control)	0.00*	0.00*
II–III (montelukast–indomethacine)	0.21	0.72
II–IV (montelukast–control)	0.78	0.03*
III–IV (indomethacine–control)	0.18	0.79

The effects of anti-inflammatory drugs, both steroids and nonsteroids, have been studied in different animal models and human with OME. Varsano et al. [13] and Abramovich et al. [14] investigated the effects of naproxen in their clinical studies. None of them has found any otoscopic difference between the naproxen and placebo group of patients with OME. Goldie et al. [10] showed that indomethacine inhibits middle ear fluid accumulation in experimental model. Cutler et al. [15] demonstrated that transtympanic steroid reduces middle ear effusion, whereas diclofenac has no effect on effusion in their experimental studies. In our study indomethacine was an ineffective agent in resolution of effusion and submucosal accumulation of neutrophil leukocytes in OME (Figs. 3 and 4).

Previous studies showed that, steroids (systemic, intranasal or transtympanic) have the beneficial effect in treatment of OME. Steroids alone or combined with other drugs (antibiotics, decongestants, etc.) can lead to resolution of OME in the short-term management [16–19]. These studies support the short-term use of steroids to significantly improve middle ear disease and decrease the need for surgical intervention. In contrast to studies mentioned above a double-blind randomized placebo-controlled study showed that use of topical intranasal steroids is very unlikely to be a clinically effective treatment for OME in the primary care setting [20]. In their guideline, NICE group states [21] that; oral steroids lead to a quicker resolution of OME in the shorter term (2

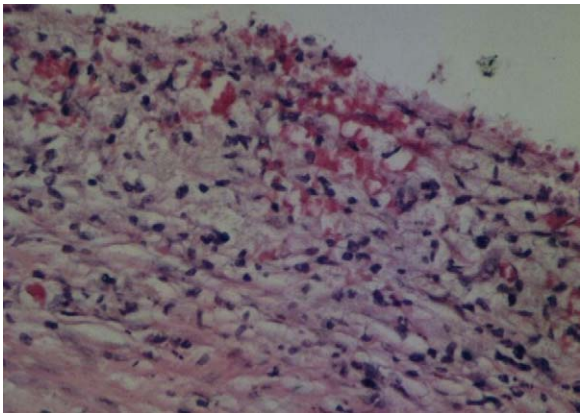

Fig. 3. Minimal neutrophil infiltration in ears of methylprednisolone group.

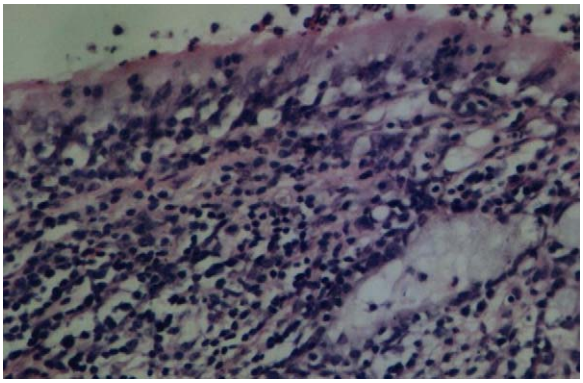

Fig. 4. Most severe neutrophil infiltration in ears of control group.

weeks) but not in the longer term. However, none of the included trials had documented hearing loss with a diagnosis of OME before starting treatment. There is insufficient good-quality evidence regarding the effectiveness of intranasal steroids. However, in our study methylprednisolone seem to be an effective agent in resolution of effusion and submucosal accumulation of neutrophil leukocytes in experimental OME.

Tada et al. [3] investigated efficacy of pranlukast in experimental OME induced by LTD4 in guinea pigs. It is suggested that LTD4 plays an important role in the formation of OME, and pranlukast may be used in treatment of OME. Balatsouras et al. [22] found that montelukast and inhaler steroids were more effective in the management of effusion than only inhaler steroids in patients with asthma and OME. Combs [23] compared the efficacy of montelukast and placebo in children with OME. He has found significant difference between the two groups and suggested that montelukast may be used in treatment of OME. In an experimental study conducted by Jung et al. [24] the levels of 5-lipooxygenase, LTC4, LTE4 and LTB4 in middle ear effusions of leukotriene inhibitor group (SCH 37224, nonselective) significantly decreased. In that study, edema, hyperemia and leukocyte infiltration partially decreased in histopathological examination despite the reduced level of LTB4. There is no knowledge about the effect of montelukast on OME in NICE guideline [21]. In our study, montelukast was effective in resolution of OME, but not in decreasing submucosal accumulation of neutrophil leukocytes in OME. This may be caused that, cysteinyl leukotriene receptors (CysLT1 and CysLT2) are important receptors for cysteinyl leukotrienes (LTD4, LTC4 and LTE4) and montelukast is only a selective CysLT1 blocker. LTC4 and LTD4 have many effects such as bronchoconstriction, increased bronchial reactivity, mucosal edema, and mucus hypersecretion. LTB4 has a potent neutrophil chemoattractant action by way of its BLT1 and BLT2 receptors. However, montelukast is ineffective for BLT1 and BLT2 receptors [11,25,26]. This explains why montelukast has beneficial effect on improvement of OME; though not on the level of neutrophil accumulation.

5. Conclusions

Methylprednisolone is an effective agent to decrease submucosal neutrophil leukocytes accumulation. Although montelukast has no effect on submucosal neutrophil leukocytes accumulation, it has beneficial effect on the resolution of OME experimentally. In order to conclude the clinical effectiveness of montelukast on OME, further clinical studies are needed.

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