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Inhibition of the development of myringosclerosis by local administration of fenspiride, an anti-inflammatory drug

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Abstract Earlier studies have revealed a relationship between the development of myringosclerosis and oxygen-derived free radicals. The latter can be blocked by the anti-inflammatory drug fenspiride. The present study was undertaken to test the ability of fenspiride to prevent myringosclerosis from developing during healing of the tympanic membrane. Myringotomized rats were treated with either topical applications or intraperitoneal injections of fenspiride for 12 days, after which the tympanic membranes were examined by otomicroscopy and studied histologically by light microscopy. Topically applied fenspiride was found to inhibit the development of sclerotic lesions, whereas intraperitoneal injections were ineffective.

Key words Tympanic membrane · Myringotomy · Myringosclerosis · Fenspiride · Rat

Introduction

An earlier study in our laboratory showed that the occurrence of experimentally induced myringosclerosis in rats was dependent on an increased oxygen concentration in the middle ear cavity [12]. Subsequent investigations demonstrated that the local application of the scavenger substances superoxide dismutase plus catalase and desferrioxamine, respectively, made it possible to prevent the development of myringosclerosis [13]. These findings have led to the hypothesis that an increased oxygen concentration in the middle ear cavity stimulates an increased production of oxygen-derived free radicals which are involved in the development of myringosclerosis.

Fenspiride (Pneumorel, I.R.I.S., Paris, France) is an anti-inflammatory drug that is used in the treatment of inflammatory conditions of the upper and lower respiratory tracts [3, 6, 14]. It possesses anti-inflammatory, anti-allergic and anti-brochoconstrictive properties [11] Its mechanisms of action have been presumed to be due to blocking of histamine-1 receptors, interaction with arachidonic acid metabolism, and inhibition of the production of oxygen-derived free radicals released by macrophages [2]. However, its mode of action is not known in greater detail. If a relationship can be assumed between myringosclerosis and oxygen-derived free radicals, fenspiride ought to reduce the frequency of myringosclerosis. To test this hypothesis, fenspiride was administered in myringotomized rats either by topical application or by intraperitoneal (IP) injections.

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Materials and methods

Thirty-five healthy adult male Sprague-Dawley rats, weighing 300–400 g, were divided into seven groups of five animals each. All animals were anesthetized with a methohexital (Brietal) via a tail vein, with this procedure also employed for daily topical treatment. Under the otomicroscope a speculum was used so that a myringotomy lancet could make a perforation in the upper rear quadrant of the tympanic membrane (TM) in both ears. The different groups were then treated as shown in Table 1.

Animals in groups 5–7 were pretreated with an IP injection of fenspiride 48 h and 24 h before being myringotomized. Twelve days after myringotomy, the status of each TM was ascertained under an otomicroscope. The animals were then sacrificed by in-

Table 1 Treatment schema for rats given either topical or intraperitoneal (*IP*) fenspiride

Group	Treatment
1	Untreated
2	Topical application of 9 g/l NaCl
3	Topical application of 20 mg/ml fenspiride, once a day
4	Topical application of 40 mg/ml fenspiride, once a day
5	IP injection of 10 mg/ml per kg body weight, fenspiride, twice a day
6	IP injection of 20 mg/ml per kg body weight, fenspiride, twice a day
7	IP injection of 40 mg/ml per kg body weight, fenspiride, twice a day

Table 2 Otomicroscopic and light microscopic findings of myringosclerosis in the pars tensa 12 days after myringotomy (*TM* tympanic membrane; *n* = total number of ears)

Group	Numbers of TMs with otomicroscopically visible myringosclerosis	Numbers of TMs with light microscopical visible myringosclerosis
1. Untreated	8 (n = 10)	9 (n = 9[b])
2. NaCl	2 (n = 10)	9 (n = 9[b])
3. Topical fenspiride 20 mg/ml	0 (n = 8[a])	1 (n = 8)
4. Topical fenspiride 40 ml/mg	0 (n = 10)	2 (n = 10)
5. IP fenspiride 10 mg/ml per kg body weight	9 (n = 10)	9 (n = 9[b])
6. IP fenspiride 20 mg/ml per kg body weight	10 (n = 10)	10 (n = 10)
7. IP fenspiride 40 mg/ml per kg body weight	8 (n = 10)	7 (n = 10)

[a] One animal died during the first week of treatment
[b] One specimen lost during dissection

Table 3 Number of closed perforations 12 days after myringotomy (n = total numbers of ears)

Group	No. of closed perforations	Comments
1. Untreated (n = 10)	1	2 perforations covered with crusts
2. NaCl (n = 10)	8	2 perforations covered with crusts
3. Topical fenspiride 20 mg/ml (n = 8[a])	7	1 perforation covered with crusts
4. Topical fenspiride 40 mg/ml (n = 10)	9	1 perforation covered with crusts
5. IP fenspiride 10 mg/mlxkg body weight (n = 10)	1	2 perforations covered with crusts
6. IP fenspiride 20 mg/ml per kg body weight (n = 10)	2	2 perforations covered with crusts
7. IP fenspiride 40 mg/ml per kg body weight (n = 10)	5	

[a] One animal died during the first week of treatment

traperitoneal injections of pentobarbital (Mebumal). After sacrifice, all animals were decapitated, each bulla tympanica was opened and the middle ears filled with a glutaraldehyde-fixative solution containing 3% glutaraldehyde in a 0.075 M Na-cacodylate buffer (pH 7.4) with 4% polyvinylpyrrolidone and 0.002 M $CaCl_2$ added. After immersion in the fixative, the pars tensa was dissected out. Immediately after removal, tissues were rinsed in cacodylate buffer and post-fixed overnight in OsO_4. After further rinsing of the specimens, tissues were dehydrated in a graded series of ethanol and embedded in an epoxy resin. Specimens were then cut into 1-µm sections and stained with toluidine blue for studies under a light microscope. Additionally, specimens were cut into 60-nm ultrathin sections and contrasted with uranyl acetate and lead citrate, for electron microscopy.

Results

Otoscopic and light microscopic findings of sclerotic lesions in the pars tensa are summarized in Table 2. The numbers of closed perforations 12 days after myringotomy are shown in Table 3.

Gross findings during otoscopy demonstrated a horseshoe-shaped whitish lesion in the anterior, uninjured part of the pars tensa of the untreated animals in group 1. Sclerotic lesions in the pars tensa of the animals in group 2 were less extensive and had a somewhat discontinuous appearance. No TM from the animals in groups 3 and 4 had any sclerotic deposits. In contrast, drums from groups 5–7 resembled those of group 1 and demonstrated comparable myringosclerotic lesions.

Under light microscopy, extensive sclerotic lesions were found in the specimens from the untreated group. Sclerotic deposits were located in the lamina propria and occurred mainly near the handle of the malleus and near the annulus on the uninjured side of the pars tensa. A similar pattern of extensive sclerotic lesions was found in the specimens from the groups treated with IP injections of either fenspiride 10 or 20 mg/ml per kg body weight (Fig. 1). Specimens from the animals treated with IP injections of 40 mg/ml per kg body weight had less extensive sclerotic lesions than those from the two other IP-treated groups. All specimens treated by topical applications of NaCl contained small myringosclerotic deposits (Fig. 2). In contrast only some drums from the animals treated by topical applications of fenspiride 20 and 40 mg/ml had few dispersed sclerotic lesions, located in the uninjured side of the pars tensa (Fig. 3).

Specimens from the untreated animals and groups treated with IP injections of fenspiride revealed marked thickening of the perforated side of the pars tensa with a readily apparent infiltration of inflammatory cells. In contrast, in specimens from the three groups treated by topical applications of NaCl and fenspiride, both the perforated and non-perforated sides of the pars tensa were slightly thickened. In these drums only a few inflammatory cells were seen present.

When examining tissue sections under the electron microscope, sclerotic lesions were found among the collagen fibers in the lamina propria in specimens from groups 1, 2 and 5–7 (Fig. 4). These occurred mainly in the outer collagenous layer with radiating fibers, but sporadic deposits were localized in the inner circular fiber layer as well. Collagen fibers were also split up at the site in which lesions had developed. Each sclerotic deposit contained a "nucleus" consisting of numerous small granules. These "nuclei" were surrounded by alternating light and dark bands. In the specimens from the rats treated with IP in-

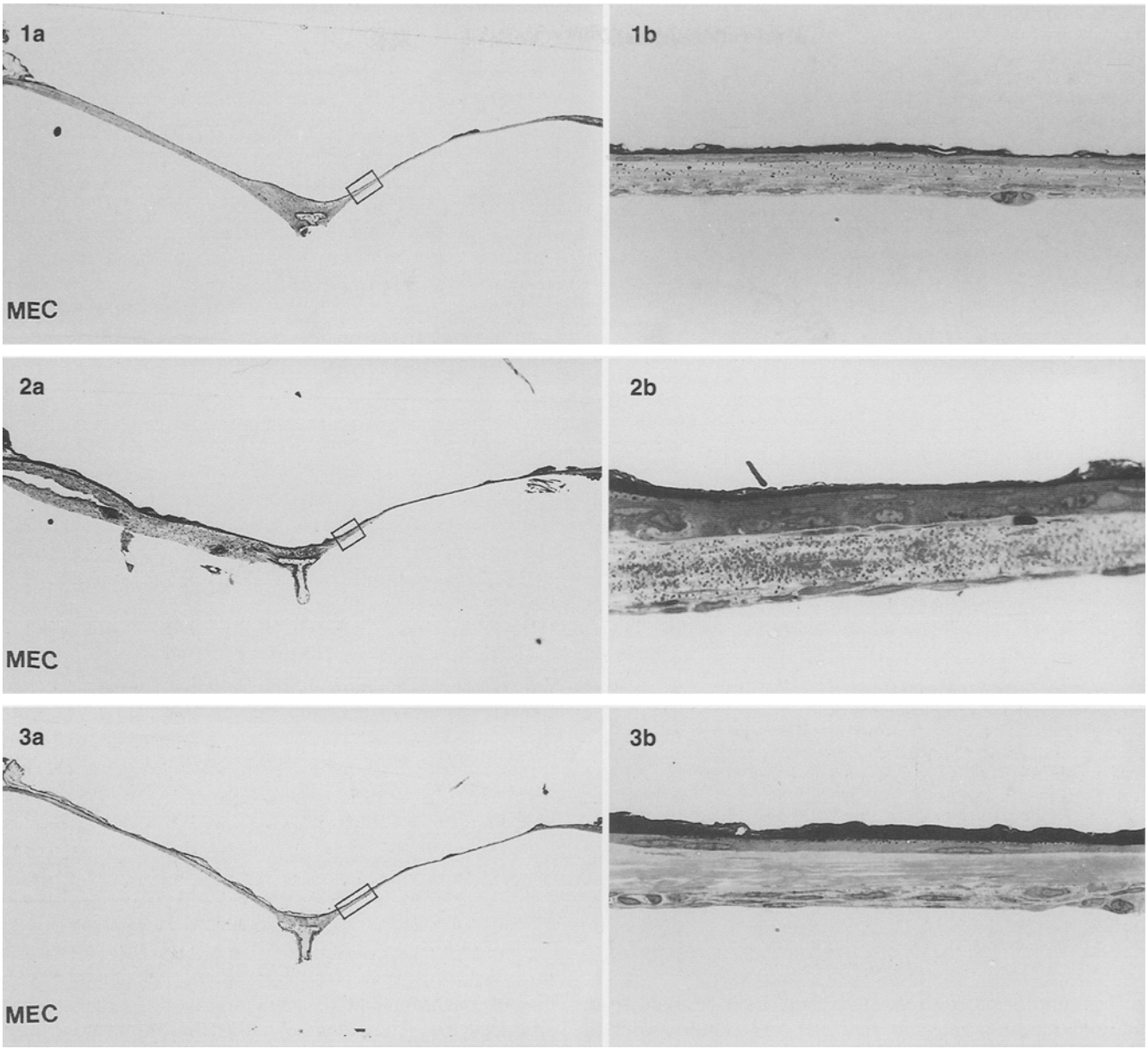

Fig. 1 **a** Light microscopic photograph of a pars tensa from a rat treated with IP injections of fenspiride 20 mg/ml per kg body weight (*MEC* middle ear cavity). Toluidine blue, × 52. **b** Detail of **a**. Only occasional sclerotic lesions are visible in the lamina propria. Toluidine blue, × 1040

Fig. 2 **a** Light microscopic photograph of a pars tensa from a rat treated with topical NaCl (*MEC* middle ear cavity). Toluidine blue, × 52. **b** Detail of **a**. Extensive sclerotic lesions are visible in the lamina propria. Toluidine-blue, × 1040

Fig. 3 **a** Light microscopic photograph of a pars tensa from a rat treated with topical topical application of 20 mg/ml fenspiride (*MEC* middle ear cavity). Toluidine blue, × 52. **b** Detail of **a**. Lamina propria is free of sclerotic deposits. Toluidine blue, × 040

jections of fenspiride, confluent lesions were also present and consisted of numerous small sclerotic deposits. Following topical applications of fenspiride, no sclerotic lesions were found.

Discussion

Previous clinical studies with fenspiride hydrochloride have shown that oral doses of 80 mg taken for 2 and 3 months were effective in treating patients with chronic otitis media by reducing inflammatory signs in the middle ear mucosa and producing a significant audiometric improvement [6, 14]. In chronic otitis with closed TMs, tympanograms improved significantly [14]. In an experimental study in which otitis media with effusion was produced in rats, the middle ear fluid and associated inflammatory elements decreased following intraperitoneal injections of the drug [8].

Myringosclerosis is a common sequela to ventilation tube treatment and has been associated with an increased production of oxygen-derived free radicals [12, 13]. It is conceivable that the effects of fenspiride on myringoscle-

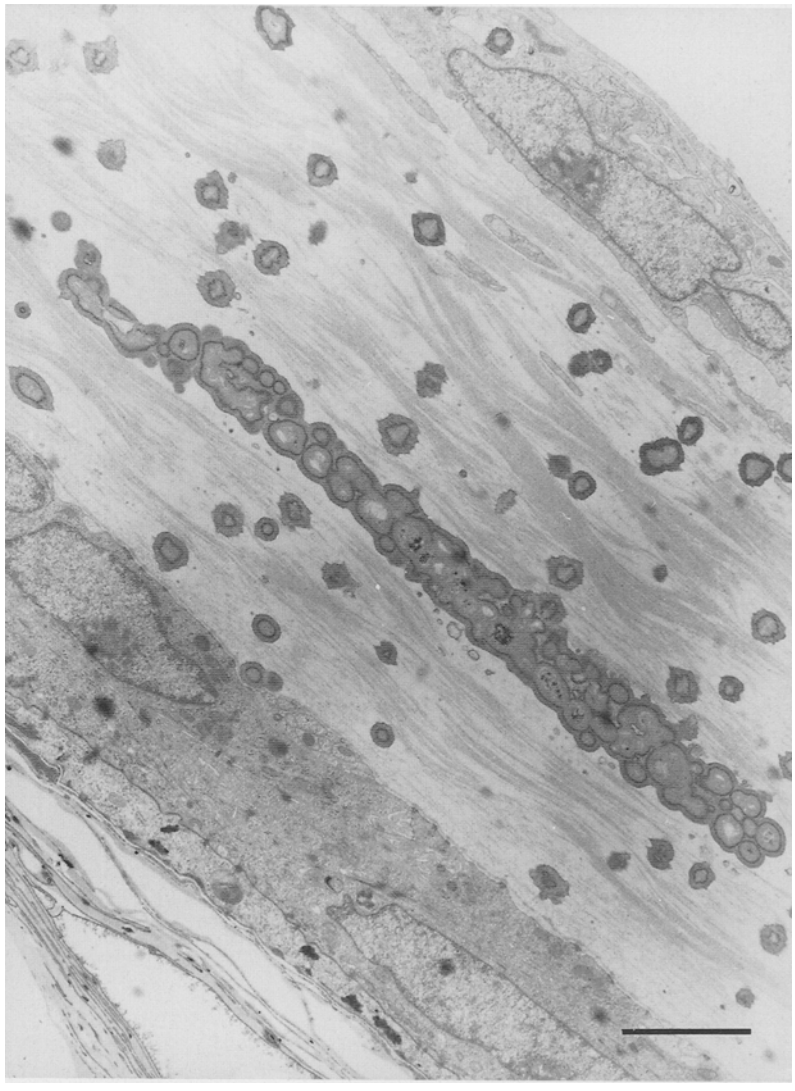

Fig. 4 Electron micrograph of a pars tensa from a rat treated with 20 mg/ml per kg body weight IP fenspiride. Both confluent and sporadic sclerotic deposits can be seen in the lamina propria. The collagen fibers are separated at sites in which the sclerotic deposits have developed. *Bar* indicates 2 µm

rosis found in our present study may have resulted from an interaction with such free radicals. Three different studies have now shown that fenspiride is able to block the production of free radicals [2, 5, 11]. In alveolar macrophages stimulated in vitro with phorbol myristate acetate or zymosan, fenspiride demonstrated a dose-dependent inhibition of free radical production as measured by chemiluminescence of the macrophages [2]. When phorbol myristate acetate was used to stimulate alveolar macrophages, fenspiride restricted the formation of H_2O_2 [5]. Although hydrogen peroxide per se is not a free radical it can be derived into the extremely reactive radical OH ·. Fenspiride has also been shown to reduce the production of malondialdehyde and to inhibit hydrogen peroxide-induced cytolysis [11]. However, fenspiride does not interfere with the production of free radicals produced by the xanthine-xanthineoxidase system and subsequently does not act as a true scavenger substance [2]. Carre et al. [2] have found that fenspiride could inhibit the production of arachidonic acid metabolites, primarily via the cyclooxygenase pathway. It is known that during the metabolism of arachidonic acid, oxygen-derived free radicals are produced [9]. Another mechanism that might contribute to the diminished production of free radicals could be a decreased neutrophil migration to an inflammatory site. An experimental study by Cunha et al. [4] showed that rats injected with endotoxin had a reduced concentration of intraperitoneal neutrophils when treated with oral fenspiride. Similar effects on neutrophils have been observed following fenspiride treatment in both experimentally induced serous and purulent otitis media (S. Hellström, unpublished data, 1993.)

During phagocytosis there is a continuous release of oxygen-derived free radicals. Modulation of the inflammatory response by a reduced concentration of neutrophils therefore restricts the release of free radicals. Consequently, fenspiride is able to reduce the production of oxygen-derived free radicals in several ways. Which of these mechanisms was responsible for the effects observed in our present study can only be speculated on.

Our rats treated with IP injections did not show any decrease in the occurrence of myringosclerosis when com-

pared with untreated animals. However, since the metabolism of fenspiride is not known when administered in this manner, it is possible that a rapid degradation of the drug takes place. The pars flaccida is the best vascularized portion of the TM, whereas the pars tensa is dependent on a passive diffusion from vessels located near the annulus and along the handle of the malleus [1]. Low drug concentrations in serum may be insufficient to reach the pars tensa. When using topical applications there is an immediate high concentration of the drug locally, without prior degradation. Our findings under light microscopy indicate that sclerotic deposits were less prevalent in animals treated with the highest dose of IP used, suggesting a possible dose-dependent effect. Although results of IP drug in our present study were not statistically significant, in vitro experiments have shown that only at high concentrations is fenspiride able to block the production of free radicals [5].

In our otomicroscopic evaluation, there was no difference between the number and extension of sclerotic lesions comparing the groups treated by topical application of fenspiride (groups 3 and 4) and the group treated with NaCl (group 2). However, in the light microscope sclerotic lesions were only noted in 3 of 18 of the sections from the locally treated groups but in all sections from the group treated with NaCl. This indicates that there is a true difference concerning the development of myringosclerosis between the two forms of treatment.

In all TMs treated by topical application with fenspiride or with NaCl the perforations closed prior to the otomicroscopic evaluation. Earlier experiments have demonstrated that the topical application of hyaluronan [10] or exogenous heparin [7] influenced the healing of a traumatized TM. Hyaluronan accelerates the healing rate and causes less structural change in the TM by interacting with the cellular processes during the healing process. Heparin treatment results in less scar tissue formation, probably by stimulating the angiogenesis. The mechanism underlying the beneficial effects of topical application of fenspriride can only be speculated on. However, when applying a solution to a perforated TM, the wounded area is kept damp, thus maintaining the original conditions in the tympanic cavity. This occurrence could be of importance to the healing of the TM.

We have indicated in our present study that locally administered fenspiride can diminish the development of myringosclerosis, possibly by restricting the formation of oxygen-derived free radicals. However, detailed knowledge of how fenspiride interferes with the production of free radicals is sparse. To our knowledge, our study is the first to demonstrate that a specific drug could have an effect on the development of myringosclerosis. Transferred to the human situation the additional use of fenspiride when inserting a ventilation tube clinically might be a tool with which to prevent the development of sclerotic lesions in the TM.

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