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An animal model for external otitis

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Abstract External otitis was produced in 12 Sprague-Dawley rats by mechanical stimulation through a plastic micropipette inserted into the right external auditory canal (EAC). The EAC was later evaluated regarding the color of the skin, swelling and the presence of fluid. Within 1 day all rats developed an external otitis that was characterized by a red, swollen ear canal containing an opalescent fluid. The tympanic membrane and middle ear cavity appeared to be normal. No healed EACs were seen within the initial 10 days of follow-up and 4 of 6 rats still exhibited external otitis at day 21. Light microscopy of biopsy specimens revealed pronounced edema of the dermis of the ear canal. Mast cells were more numerous in the early phase of the otitis present, although very few inflammatory cells were found in tissues despite the marked inflammatory reaction produced. Findings show that this animal model for external otitis can be used to investigate pathogenesis as well as to test various treatment strategies.

Key words External auditory canal · External otitis · Pathogenesis · Rat

Introduction

External otitis is one of the most common diagnoses in clinical practice. However, its definition can be vague and a variety of therapies exist. A literature survey revealed 11 different strategies for treatment, including topical glucocorticoids, glucocorticoids with antibiotics, an acid, an acid with antibiotics, an ethanol mixture, antimycotics, oils and dyes (e.g. gentian violet). Systemic treatment has employed various antihistamines, antimycotics and antibiotics. Irrespective of the treatment strategy for external otitis, there is a high recovery rate [4, 13]. However, it appears that systemic antibiotics are usually unnecessary, whether an infection is involved or not. Nonetheless, it would be of great interest to find an ideal therapy for this clinical condition. Testing treatment strategies in an animal model is one way to show whether or not management is useful.

Numerous studies have demonstrated histological similarities between the human middle ear and that of the rat [1, 8, 15]. When challenged by pneumococcal infection, the rat ear also reacts similarly to that of the human ear [10]. An earlier study on mechanisms involved in the development of otitis media with effusion showed that sufficient mechanical stimulation of the external auditory canal (EAC) will cause an inflammatory reaction of the ear canal dermis [3]. This finding raised the question whether this procedure could be used to produce an animal model for studies on external otitis.

The purpose of the present study in the rat was to ascertain whether a standardized procedure for mechanical stimulation of the EAC would elicit a reaction resembling that of human external otitis. If so, such an animal model for external otitis could be used for studies on the pathogenesis of otitis, as well to test various treatment stategies.

Materials and methods

Fifteen healthy adult male Sprague-Dawley rats, weighing 250–300 g, were anesthetized by intravenous administration of sodium methohexital (Brietal, Lilly, Indianapolis, Ind., USA) through one of the tail veins. In 12 of the rats the lateral part of the EAC was exposed to 400 rotations of a conical micropipette, using an otomicroscope. The diameter of the cut tip of the micropipette was 4 mm. This size prevented the pipette from contact with the most medial 5 mm of the ear canal, thus avoiding damage to the tympanic membrane. The speed of rotation was limited to 80 rpm to avoid thermal effects and ulceration. The opposite (left) untreated ear served as control. The 3 remaining animals were left with both ears unstimulated and were used as untreated controls. No animal died accidentally during the investigation or showed any systemic reaction to the treatment.

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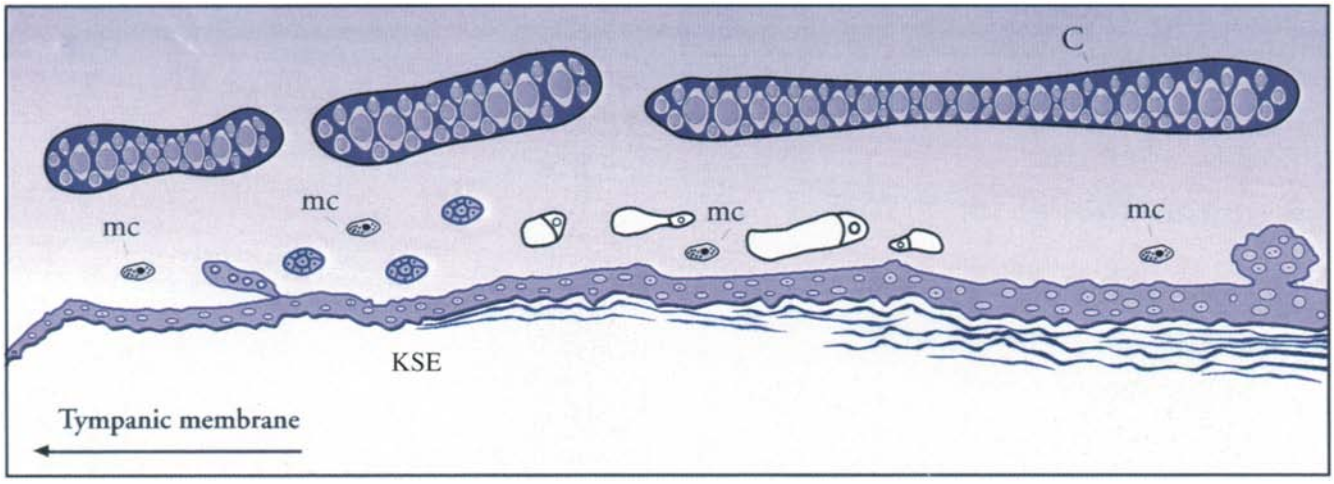

Fig. 1 Survey histology of the typical rat external auditory canal (EAC). Note the cartilage (*C*) supporting the EAC in almost its entire length. The keratinizing squamous epithelium (*KSE*) becomes thinner in the direction of the tympanic membrane. Blood vessels (*V*) and mast cells (*MC*) are located in the subepidermal connective tissue

During anesthesia the EAC status was observed once daily from day 1 to day 4 and on days 7, 11 and 20. Color, swelling and effusion were evaluated according to a numerical grading system. The color was graded as: 0 = normal, 1 = red, and 2 = purple. Swelling was determined by use of funnels of varying size, i.e. 1–4 mm. Swelling was graded as: 0 = absent or an EAC measuring at least 4 mm; 1 = a canal 3 mm or less; 2 = 2 mm or less, and 3 = 1 mm or less. The occurrence of effusion was classified as: 0 = dry, 1 = moist, 2 = fluid in the EAC and 3 = otorrhea.

On days 3, 7 and 11, two rats each were sacrificed for histology. The remaining rats were sacrificed on day 21. A well-defined piece of skin from the EAC that was 5–15 mm from the tympanic membrane was biopsied for light microscopy.

Light microscopy

The EAC skin was divided into one medial portion and one lateral portion. From each of these two portions, two specimens were prepared and processed separately for either paraffin embedding or plastic embedding.

Paraffin sections

Fresh tissue samples were transferred to saline and within 30 min placed in a mixture of 2% formaldehyde mixed with 0.5% glutaraldehyde in a phosphate buffer. They were then fixed in a microwave oven set at 45°C. After a rinse in 0.1 M phosphate buffer specimens were dehydrated in a graded series of ethanol and embedded in paraffin wax. Sectioning was done in 5-μ m sections. The paraffin sections were stained in hematoxylin-eosin for routine examination. Other sections were stained with 0.5% toluidine blue solution (pH 2.0) for the detection of mast cells. Sections were also reacted with a biotinylated hyaluronan-binding protein probe for localization of hyaluronan. The probe was then visualized by the avidin-peroxidase technique [9] and reactions studied in a Zeiss Axiophot light microscope. The toluidine-blue-stained sections were analyzed for mast cells by use of a point-counting technique [14]. Morphometric measurements were made on 4–5 areas from each section, using a graticule within the eyepiece of a light microscope. The measurements were made at an objective lens magnification of × 40.

Plastic sections

For plastination, skin specimens were fixed for at least 24 h in a 3% glutaraldehyde solution in 1.0 M cacodylate buffer with 4% polyvinylpyrrolidone and 0.002 M $CaCl_2$ added. Specimens were then postfixed in 1% osmium tetroxide in the same buffer, followed by dehydration in increasing concentrations of acetone. Samples were next embedded in an Epoxy resin (Polybed 812; Polysciences, Warrington, Pa., USA) and sectioned with an ultramicrotome in 1-μ m sections. The sections were stained with toluidine blue and analyzed and photodocumented in a Zeiss Axiophot light microscope.

Results

Normal appearance of the EAC

The adult rat EAC is about 20 mm in length. The bony portion is very short, extending only 2–3 mm from the tympanic membrane area. The remaining portion is supported by a wrinkled cartilage (Fig. 1). The lumen of the EAC is approximately 4 mm in diameter but widens close to the tympanic membrane. The EAC skin, normally pale in color, is covered by a keratinizing stratified squamous epithelium, which is 3–4 layers thick in its medial portion and 4–8 layers thick in the lateral portion (Fig. 2). Between the epidermal layer and cartilage there is a loose, collagen-rich connective tissue containing numerous vessels and mast cells. Sebaceous glands are found and are more frequent in the medial portion of the EAC. Close to the external meatus hair follicles are seen.

Otomicroscopy

Mechanical stimulation elicited redness, swelling and fluid collection in the EAC in all 12 animals (Table 1). Changes were most pronounced on day 2, whereafter they declined. By day 21 the EAC had almost normalized, although slight redness and swelling persisted in 4 of 6 rats.

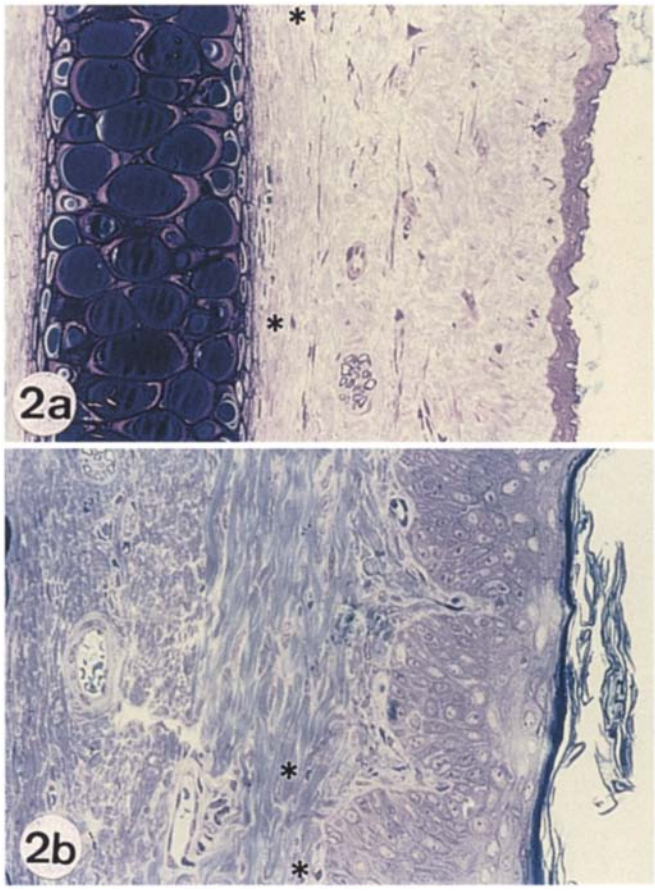

Fig. 2 Light photomicrographs of a normal rat EAC showing **a** the medial portion and **b** the lateral portion. Below the keratinizing squamous epithelium is a loose connective tissue. Mast cells (*) are present. Glands occur more frequently in the medial than in the lateral portion. Toluidine blue staining, Epon embedding, × 240

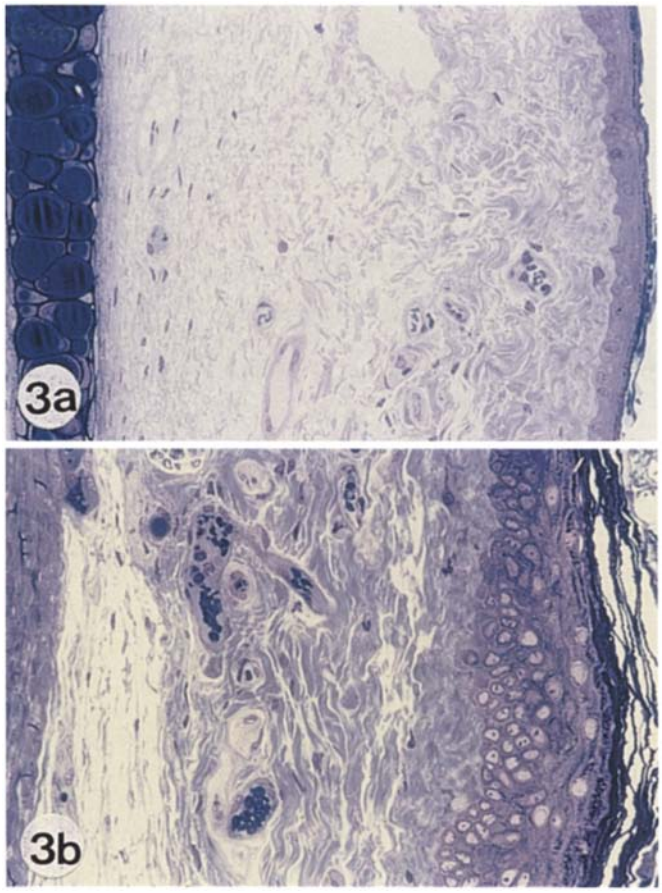

Fig. 3a, b Light photomicrographs of the rat EAC with external otitis 3 days after mechanical stimulation. **a** Medial portion, **b** lateral portion. There is pronounced edema in the connective tissue layer. Note the lack of inflammatory cells within the tissue. Toluidine blue staining, Epon embedding, × 240

Table 1 Otoscopic grading of color, swelling and effusion of the EAC skin in experimentally induced otitis externa (± shows the mean of the various gradings)

		Day								
		0	1	2	3	4	5	7	11	21
Color	0	12			1	1	1	3	4	2
	1		12	12	11	9	9	7	4	4
	2									
	±	0	1.0	1.0	0.9	0.9	0.9	0.7	0.5	0.7
Swelling	0	12		2	2	0	1	1	0	2
	1		4	6	7	10	9	9	8	4
	2		8	4	3	0	0	0	0	
	3									
	±	0	1.7	1.2	1.1	1.0	0.9	0.9	0.8	0.7
Effusion	0	12				0	6	10	8	5
	1		1	7	11	9	4	0	0	1
	2		1	5	1	1	0	0		
	3									
	±	0	1.9	1.4	0.9	1.1	0.4	0	0	0.2

Light microscopy

A pronounced hyperkeratosis of the EAC skin occurred in all stimulated rats. On day 3 there was pronounced edema in the subepidermal connective tissue (Fig. 3). The blood vessels were dilated and the number of mast cells was increased (Fig. 4, Table 2). Leukocytes were sparse. An increased immunoreactivity to hyaluronan was observed (Fig. 5). Findings were similar on day 7. By day 11 edema was reduced and stainability for hyaluronan resembled that of the controls. Occasionally, some areas of the cartilage seemed to have become transformed into a more bone-like tissue. By day 21 tissues of the EAC were found to be normal when viewed by light microscopy. Throughout the study, all 12 stimulated rats had a normal left ear, both clinically and when tissues were examined under light microscopy.

Discussion

All rats in which the EAC was subjected to mechanical stimulation developed external otitis. The inflammatory

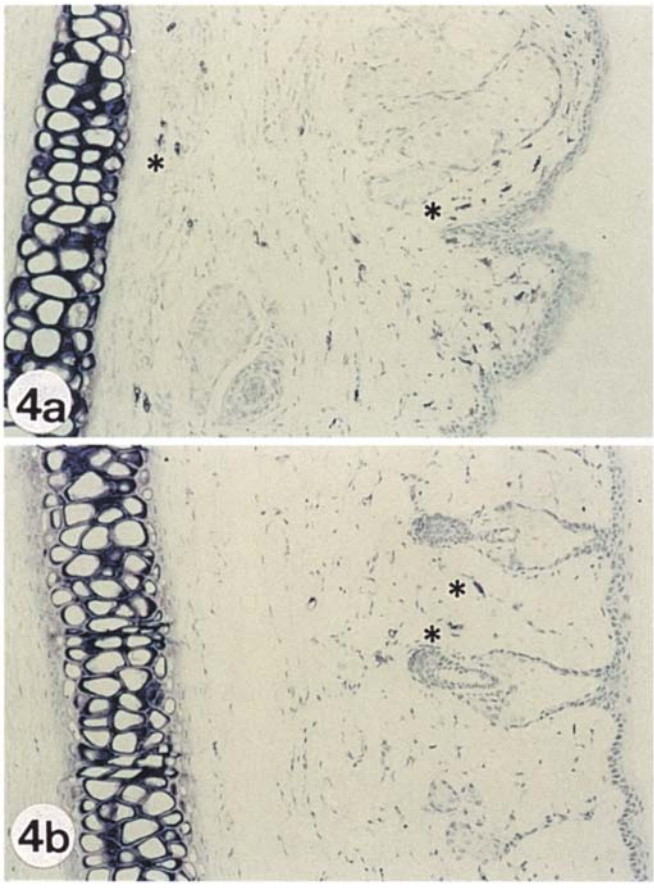

Fig. 4a,b Light photomicrographs of EAC tissue prepared for staining of mast cells (*). **a** External otitis 3 days after mechanical stimulation; **b** control. The number of mast cells appears to be increased in the ear with external otitis. Toluidine blue staining, paraffin embedding, × 120

Table 2 Mast cell numbers in ear canal skin in experimentally induced external otitis. The number of mast cells is presented as the mean ± SD on day 0 and day 21. The values for the remaining days are presented as means without SD, since tissue from only 2 animals in each group was available for study after planned sequential sacrifices of the other animals. * Significantly different compared with mast cell numbers in controls ($P < 0.05$)

		Mast cell numbers as percentage of stromal tissue
Control	Day 0	1.35 ± 0.24 ($n = 5$)
	Day 3	1.50 ($n = 2$)
	Day 7	1.99 ($n = 2$)
	Day 11	1.60 ($n = 2$)
	Day 21	1.73 ± *0.39 ($n = 4$)

reaction seen appeared to be uniform, both clinically and histologically. The condition was characterized by a pronounced edema of the connective tissue. Surprisingly few inflammatory cells were observed in the edematous tissue, whereas the ear canal fluid contained numerous inflammatory cells, which were mainly polymorphonuclear leukocytes and macrophages. An increased stainability for

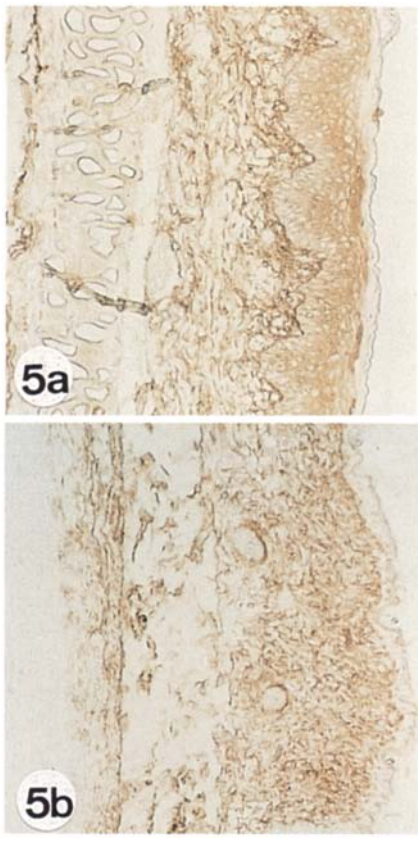

Fig. 5a,b Light photomicrographs of the EAC showing staining for hyaluronan. **a** External otitis 3 days after mechanical stimulation; **b** control. There is an increased staining reaction for hyaluronan in the external otitis ear. Avidin-peroxidase, paraffin embedding, × 120

hyaluronan occurred concomitant with the edema. Hyaluronan has strong osmotic properties that can attract and retain water in swollen tissues. Since hyaluronan is an important matrix component involved in wound healing processes, its increase may indicate a reactive reparative process with fibroblast activation in the interstitium [11].

The epidermal layer seemed to become thinner during the external otitis and was presumed due to a distention of the skin cover. In the early phase of the otitis, the hyaluronan content appeared to increase between the cells of the keratinizing epithelium. Here, the lubricating properties of hyaluronan were presumed to support distention of the epithelium, as has been suggested for other tissues [12].

In earlier studies, mechanical stimulation of the EAC was shown to create effusion in the middle ear cavity [3]. In the present study most rats demonstrated a slightly thickened pars flaccida, but no signs of effusion. The reason for this conceivably was that the most medial 5 mm of the EAC of the ear had been shielded from mechanical stimulation.

The ear canal skin is contiguous with the pars flaccida of the tympanic membrane, which is extremely rich in mast cells [2]. Hellström and Goldie [7] have suggested that these mast cells are involved in the development of otitis media by neurogenic inflammation. In the present investi-

gation, numerous mast cells were found in the EAC skin, suggesting that a similar mechanism may also be involved in the development of external otitis in our animals.

Mast cells store a variety of inflammatory mediators that are already manufactured or under de novo generation. These have both vasoactive and chemotactic properties. The involvement of mast cells may well explain the increased vascularity influencing the edema.

Regarding possible chemotactic effects, the sparse occurrence of leukocytes was surprising to us. However, in otitis media with effusion evoked by mechanical stimulation, the effusion fluid in the middle ear cavity again contained very few inflammatory cells [6]. The fluid in earlier studies was thus suggested to be a true transudate. This could also be the case in our present study.

It would appear that mechanical stimulation of the rat ear canal may be a good and reproducible animal model for external otitis. Furthermore, the rat is relatively easy to handle and rather inexpensive as a laboratory animal. It is tempting to suggest that this animal model for otitis externa could be utilized to investigate the pathogenesis of inflammation and to assess various treatment strategies. Experiments are already in progress regarding the involvement of bacteria in the development of external otitis, as well as the efficacy of steroids in its treatment.

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