

Evolution of Middle Ear Changes After Permanent Eustachian Tube Blockage

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Objective: To develop a valid animal model for otitis media with effusion (OME).

Design: Forty specific pathogen-free Wistar rats underwent a procedure based on the permanent obstruction of pharyngeal eustachian tube by means of electrocoagulation without any manipulation.

Setting: Ear Research Group, Department of Otorhinolaryngology, Puerta de Hierro Hospital, Universidad Autónoma de Madrid, Madrid, Spain.

Main Outcome Measures: The assessment of OME by otoscopy and tympanometry. The rats were hu-

manely killed at 15 and 90 days, and temporal bones were obtained and processed for histopathologic study.

Results: The histopathologic study of the temporal bones demonstrated the occurrence of chronic effusion and mucosal changes owing to mucoperiosteal enlargement.

Conclusions: Comparison with other experimental models was made. Our animal model was consistent and reproducible and resembled human OME.

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OTITIS MEDIA WITH EFFUSION (OME) is the most common reason for surgical admission during childhood in the Western world and is the second most common disease (the common cold is first).¹ The pathologic characteristics have a sudden beginning and a variable course, usually with spontaneous resolution. In a number of cases, OME is recurrent and may evolve into different complications or sequelae (eg, otorrhheic sequelae, purulent otitis media, cholesteatoma).²

The physiopathologic characteristics are only partially understood. Many clinical and experimental studies have demonstrated that although eustachian tube (ET) obstruction is an important factor,³ it is not the only one. The pathogenesis of OME is multifactorial and includes ET dysfunction, bacterial infection (isolated forms or organized in biofilms),⁴ an immature immune system, allergy,⁵ the presence of inflammatory mediators,⁶ or middle ear anatomical features.⁷

Our objective was to develop an experimental model that reproduced the human physiopathological patterns of OME and to analyze short- and long-term histologic changes.

METHODS

Forty female Wistar rats weighing 240 to 260 g underwent a permanent blockage of the right ET by means of electrocoagulation of the pharyngeal orifice as described previously.⁸ Otoscopic evaluations and tympanograms were performed in all cases before the procedure and again before the rats were humanely killed. The animals were handled according to the National Council for the Care of Animals guidelines.

GROUP ASSIGNMENT

Nineteen rats were randomly assigned to the short-survival group and examined and humanely killed at 15 days after cauterization to evaluate early tissue changes. The remaining 21 animals assigned to the long-survival group were examined and humanely killed at 90 days

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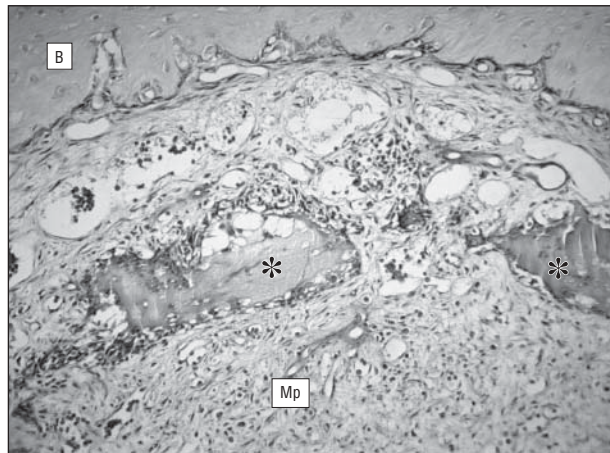


Figure 1. Inflammatory reaction in mucoperiosteum (Mp) at 15 days. Note the fibrous and new bone (B) formation, neovascularization, and serous effusion in the cavity (asterisks) (hematoxylin-eosin, original magnification $\times 125$).

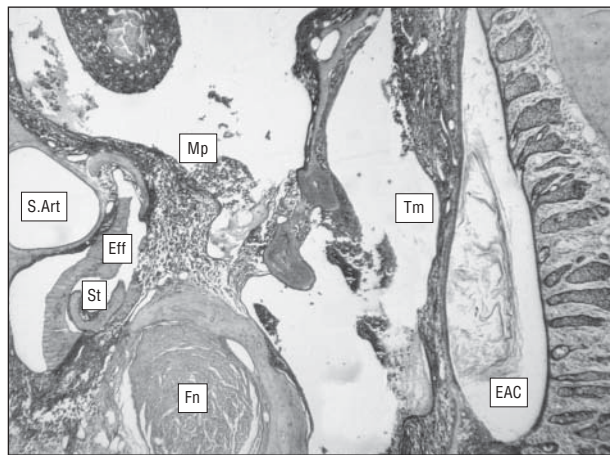


Figure 2. Tympanic membrane (Tm) and ossicular changes (St, stapes) at 15 days. Note serous effusion (Eff) and mucoperiosteal (Mp) inflammatory reaction. S. Art indicates stapedial artery; Fn, facial nerve; and EAC, external auditory canal (hematoxylin-eosin, original magnification $\times 35$).

after cauterization to evaluate long-term responses. All of the animals were housed under hygienic conditions in a laminar cross-flow cabinet as specific pathogen-free rats.

ANESTHETIC PROCEDURES

Before the procedure the animals were intraperitoneally injected with ketamine (75 mg/kg), diazepam (2.5 mg/kg), and atropine (0.02 mg/kg). The animals' temperatures were monitored by rectal thermometry and maintained at 37°C throughout the anesthetic and surgical procedures (10 minutes) with an electrical blanket. Subcutaneous buprenorphine (0.05 mg/kg injected every 8 hours) was used for postoperative analgesia.

CAUTERIZATION OF THE ET

The mouth of the rat was kept open with a retractor. An electrical cautery needle, 0.6 mm in diameter and curved at its end, was inserted at the soft and hard palate junction and after being introduced approximately 5 mm, was rotated to the right. Finally, the right ET nasal orifice was cauterized (for four 0.5-second pulses at 30 W). The left ear was the control.

The animals were humanely killed under anesthesia, and the temporal bones were removed. Then the bullae were fixed in 10% formaldehyde, decalcified in Microdec (Microstain; Granardo, Italy) for 2 days, and finally embedded in paraffin. Next, 5- μ m-thick slices were stained with hematoxylin-eosin and Masson trichrome stain for light microscopic study.

RESULTS

SHORT-SURVIVAL GROUP

Otoscopic evaluation showed eardrum retraction with medial displacement of the handle of the malleus and dullness of the tympanic membrane in the challenged right ears of all the animals. Four ears (21%) were found to have air-fluid levels, 1 (5%) presented with adhesive otitis, and 5 (26%) had purulent otorrhea. Findings from otoscopic examination of the control ears were within reference range in all animals.

Findings from the tympanogram showed flat curves in the 4 animals with air-fluid levels and purulent otorrhea, whereas the rest of the challenged ears showed values of 0.4 mL at ± 200 mm H₂O and 0.7 mL at 0 mm H₂O. The control ears showed values within the reference range.

Macroscopically, the challenged ears, except in the 1 rat with adhesive otitis, contained a yellow, transparent serous fluid. The macroscopic findings in the control ears were within reference range.

Microscopically, in the challenged ears a leukocyte infiltrate was found in the mucoperiosteum and inside the middle ear. The mucoperiosteal layer was enlarged owing to vascular dilatation and proliferation, edema, and hyperplasia of the lamina propria. Mild lymphocyte and erythrocyte vascular infiltration was observed. In the epithelium there was hyperplasia that appeared cuboidlike, with several layers of distended and vacuolated cells and increased numbers of fibroblasts and collagen fibers, forming polypoid masses within a serous proteinlike substance (**Figure 1**). Most of the middle ear was occupied by the enlarged mucoperiosteum covered by a thin layer of a nonciliated epithelium with a few goblet cells. The walls showed bone neoformation with increased numbers of osteocytes and osteoclasts and periosteum hyperplasia. These bony changes were also present in the ossicles. The tympanic membrane was slightly enlarged at the expense of the internal layer (**Figure 2**).

The effusion appeared as a serous fluid filling the middle ear cavity and mucoperiosteal invaginations (indicated by the asterisks in Figure 1) with a scarce mucous component. No macrophages or polymorphonuclear cells were observed except in the animals presenting with otorrhea.

LONG-SURVIVAL GROUP

Otoscopic evaluation showed persistent tympanic retraction in the challenged ears of all but 4 animals: 1 rat developed a perforation, 1 rat had OME that had healed by the time it was killed, and 2 animals developed chronic otitis media (**Figure 3**). Tympanometric findings in the

remaining 17 animals were similar to those of the short-survival group.

The bullae of all animals were filled with a seromucous exudate, and non-foul-smelling otorrhea was found in 2 of them. The bullae of control ears were normal.

Light microscopic examination showed a thinner mucoperiosteum in this group than in the short-survival group, with less cell infiltration but with a larger amount of fibers and new vessels. Secretory and ciliated epithelium and hyperplastic submucosal glands were observed. The effusion was more dense and mucouslike and contained isolated cells. The bony walls were more enlarged and showed a laminar pattern with low cell activity and scarce osteocytes. The tympanic membrane was thicker at the expense of both fibrous and internal layers, with retractions and adhesions to the ossicles.

In the animals that had developed infection, inflammatory changes were more pronounced, with polymorphonuclear leukocyte abscess formation and the presence of foamy histiocytes. A strong inflammatory and osteogenic reaction had occurred in the ossicles, with erosion and fibrotic adhesion.

COMMENT

Histopathologic changes in human OME consist of epithelial enlargement and edema,⁹ just as OME in rats is also characterized by a subepithelial enlargement owing to edema associated with a certain degree of capillary dilatation and vascular congestion and few inflammatory cells. In our study in the mucoid effusion type, the epithelium became pseudostratified and goblet cells were increased as were the intraepithelial and subepithelial secretory glands. There was subepithelial enlargement owing to fibrous tissue, edema, vascular dilatation and neoformation, and infiltration of lymphocytes, macrophages, and plasma cells.

We have developed an experimental model of ET cauterization in the Wistar rat with the aim of stimulating and maintaining a chronic otologic disease while avoiding development of more destructive forms of otitis media. The experimental OME developed in our model is similar to the OME described in humans^{3,9-12} and was reproducible in most of the animals in our study.

In Wistar rats, ET cauterization induced a middle ear inflammatory reaction, showing an intense mucoperiostitis at 15 days, with a small leukocyte infiltration mainly around the vessels, a remarkable fibrous tissue hypertrophy (immature collagen fibers), with vascular dilatation and neoformation, and cavitations of the mucoperiosteum in the areas closest to bone. The tympanic membrane was less affected than the rest of the middle ear mucosa in this acute phase, showing only a slight thickening of the internal layer. The bony walls and the ossicles showed signs of osteogenic activity. The acute inflammatory response mainly affected the mucoperiosteum with an increased vascular dilatation and neoformation. Because there was no evidence of gland hypertrophy and metaplasia involvement, the serous effusions must have been the result of transudation.

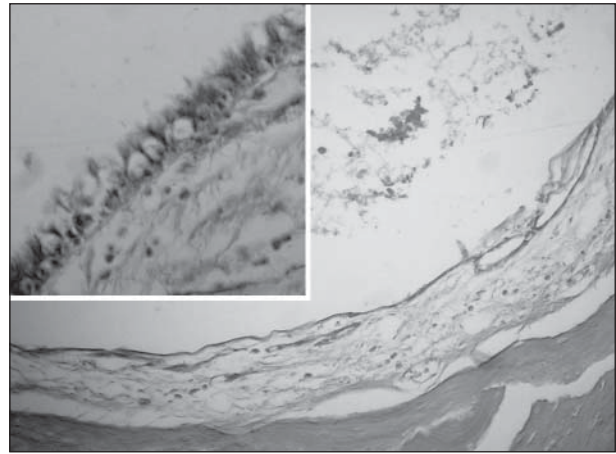


Figure 3. Otitis media with effusion at 90 days. Note mucoperiosteum hypertrophy with reparative process and polystratified and secretory epithelial layer (inset, hematoxylin-eosin, original magnification $\times 250$) and mucous effusion (hematoxylin-eosin, original magnification $\times 125$).

After 90 days of permanent blockage of the ET, some changes tended to consolidate, although they were less intense than at 15 days, and new features could be seen. Mucoperiostitis decreased, and fibrotic changes were more marked, with decreasing cellularity and maturing collagen fibers. Vessels and cavitations were enlarged. In contrast to the changes observed in the epithelial layer at 15 days, at 90 days it was of a ciliated pseudostratified type with an increased number of goblet cells and intraepithelial and organized submucosal secretory glands. The middle ear cavity was more spacious because there was less edema of the mucoperiosteum, which showed fibrous adhesions. The middle ear cavity was filled with a mucoid effusion and cellular rests. A laminar bone without osteoblastic activity was seen. Chronic histologic changes were noted: organization and maturing of fibrous tissue, retraction of tympanic membrane, and also adhesion to the ossicles, and epithelial metaplasia to a hypertrophied, ciliated, and secretory epithelium. Consequently, chronic effusion was an exudate rather than an acute transudate.

When comparing infected vs noninfected ears at 15 days, it was interesting to see that the histopathologic changes were qualitatively similar but were more intense in the infected cases, with a higher polymorphonuclear infiltration in the mucoperiosteum and effusion of the middle ear cavity. The same changes were observed at 90 days when comparing infected vs noninfected ears. These similarities support the continuum theory of otitis media.⁹

There exist many experimental models of OME with varied histopathologic findings.^{10,13-20} In some cases, pathologic changes were intense and affected all middle ear structures, whereas in some cases only a mild inflammation of the mucoperiosteum and a serous effusion were observed. Comparison of our results at 15 days with other experimental models^{10,13-17,20} is shown in **Table 1**, and our findings at 90 days are also compared with other experimental models^{10,14,15,20} in **Table 2**.

Several animal models in which the middle ear was manipulated or isolated from the pharynx have shown

Table 1. Short-Term (15-Day) Histopathologic Changes in Otitis Media With Effusion (OME) Developed in Different Animal Models

Location of Change	Animal/Technique							
	Wistar Rat, Germ-Free/ Extratympanic ET Cauterization (Ventral Approach) ^{10,15}	Mongolian Gerbil/Tympanic ET Obstruction With Gutta Percha Interposition ¹⁴	Wistar Rat, Conventionally Raised/ET Isthmus Section and Muscle or Gutta Percha Interposition ^{13,16}	Chinchillas/Intratympanic Inoculation ¹⁷			Mongolian Gerbil/ Transpalatal External ET Cauterization ^{14,20}	Wistar Rat, Pathogen-Free/ Transpalatal External ET Cauterization (Present Study)
				MEE	LPS <i>H Infl</i>	KLH, CFA		
Epithelium								
Goblet cell	Oval window and ET+	NA	+	+	++	+++	+	+
Hyperplasia		Cuboidlike cell +	++	+	++	+++	+	+
Edema	++	NA	+	+	++	++	+	+
Pseudopolyps	+	NA	Nonkeratinized polystratified epithelium (squamous metaplasia)		+	+	+	+
Other	Flat to secretory epithelial change	NA	NA	NA	NA	NA	NA	Unciliated epithelium
Mucoperiosteal								
Vascular DN	+	+	+	+	++	+++	++	++
Edema	+	NA	+	+	+++	++	+	+
Cavitations	++	NA	+	+	++	++	++	+++
Cell infiltration	Round cells (perivascular)	NA	Lymphocytes and macrophages	+	+++	+	PMN	Round cells, perivascular
Fibrous tissue	++	+		+	+	+	++	++
Bone								
Osteoblasts present	First week	NA	NA	No	Yes	Yes	Yes	Yes
Hypertrophy	++				+++	+	+	+++
Effusion (S/M) Type	S (sterile) ++	+	+	S+	S++	S++	+++	+
Tympanic membrane	No changes	S PF, retraction and hypertrophy of the mucosa of the tympanic PF	S Retraction	20†	99†	99†	S Retractions and adhesions, hypertrophic. PF	S Retraction and hypertrophy of the mucosa of the tympanic PF, and PF mucosa
Comments*	Different pathologic characteristics than in human OME		Tracheotomy; difficult technique				OM; cholesteatoma	Easy and inexpensive technique

Abbreviations: CFA, complete Freund's adjuvant; DN, dilatation and neoformation; ET, eustachian tube; *H Infl*, *Haemophilus influenza*; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; M, mucous; MEE, middle ear effusion; NA, not available; OM, otitis media; PF, pars flaccida; PMN, polymorphonuclear leukocyte; S, serous; + indicates the intensity of the changes, with + being the smallest and +++ the largest.

*Animals with purulent otorrhea presented with similar but more intense tissue responses and with high polymorphonuclear infiltration.

†Percentage of the animals in the study that showed S effusion.

features similar to those found in human OME.^{10,13-21} In a study by Tos,¹⁰ germ-free rats developed only a sterile serous effusion with an intense goblet cell hyperplasia after ET obstruction. However, specific pathogen-free Wistar rats with saprophyte germs in the nasopharynx showed humanlike histopathologic changes when developing OME without infection. Kuijpers et al¹⁵ defined these as animals free of pathogenic microorganisms but with nonpathogenic symbiotic germs, reared under hygienic conditions, and housed in a laminar cross-flow cabinet. Therefore, it may be possible that what we are studying is a pathologic entity with serous effusion but different from human OME, because animals with an anatomical disruption or nasopharynx isolation do not reproduce anatomical or physiopathological human conditions. Thus, ET blockage with plastic materials but with-

out local inflammatory procedures^{14,16} provokes just a slight sterile serous effusion with a nonmetaplastic cuboidlike epithelium but without long-term changes.

Mucoperiosteal responses at 15 days were more remarkable in those models in which the inflammatory reaction was promoted by endotoxin intratympanic inoculation or by keyhole limpet hemocyanin and complete Freund adjuvant.¹⁷ Epithelial changes were also significant (edema, hyperplasia, and a higher number of leukocytes). In comparison with these models, in which inflammation was induced by external substances,¹⁷ mucoperiosteal responses were lower in isolated ET obstruction models^{13,14,16} without additional inflammatory stimulation and in models with isolation of the nasopharynx from the middle ear.^{10,14-16,20} These responses were slighter, with an abundant sterile serous effusion but mi-

Table 2. Long-Term (90-Day) Histopathologic Changes in Otitis Media With Effusion (OME) Developed in Different Animal Models

Location of Change	Animal/Technique		
	Wistar Rat, Germ-Free/ Extratympanic ET Cauterization (Ventral Approach) ^{10,15}	Mongolian Gerbil/ Transpalatal External ET Cauterization ^{14,20}	Wistar Rat, Pathogen-Free/ Transpalatal External ET Cauterization (Present Study)
Epithelium			
Hyperplasia	PC +++	Flat to keratin epithelial metaplasia	PC
Edema	++	Cholesterol granulomas	++
Pseudopolyps	++	++	+
Goblet cell	Oval window and ET ++		+
Other	Intraepithelial and subepithelial cylindrical glands		Intraepithelial and subepithelial cylindrical glands
Mucoperiosteal			
Vascular DN	+	+	+
Edema	+	+	+
Cavitations	+		Confluent cavitations
Cell infiltration	Round cells (perivascular) +	++	A few cavitations +
Fibrous tissue	++	+++	++
Bone			
Osteoblasts	Inactivity	Bone erosions	Inactivity
Hypertrophy	++		++
Effusion S/M	++	+++	++
Type	M (sterile)	M	M; cell remainder
Tympanic membrane	Mucosal hypertrophy ++, pseudostratified epithelium	Retractions and adhesions, keratin deposits	Enlarged ++, retraction and adhesions
Comments	Different pathologic characteristics than in human OME	Cholesteatoma develops	Easy and inexpensive technique

Abbreviations: DN, dilatation and neoformation; ET, eustachian tube; M, mucous; PC, pseudostratified and ciliated; S, serous; + indicates the intensity of the changes, with + being the smallest and +++ the largest.

nor vascular dilatation or edema, although an increased goblet cell population was shown.^{10,15} This abundant, serous effusion seems to be related more to an increased secretory activity rather than to the increased permeability that occurs with inflammatory stimulation models or in human OME,⁹ in which vascular dilatation and permeability are related to the presence of endothelial vascular growth factor.²² This factor is released in the inflammatory response.

Fibrous tissue was composed of immature collagen fibers and had a high cellularity in all models at 15 days. Bone and tympanic membrane changes were also simi-

lar in the different models. Therefore, it is reasonable to conclude that these pathologic changes are a consequence of unspecific repairing processes rather than the original cause of the acute inflammation.

At 90 days, mucoperiosteum had decreased and repairing processes were dominant, and an important epithelial metaplasia to pseudostratified epithelium was seen, with edema and increase in secretory cells.^{10,14,15,20}

It is remarkable to see that when ET obstruction procedures were performed in certain animal species such as rabbits, chinchillas, or mongolian gerbils,^{14,20} most of these animals developed a cholesteatoma with keratin accumulation and not OME.

When specific pathogen-free Wistar rats^{10,15} were used instead of germ-free rats, the changes were more similar to those in human OME. Findings from culture samples from germ-free rats were negative for organisms, and symbiotic germs were detected in specific pathogen-free rats. This observation is in accord with recent studies in humans where bacterial colonization in human OME was detected by polymerase chain reaction techniques in all cases.⁴

In conclusion, short- and long-term histopathologic results in our OME experimental model are similar to those in human OME. The procedure presented in this study overcomes weak points found in other experimental models and fairly reproduces physiopathological conditions found in human OME.

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Author Contributions: Drs Vicente, Trinidad, Ramírez-Camacho, García-Berrocal, and Pinilla had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Vicente, Trinidad, Ramírez-Camacho, García-Berrocal, and Pinilla. *Acquisition of data:* Vicente, Trinidad, González-García, Ibáñez, and Pinilla. *Analysis and interpretation of data:* Vicente, Trinidad, Ramírez-Camacho, García-Berrocal, and Ibáñez. *Drafting of the manuscript:* Vicente, Trinidad, Ramírez-Camacho, García-Berrocal, González-García, and Ibáñez. *Critical revision of the manuscript for important intellectual content:* Vicente, Trinidad, Ramírez-Camacho, and García-Berrocal. *Statistical analysis:* Vicente. *Obtained funding:* Vicente and Ramírez-Camacho. *Administrative, technical, and material support:* Vicente, Trinidad, González-García, and Ibáñez. *Study supervision:* Vicente, Ramírez-Camacho, García-Berrocal, and Pinilla.

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