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ORIGINAL ARTICLE

Spontaneous healing of the tympanic membrane after traumatic perforation in rats☆,☆☆

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KEYWORDS
Tympanic membrane perforation;
Wound healing;
Histology

Abstract The most common etiologies of tympanic membrane perforation are infections and trauma.
Objective: The objective of the present study was to assess the healing of traumatic tympanic membrane perforation in rats.
Methods: The tympanic membrane from male Wistar rats was perforated in the anterior and posterior portions to the handle of the malleus. Five tympanic membranes were evaluated 3 days after tympanic perforation; 5 after 5 days; 5 after 7 days; 3 after 10 days; and 4 after 14 days. The tympanic membranes were submitted to histopathological evaluation after hematoxylin–eosin staining.
Results: Tympanic membrane closure occurred at about 7–10 days after injury and the healing process was complete by day 14. The proliferative activity of the outer epithelial layer was present close to the handle of the malleus and to the tympanic annulus.
Conclusion: The spontaneous healing process of the tympanic membrane starts from the outer epithelial layer, with later healing of the lamina propria and the mucosal layer.

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PALAVRAS-CHAVE
Perfuração da membrana timpânica;
Cicatrização;
Histologia

Cicatrização espontânea da membrana timpânica após sua perfuração traumática: estudo experimental em ratos

Resumo As causas mais comuns de perfurações de membrana timpânica são infecções e trauma.
Objetivos: Avaliar o reparo cicatricial de perfurações traumáticas da membrana timpânica em ratos.
Método: A membrana timpânica de ratos Wistar machos foram perfuradas nas porções anterior e posterior ao cabo do martelo. Cinco membranas timpânicas foram avaliadas 3 dias após perfuração timpânica; 5 após 5 dias; 5 após 7 dias; 3 após 10 dias; e 4 após 14 dias. As membranas timpânicas foram submetidas à avaliação histopatológica após coloração com hematoxilina-eosina.
Resultados: O fechamento da membrana timpânica ocorreu em torno de 7 a 10 dias após perfuração traumática, e o processo de cicatrização estava completo no 14° dia. A atividade proliferativa da camada epitelial externa foi identificada próxima ao cabo do martelo e ao ânulus timpânico.
Conclusão: O processo de cicatrização espontânea da membrana timpânica se inicia com a camada epitelial externa, com posterior cicatrização da lâmina própria e da camada mucosa.

Introduction

The tympanic membrane (TM) is an anatomical structure that separates the outer ear from the middle ear. The TM is responsible for sound amplification and transmission through the ossicular chain to the oval window and vestibular ramp, in addition to the protection of the round window and the tympanic ramp.[1]

The ultrastructural anatomy of TM consists of 3 layers: the outer layer, of epithelial (ectodermal) origin; the middle layer or lamina propria, of mesodermal origin; and the inner layer, of endodermal origin, comprising the middle ear mucosa.[2]

The outer layer consists of keratinized stratified squamous epithelium.[3–5]

The middle layer or lamina propria consists of loose subepithelial connective tissue, organized dense connective tissue and submucosal loose connective tissue. Loose subepidermal and submucosal connective tissue consists of loosely arranged collagen fibers, fibroblasts, nerve fibers and capillaries. The dense connective tissue consists of more externally organized radial collagen fibers and more internally organized circular collagen fibers.[2–5]The inner layer consists of simple columnar epithelial tissue, which is continuous with the middle ear mucosa.[2–6]

The most common etiologies of TM perforations are otitis media and trauma.[7] Traumatic TM perforations have a 78.7% rate of healing.[8] Factors that prevent adequate repair of TM in the absence of infection are yet to be defined.[7,8]

The objective of this study is to develop the two-dimensional study of scar repair in traumatic TM perforations in rats.

Method

The experimental study was carried out with 19 male Wistar albino rats (*Rattus norvegicus*), weighing on average 280 g (range 270–290 g). The study followed the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee on Animal Experimentation (CETEA) on 08/27/2007 – protocol number for use of animals in research No. 082/2007.

Before the procedure, all animals were anesthetized with intramuscular ketamine hydrochloride (40 mg/kg) (Ketamine 50 mg/mL, Cristália Laboratory, São Paulo, Brazil) and intramuscular xylazine hydrochloride (5 mg/kg) (Dopaser 20 mg/mL, Hertape Calier, Minas Gerais, Brazil). The ears of all animals were assessed using a DFV MU-M19 otomicroscope (DFV, Rio de Janeiro, Brazil) before the procedure to rule out infection. A total of 19 animals were included in the study, equivalent to 23 bullae with normal TMs. Bullae with infection were excluded from the procedure.

Traumatic perforation of the tympanic membrane was performed with a 30 mm × 0.8 mm BD needle (Becton Dickinson, New Jersey, USA) anterior and posterior to the malleus handle, in the pars tensa region of the TM (Fig. 1). For histological evaluation, 3 animals were euthanized 3 days after the perforation (5 bullae), 4 animals after 5 days (5 bullae), 5 animals after 7 days (5 bullae), 3 animals after 10 days (3 bullae) and 3 animals after 14 days (4 bullae). One animal (1 bulla) with intact TM was assessed as control. The animals were euthanized with an intraperitoneal injection of an overdose of thiopental (Thionembutal, Abbot, São Paulo, Brazil).

The bullae were removed from the animals and fixed for 24 h in 10% formalin (Merck) diluted in phosphate buffer solution and then decalcified in an aqueous solution of 4.13 g EDTA (Merck) and 0.55 g NaOH (Merck) for approximately 50 days. After decalcification, the samples were dehydrated in ethanol, xylene and embedded in paraffin. Histological sections of 6 μm thickness were performed with Leica Jung RM2065 microtome (Leica Microsystems GmbH, Wetzlar, Germany). Sections were oriented using the malleus