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Rat model of chronic tympanic membrane perforation: A longitudinal histological evaluation of underlying mechanisms

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ABSTRACT

Objective: To evaluate histologically the progressive development and underlying mechanisms of chronic tympanic membrane perforation (TMP) in a rat model using a two-weeks ventilation tube (VT) treatment combined with topical application of mitomycin C/dexamethasone (VT-M/D), compared with normal tympanic membrane and acute TMPs.

Methods: Fifty male Sprague-Dawley rats were divided into three experimental groups: a normal control group (n = 5), an acute TMP group (n = 5) (i.e. 3 days post-myringotomy) and a VT-M/D group (n = 40). The TMs were regularly assessed by otoscopy. The normal control animals were sacrificed on day 0 and the acute TMP group was sacrificed 3 days post-myringotomy for histological and immunohistochemical evaluations. The VT-M/D group was sacrificed at various time points - 14 and 17 days, 3, 4, 6, 8 and 10 weeks.

Results: On longitudinal histological examination, compared with normal TM and acute TMP, the perforation edges at the later time points illustrated thickened stratified squamous epithelium rimming around the edges, significant increase in keratin and collagen deposition, increased macrophage infiltration as well as reduced cellular proliferation. Three phases of TMP healing process were identified - the acute healing phase (3–17 days), the transition phase (3–4 weeks) and the chronic phase (6–10 weeks).

Conclusion: Based on the histological results of this study, the progressive development of chronic TMPs appeared to be associated with increased epidermal thickening, collagen and keratin deposition, macrophage infiltration and reduced cellular proliferation. After the 3–4 weeks of transition phase, the TMPs seemed to have transformed into a non-healing chronic TMP between 6 and 10 weeks.

1. Introduction

Tympanic membrane perforation (TMP) is a common clinical pathology worldwide, usually resulting from otitis media, trauma or ventilation tube (VT) treatment. A majority of acute TMPs heal spontaneously within 10 days [1,2] but those that fail to heal and stay patent are defined as chronic. In cases of chronic TMPs, surgical grafting is an option for treatment. In order to evaluate novel graft or repair treatments, it is essential to develop a chronic TMP animal model that would mimic the clinical condition of chronic TMPs.

The current level of evidence in the literature on chronic TMP animal models has been recently brought to attention [3–5]. Various animal species have been utilized in the creation of models for chronic TMPs including chinchilla [6,7], guinea pig [8,9], rat [10,11], dog [12] and mouse [13]. Numerous techniques have been trialed including infolding methods [6,14], thermal injury [15,16],

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re-myringotomy [17,18], application of topical agents (e.g. mitomycin C [11,19], steroids [10,20]) and by genetic modification [13]. Controversies and discussions have been generated regarding both the reproducibility and validity of previously reported methods [21–26]. Topical applications of mitomycin C and dexamethasone (M/D) have been shown by another group [11,19] in creating chronic TM perforations in a rat model. However, our research group recently found that topical M/D delayed healing but not enough to produce a chronic TMP in rats [26].

A strong association of increased chronic TMP incidence in patients with long-term VT treatment (i.e. several years) compared with short-term VT treatment (i.e. 8–14 months) has been reported in the literature. Previously reported rates of persistent TMPs in patients after long-term VT treatment ranged between 10 and 30% [27–36]. Based on this clinical observation, our research group embarked on a rat study and found that chronic TMPs staying patent for up to 10 weeks were successfully created by VT treatment only. The success rate, however, was only 20%. When VT treatment was combined with topical application of M/D (VT-M/D) the success rate at 10 weeks was increased to approximately 70%, which is a rate suitable for experimental evaluation of potential preventions or remedies [37]. We also showed that these persistent TMPs depicted the classic histological features of chronic TMPs as described in the literature [6].

Acute TMPs have been investigated for healing mechanisms at histological [38–42] and genetic levels [43,44], however, the mechanisms involved in delayed or arrested healing of a TMP leading to the formation of chronic TMPs in patients are still not clear. Why do certain TMPs close spontaneously yet others stay patent as chronic TMPs? Twenty years ago, a pioneering study by Spandow et al. [45] investigated the perforation edges histologically in chronic TMP from 25 patients selected for myringoplasty. Nevertheless, further experimental mechanistic studies on the formation of chronic TMPs have not yet been described in the literature.

The aim of this study was to evaluate longitudinally the histological development of chronic TMPs in a rat model utilizing VT-M/D at various time points to assess the underlying mechanisms in the progressive development of chronicity, in comparison with normal controls and acute TMPs.

2. Materials and methods

2.1. Animals

Fifty male Sprague-Dawley rats, weighing 250–300 g, were obtained from the Animal Resources Centre (Murdoch, Western Australia, Australia). Experiments were approved by the Animal Ethics Committee of The University of Western Australia (No. 100/1239). Experiments were performed in accordance with the National Health and Medical Research Council of Australia Code of Practice for Care and Use of Animals for Scientific Purposes. Rats were maintained in a room with twelve-hour light/dark cycles and provided with food and water *ad libitum*.

2.2. Materials

Fluoroplastic Collar Bobbin VTs were utilized with an inner diameter of 0.75 mm (Olympus, Australia; Fig. 1A). Five mg of Mitomycin C (Sigma-Aldrich, USA) was dissolved in sterile water to a concentration of 0.5 mg/ml. Dexamethasone solution (5 mg/ml) was purchased from Ilium, Australia. Gelfoam® was purchased from Ethicon Inc (Somerville, USA). Microsurgical instruments (Karl Storz Ltd., Germany) were autoclaved routinely before use. The otomicroscope was Stativ S3 from Zeiss (Sydney, Australia) and the digital video-otoscope was from MedRX (USA).

2.3. Experimental design

A total of fifty rats were assigned to three treatment groups: a normal control without any intervention (n = 5), an acute TMP group (n = 5) (i.e. day 3 post-myringotomy) and a VT-M/D group (n = 40). Only the right TM of each animal underwent a procedure while the left TM was untouched. Post-operatively, all ears were observed with otoscopy regularly. The normal control group animals were sacrificed on day 0 while the acute TMP group animals were sacrificed on day 3 post-myringotomy for histological and immunohistochemical evaluations. In the VT-M/D group, three to four animals with patent TMPs were sacrificed longitudinally at each of the various time points – 14 and 17 days, 3, 4, 6, 8 and 10 weeks. Animals with closed TMPs on routine otoscopy at each of various time points were excluded from the study.

2.4. Surgical procedure

Before commencement of experiments, both ears were examined with an otomicroscope to exclude middle ear disease. Debris from the external auditory canal was removed when needed. The animals were put under general anesthesia with isoflurane (Bomac, New Zealand) (4% induction, 2% maintenance in 100% oxygen) throughout all surgical procedures. Unilateral right side myringotomy was performed via a transcanal approach using a Wullstein needle. The posterior half of the pars tensa was perforated to an approximate diameter of 0.8 mm, gauged using the tip of the Wullstein needle. The primary author (AYW) performed all surgeries to ensure consistency.

2.4.1. Normal control group

The TMs underwent no intervention at all and animals were sacrificed on day 0.

2.4.2. Acute TMP group

After myringotomy, there was no further intervention (Gelfoam was not applied to the TMP), allowing the TMPs to start spontaneous healing. The animals were sacrificed on day 3 post-myringotomy.

2.4.3. VT-M/D group (ventilation tube and mitomycin C/dexamethasone)

Immediately following myringotomy on day 0 (Fig. 1B), a small piece of Gelfoam soaked with mitomycin C (0.5 mg/ml) was placed over the TMP for ten minutes and then removed. Next, a second small piece of Gelfoam soaked with dexamethasone (5 mg/ml) was placed over TMP for ten minutes and then removed. Immediately thereafter, a VT was inserted into the TMP (Fig. 1C) using a Wullstein needle. On day 14, immediately after removal of the VT (Fig. 1D), a second dose of M/D was topically applied on TMP according to the same protocol as described above. The animals were sacrificed at various time points - 14 and 17 days, 3, 4, 6, 8 and 10 weeks.

2.5. Otoscopy

All rats underwent regular otoscopic observation under general anesthesia postoperatively until the time of sacrifice using an otomicroscope and a digital video-otoscope. The TMPs were judged as either closed or patent. Digital images were recorded using Aurisview software (Ear Science Institute Australia, Australia).