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Searching for a rat model of chronic tympanic membrane perforation: Healing delayed by mitomycin C/dexamethasone but not paper implantation or iterative myringotomy

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ABSTRACT

Objectives: Surgical intervention such as myringoplasty or tympanoplasty is an option in the current clinical management of chronic tympanic membrane perforation (TMP). Animal models of chronic TMP are needed for pre-clinical testing of new materials and to improve existing techniques. We evaluated several reported animal model techniques from the literature for the creation of chronic TMPs. The aim of this study was to evaluate production of chronic TMPs in a rat model using topical mitomycin C/dexamethasone, paper insertion into middle ear cavity (MEC) or re-myringotomy.
Methods: Forty male Sprague–Dawley rats underwent myringotomy of the right tympanic membrane (TM) and were randomly divided into 3 experimental groups: application of topical mitomycin C/dexamethasone, paper insertion into middle ear cavity, or re-myringotomy. Control perforations were allowed to close spontaneously. TMs were assessed regularly with otoscopy for 8 weeks. At the end of 8 weeks, animals were sacrificed for histology.
Results: The closure of TMPs was significantly delayed by mitomycin C/dexamethasone (mean patency, 18.9 days; $P \leq 0.01$) compared with the control (mean patency, 7 days), but was not significantly delayed in the paper insertion group (mean patency, 9.4 days; $P = 0.74$). Repeated myringotomy of closed perforations (mean number of myringotomies, 8.9 per ear) stimulated acceleration of closure rather than delay. Histologically, the mitomycin C/dexamethasone group had almost normal TM morphology, while the paper insertion group revealed inflammatory and granulomatous responses. The re-myringotomy group had a thickened TM fibrous layer with collagen deposition.
Conclusions: Mitomycin C/dexamethasone delayed TMP closure in rats but the effect was not sufficiently long-lasting to be defined as a chronic TMP. Neither paper insertion into middle ear cavity nor re-myringotomy created chronic TMP in rats.

1. Introduction

Tympanic membrane perforation (TMP) is a common clinical presentation worldwide, caused mainly by trauma, infections (e.g. otitis media) or ventilation tube removal/extrusion. Although the majority of acute TMPs heal spontaneously without intervention [1], those which fail to heal within three months can be defined as chronic TMP. Chronic TMPs often stay permanently patent and are unlikely to close spontaneously. In these cases, surgical grafting is an option for treatment. Currently, the most preferred graft materials are autologous temporalis fascia, cartilage and perichondrium.

The development of novel graft materials for TM repair is required to undergo extensive in vitro and in vivo (i.e. animal

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models) evaluations prior to clinical trials. In order to fully examine the efficacy of a novel graft, a chronic TMP animal model that mimics the clinical condition of chronic TMPs in patients is paramount. In the current literature, various animal models have been reported, including rat [2], mouse [3], chinchilla [4], guinea pig [5] and dog [6]. However, these models are mostly of acute TMPs and not useful for assessment of materials for myringoplasty or tympanoplasty on chronic TMPs. Up to 94% [7,8] of acute TMPs heal spontaneously without intervention, thus accelerating the healing of acute TMPs is of less practical value. In contrast, a chronic TMP animal model has more clinical relevance but an 'ideal' animal model has not yet been found. Currently, the most popular preference on chronic animal models defines a minimum duration of TMP patency to be at eight weeks [9–12] and so we look to evaluate published models to test for TMP of eight weeks duration in rats.

The combination of mitomycin C and steroid (dexamethasone or hydrocortisone) has been shown recently to be efficacious in creating chronic TMP animal models. Seonwoo et al. [13] utilized topical mitomycin C (0.5 mg/ml) and dexamethasone (5 mg/ml) in rats with a reported chronic TMP success rate of 100% after one week. Cui et al. [14] tested topical mitomycin C (0.2 mg/ml) with hydrocortisone (5 mg/ml) in rats having a success rate of 87% after eight weeks. A recent study [15] applying topical hydrocortisone with ciprofloxacin antibiotic and mitomycin C (0.2 mg/ml) on guinea pigs achieved a success rate of 80% at six weeks.

Paper insertion into middle ear cavity (MEC) involves inserting cigarette paper strips through TMPs into the MEC. Shen et al. from our laboratory [16] conducted a biocompatibility study of numerous materials by inserting paper, silk fibroin, collagen and Gelfoam® into MEC of rats. In the paper group, six of nine ears (66.7%) were found with persistent patent TMPs with serous otorrhea in two ears at each of the three time points—2, 4, and 12 weeks postoperatively. Thus, in this study we hypothesized that the incorporation of paper into MEC could be a chronic TMP animal model, by inducing a local inflammatory response to delay closure of TMPs and modeling suppurative otitis media.

The re-myringotomy technique entails re-perforation of an initial myringotomy site upon closure at follow-up otoscopy. Laidlaw et al. [17] applied re-myringotomy in chinchillas and reported chronic TMP in 85% of animals after a period of five to eight weeks. Numerous studies have evaluated re-myringotomy technique in combination with thermal injury and/or infolding technique showing variable success rates ranging from 69–100% [18–26].

Hence, the aim of this study was to evaluate and validate the efficacy of three techniques in creating chronic TMP in a rat model: (1) combination of topical mitomycin C/dexamethasone, (2) paper insertion into MEC, and (3) re-myringotomy.

2. Materials and methods

2.1. Materials

Mitomycin C from *Streptomyces caespitosus* in 5 mg powder (Sigma-Aldrich, St Louis, USA) was dissolved in sterile water for injection (Ilium, Australia) to a concentration of 0.5 mg/ml. Dexamethasone sodium phosphate solution (5 mg/ml) was purchased from Ilium, Australia. Gelfoam® (absorbable gelatin sponge) was purchased from Ethicon Inc (Somerville, USA) and supplied sterile in original packaging. Paper strips were prepared from cigarette paper (Tally Ho, Imperial Tobacco, Australia) packaged and sterilized with ethylene oxide gas prior to use. Microsurgical instruments (Karl Storz Ltd., Tuttlingen, Germany) were autoclaved routinely before use. The otomicroscope was Stativ S3 from Zeiss (Sydney, Australia) and digital video-otoscope was from MedRX (Largo, USA).

2.2. Animals

Forty male Sprague–Dawley rats (*Rattus norvegicus*), weighing 250–300 g, seven to eight weeks old, were obtained from Animal Resources Centre (Murdoch, Western Australia, Australia) and allowed to acclimate for seven days. The experiments were approved by Animal Ethics Committee of The University of Western Australia (UWA) (approval number: 100/1239). Experiments were performed in accordance with National Health and Medical Research Council of Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Dedicated barrier animal resources facility located on campus of UWA was used to house animals. Twelve-hour light/dark cycles were maintained in a room and provided with food and water ad libitum.

2.3. Experimental design

A total of 40 rats were randomly assigned to four treatment groups: control (initial myringotomy only) ($N = 10$), topical application of mitomycin C/dexamethasone ($N = 10$), paper insertion into MEC ($N = 10$) and re-myringotomy ($N = 10$). Only the right TM of each animal underwent procedures while the left TM were untouched and used as normal controls. Postoperatively, all 40 rats were observed via otoscopy on a regular basis throughout the total duration of eight weeks. All animals were sacrificed at a single final time point at the end of eight weeks for histology.

2.4. Surgical procedure

Prior to commencement of the experiment, both ears of each rat were examined with an otomicroscope to exclude any middle ear disease. Debris from external auditory canal (EAC) was removed using a 1.0 mm Baron suction tube and 4.0 mm aural speculum when needed.

Animals were put under general anesthesia using an inhalational technique with isoflurane (Bomac, New Zealand) (4% induction, 2% maintenance in 100% oxygen) throughout all surgical procedures. Prior to surgery, ear canals and surrounding skin were swabbed and cleaned with povidone iodine solution. Under aseptic conditions, unilateral right myringotomy was performed via transcanal approach using a Wullstein needle. The posterior half of the pars tensa was perforated to a diameter of 0.8 mm. The tip of the Wullstein needle (0.2 mm diameter) was used as a gauge to ensure that the diameter of the TMP was consistently 0.8 mm. The primary author of this study (AYW) performed all the animal surgeries in this experiment in order to ensure consistency.

2.4.1. Control group

Once initial myringotomy was performed (described above), there was no further intervention allowing TMPs to close spontaneously.

2.4.2. Mitomycin C/dexamethasone group

Immediately following myrinogtomy, a Gelfoam soaked with mitomycin C (0.5 mg/ml) was placed on perforation margin for ten min and was then removed. Subsequently, a second Gelfoam soaked in dexamethasone (5 mg/ml) was placed on TMP which was left in-situ before removal on day seven. This exact method has been reported to be efficacious by Seonwoo et al. [13]. Upon examination by routine otoscopy during the first week, if dexamethasone soaked Gelfoam was found to be absent or displaced from TMP, a new Gelfoam soaked with dexamethasone was re-applied. Upon examination by routine otoscopy during the first week, dexamethasone soaked Gelfoam was found to be absent or displaced from the TMP in one instance, so a new Gelfoam soaked with dexamethasone was re-applied.