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Review Article

Animal models of chronic tympanic membrane perforation: A 'time-out' to review evidence and standardize design☆

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ABSTRACT

Objective: To review the literature on techniques for creation of chronic tympanic membrane perforations (TMP) in animal models. Establishing such models in a laboratory setting will have value if they replicate many of the properties of the human clinical condition and can thus be used for investigation of novel grafting materials or other interventions.

Methods: A literature search of the PubMed database (1950–August 2014) was performed.

The search included all English-language literature published attempts on chronic or delayed TMP in animal models. Studies of non English-language or acute TMP were excluded.

Results: Thirty-seven studies were identified. Various methods to create TMP in animals have been used including infolding technique, thermal injury, re-myringotomy, and topical agents including chemicals and growth factor receptor inhibitors. The most common type of animal utilized was chinchilla, followed by rat and guinea pig. Twenty three of the 37 studies reported success in achieving chronic TMP animal model while 14 studies solely delayed the healing of TMP. Numerous experimental limitations were identified including TMP patency duration of <8 weeks, lack of documentation of total number of animals attempted and absence of proof for chronicity with otoscopic and histologic evidence.

Conclusion: The existing literature demonstrates the need for an ideal chronic TMP animal model to allow the development of new treatments and evaluate the risk of their clinical application. Various identified techniques seem promising, however, a need was identified for standardization of experimental design and evidence to address multiple limitations.

Contents

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1. Introduction

Tympanic membrane perforations (TMP) are common, usually resulting from trauma and infection. Most TMP heal spontaneously in 7–10 days [1,2] by a complex series of events including epithelial migration, fibroblastic activity and vascular proliferation. However, some TMP fail to heal within 3 months and are associated with conductive hearing loss and recurrent infection. These chronic TMP attract surgical interventions (e.g. myringoplasty), typically with a human autograft (e.g. temporalis fascia). With the advent of modern biomaterials, new options are being considered to increase success rate and to address deficiencies in autografts for repair of chronic TMP, such as increased intra-operative time and general anesthetic risks. Many novel materials have an unknown safety and efficacy profile, so need to undergo evaluation (i.e. animal models) prior to clinical trial [3]. A variety of animal models have been used, including rat [4,5], mouse [6], chinchilla [7], guinea pig [8,9] and dog [10]. However, their relevance has been hampered by the acute nature of most TMP animal models rather than the chronic TMP we treat in patients. Up to 94% of acute TMP [1,2] heal spontaneously without intervention, potentially reducing their clinical relevance [11]. Testing interventions on sub-optimal or unreliable chronic TMP animal models could provide inaccurate and misleading interpretations, with increased risk when transitioning treatments to clinical trials.

Our research group reviewed the literature on TMP in 2007 and identified the need for chronic TMP animal models [12]. Similar conclusion was made in a recent review by Hong et al. [11]. Since 2007, there have been 18 additional studies attempting to produce chronic TMP, with both positive and negative outcomes. Importantly, there have been studies where the concept of chronicity was only weakly developed and whether the methods used did create a 'true' chronic TMP is still controversial [13–15]. In a recent commentary [16], we further highlighted this need for standardized experimental design and evidence for chronic TMP. Currently, there is no consensual definition for a chronic TMP animal model published.

In this review, we update our literature review on the topic, discuss the mechanisms of chronic TMP formation, evaluate the evidence of different methods and identify limitations in experimental design and methodology for chronic TMP animal models. We aim to provide a standardized protocol for future studies.

2. Methods

A search of the online database of the National Library of Medicine (PubMed) was performed from 1950 to August 2014 to identify all publications regarding attempts on chronic or delayed TMP in animal models. The key terms "chronic", "delayed", "tympanic membrane", "perforation" and "animal model" were searched and combined to identify 44 titles and abstracts. These were reviewed and the inclusion and exclusion criteria were applied to narrow the list of studies to 37. The inclusion criteria were as follows: (1) methods in delaying TMP in animal model or (1) methods in creating chronic TMP in animal model. Exclusion criteria included: (1) testing acute TMP in animal model or (2) non English-language publication.

3. Review

3.1. Mechanism of chronic tympanic membrane perforation in humans

The mechanism resulting in persistent patency of chronic TMP in man is poorly understood. It is believed that the spontaneous healing capability of the TM has been impeded by numerous factors [17]. According to previous literature, we have classified hypotheses into structural, histologic, infectious and growth factor-related mechanisms. In terms of structural factors, a large sized perforation might have insufficient structural support to allow bridging epithelium to span the defect [18]. Nonetheless, some argued [4,19] against structural deficits given that many large traumatic perforations heal spontaneously, while other small sized TMP persist. In very large TM defects, the epithelial germinating centers on the remnant TM and middle ear might be disrupted resulting in impaired re-epithelialization. From a histologic point of view, instead of the epithelium migrating across the perforation edge to bridge the defect, as in normal TMP healing, in chronic TMP a mature epithelial rim is formed around the edge of the perforation to meet with the medial mucosal layer of TM [20]. This epithelialization pattern has been suggested to be a barrier to further healing [7,17]. Infectious factor such as otitis media are closely associated with many of the chronic TMP, however exactly how infection hinders healing of TM is poorly understood. Presumably epithelial proliferation or migration is inhibited leading to epithelial rim formation [4]. Others believe that infection causes growth inhibition by promoting epithelialization of TMP edge [17,21,22], inadequate supply of growth factors at TMP edge [21,23] or deficient blood supply [24]. In addition, during healing many growth factors regulate inflammation, angiogenesis, re-epithelialization and tissue regeneration [25]. Impairment of growth factor pathways may be involved in chronic TMP. Animal studies have shown that growth factor receptor inhibitors delay healing of TMP [26–28] while exogenous growth factors may enhance the rate of healing [29–31].

3.2. Efficacy of chronic TMP animal model methods

Various methods have been used to create chronic TMP animal models (Tables 1 and 2). We have categorized these into three groups according to a reported level of efficacy, from the weakest delayed perforation healing outcomes to the most promising chronic perforations. These methods are summarized in Table 1 (reported successful) and Table 2 (delayed).