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Animal models of chronic tympanic membrane perforation: in response to plasminogen initiates and potentiates the healing of acute and chronic tympanic membrane perforations in mice

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Abstract

Tympanic membrane perforations (TMP) are relatively common but are typically not treated in their acute stage, as most will heal spontaneously in 7–10 days. Those cases which fail to heal within 3 months are called chronic TMP which attract surgical intervention (e.g. myringoplasty), typically with a temporalis fascia autograft. New materials for the repair of chronic TMP are being developed to address deficiencies in the performance of autografts by undergoing evaluation in animal models prior to clinical study. However, there is currently a lack of ideal chronic TMP animal models available, hindering the development of new treatments. Various techniques and animal species have been investigated for the creation of chronic TMP with varied success. In the present commentary, we bring to the attention of readers the recent report by Shen et al. in Journal of Translational Medicine. The study reported the creation of a chronic TMP animal model in plasminogen gene deficient mice. However, the short observation time (9, 19 days), lack of success rate and the scarcity of solid evidence (e.g. otoscopic & histologic images) to confirm the chronicity of TMP warrant a more thorough discussion.

Keywords: Tympanic membrane perforation; Chronic; Animal model; Otology; Plasminogen; Gene deficiency

Background

Tympanic membrane perforations (TMP) are a common problem in otology, resulting from trauma, infection (e.g., otitis media) or a sequel after extrusion of tympanostomy tubes. A major proportion of the acute TMP heals spontaneously in 7–10 days [1,2] without further intervention by epithelial migration, fibroblastic activity and vascular proliferation. A minority of cases fails to heal within 3 months which are called chronic TMP associating with morbidities such as conductive hearing loss, recurrent chronic infections and cholesteatoma formation. Chronic TMP attract surgical intervention (e.g. myringoplasty), typically with a temporalis fascia autograft.

New materials for the repair of chronic TMP are being developed to address deficiencies in the performance of autografts by undergoing evaluation in animal models prior to clinical study [3]. These studies have been investigated on a variety of animal models, including rat [4,5], mice [6], chinchilla [7], guinea pig [8,9] and dog [10]. However their relevance has been hampered by the utilization of acute TMP models rather than chronic. The major inadequacy of the acute model is that up to 90% [1,2] heal spontaneously without intervention and therefore accelerating the healing of acute TMP is of little practical value. Hence, a chronic TMP animal model would have more clinical relevance [11].

The lack of an ideal chronic TMP animal model was initially brought to attention in 2007 [12], as this issue hinders the development of new treatment. Since then, there have been additional studies in the literature involving creation of chronic TMP animal model associated with various techniques, animal species and success

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rates. However, whether these existing methods do in fact create a 'true' chronic TMP is still controversial.

Commentary

We read with great interest the recent research article by Shen et al. [13] investigating the efficacy of injections of plasminogen (plg) on the healing of both acute and chronic tympanic membrane perforations (TMP) in mice with plg gene deficiency ($plg^{-/-}$). This intervention significantly improved healing rates of chronic TMP in $plg^{-/-}$ mice and indicates an important role of plasminogen in TMP healing.

We would like to draw attention to the labeling of this model as a chronic perforation by Shen et al. [13], and the indicated potential for translating plasminogen injection treatment to clinical use. The tympanic membranes were perforated on day 0, left untreated for a period of 9 days, and thereafter treatments applied for comparison of effects on healing rate over a further period of 10 days. However, the most popular consensus in the current literature defines the minimum duration of chronic TMP patency to be at least 8 weeks in animal models [14-24]. Can this short time period of 9 days be defined as chronic or rather as delayed? Indeed, many publications of chronic TMP animal model have conservatively defined their TMP failing to reach the maximum patency time up to 8 weeks as delayed healing [4,25-31], including the recently reported study on mice with deficiency in urokinase-type plg activators [25].

More detailed information may help to resolve this question, specifically to the methodology of assessing a chronic TMP animal model. The success rate of this chronic TMP method (i.e. number of successful chronic TMP before plg injection divided by the total number of ears initially attempted) was not provided. Thus, it is difficult to assess the reliability and stability of this particular chronic method. This study has employed an animal model (i.e. $plg^{-/-}$ mice) previously described by Li et al. [32] demonstrating that in $plg^{-/-}$ mice, 2 of 10 TMP (success rate of 80%) have closed on day 8; 21 of 26 TMP (success rate of 19%) have healed by day 50 (i.e. just after 7 weeks) and all TMP have closed by day 143. Given the relatively low success rate at day 50, this seems to be a delayed model under the conservative definition, rather than a chronic model.

It may be of benefit to illustrate chronicity of the TMP with morphologic evidence, such as otoscopic and histologic images. Otoscopic examination is necessary to evaluate the patency of a chronic TMP with typical features of thickened and opaque margins compared to an acute TMP. Microscopically, a chronic TMP is featured by squamous epithelium growing over the perforation edge to join with the medial mucosal layer [7,33]. This evidence was not included in this article to confirm that chronicity of TMP was achieved before the intervention of plg injections. Previous studies with success have presented this important piece of histologic evidence [7,10,14,16]. Thus, whether 'true' chronic TMP were produced in this mouse model given the absence of otoscopic and histologic proof is uncertain.

Discussion

The absence of an ideal chronic TMP animal model in the current literature has been brought to attention in recent years [3,11,12], as this issue hinders development of treatments for chronic TMP in a clinical setting. Since then, there have been several studies in the literature involving creation of chronic TMP animal models associated with various techniques (e.g. infolding technique [7,14,16], thermal injury + reperforation [22,23,34], mitomycin C + steroids [21,24,35]), animals (e.g. chinchilla, rat, guinea pig, mouse) and success rates. However, whether these existing methods do in fact create a 'true' chronic TMP is still controversial [36,37]. In addition, in terms of TMP healing, there is a significant discrepancy between an acute and chronic TMP in terms of their underlying healing mechanisms and responses to the same treatment [9,38]. A major proportion of acute TMP in both human and rodents heal spontaneously without any intervention within 7–10 [1,2] and 5–7 days [39,40] respectively via otoscopic observation. Thus, mislabeling an actual acute TMP model as chronic may give an ambiguous interpretation.

Conclusion

In conclusion, we value the potential of $plg^{-/-}$ mice as an animal model of TMP healing mechanisms [13], but an observation period of at least 8 weeks before treatment is commenced may ensure a chronic condition is being treated. The outcome of this novel injection of plg for TMP repair seems ultimately to be promising for clinical therapy. However, caution needs to be paid to the reliability of this animal model to provide an accurate prediction of efficacy for chronic TMP, otherwise the success of future transition to human clinical trials may be doubtful and even potentially risky.

Abbreviations

Plg: Plasminogen; $plg^{-/-}$: Plasminogen deficient; TMP: Tympanic membrane perforation.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

AYW, YS, JTW, RHE and RJD made intellectual contributions and drafted the manuscript. All authors read and approved the final manuscript.

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