The Journal of Laryngology & Otology (2013), ***127***, *359–363*.

doi:10.1017/S002221511300011X

MAIN ARTICLE

Effect of intratympanic mitomycin C on the development of cholesteatoma and otitis media in rats

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Abstract

Objective: To determine whether the administration of mitomycin C prevents propylene glycol exposure from inducing middle-ear cholesteatoma and otitis media, in a rat model.

Methods: Twenty-four Wistar rats underwent intratympanic injections on days 1, 8 and 15, via the tympanic membrane pars tensa, in both the right and left ears. The right ear injection solution contained 50 per cent propylene glycol, gentamicin and saline (0.9 per cent), while the left ear solution contained 50 per cent propylene glycol, gentamicin and mitomycin C. Animals were sacrificed and examined.

Results: There were statistically significant differences between the control and experimental groups for tympanic bulla mucosal thickness ($p = 0.004$) but not for tympanic membrane thickness ($p = 0.371$), otomicroscopic findings ($p = 0.262$), or the presence of exudate ($p = 0.125$), fibrosis ($p = 1.000$) or cholesteatoma ($p = 0.687$).

Conclusion: Intratympanic mitomycin C was ineffective in preventing middle-ear cholesteatoma and otitis media in this rat model.

Key words: Mitomycin C; Cholesteatoma; Otitis Media; Propylene Glycol; Rats, Wistar

Introduction

It has long been known that it is possible to induce otitis media and cholesteatoma in the middle ear of experimental animals via various means.[1–3]

In the last 20 years, experimental and clinical use of mitomycin C has been introduced. This drug acts by inhibiting fibroblast proliferation and has been used to treat recurrent conjunctival and corneal squamous cell carcinoma.[4–6] The pharmacological properties of mitomycin C may give it a potential role (as yet uninvestigated) in the process of cholesteatoma formation.

The current study aimed to analyse the effect of mitomycin C on the development of cholesteatoma and otitis media in rats receiving intratympanic injection of propylene glycol. This was done by assessing the following histopathological features: presence of keratinised epithelium (cholesteatoma) in the middle ear; type and extent of inflammation of the middle-ear mucosa as well as the presence of exudate and fibrosis; and tympanic membrane morphology.

This research is justified by the possibility of therapeutic use of mitomycin C in humans, should the drug prove to be effective.

Methods

Population

Twenty-four healthy, male, albino, Wistar rats weighing 220 to 480 g were used. The study was performed between December 2004 and March 2005. The study was approved by our institution's ethical committee for animal experimentation.

Two animals with normal appearance on otomicroscopy, who had undergone no previous procedure, were sacrificed to establish normal parameters for tympanic membrane morphology and cross-sectional middle-ear mucosal thickness.

Study design

The study was designed as an experimental, controlled, paired study.

Anaesthesia

Procedures were performed under general anaesthesia using 10 per cent chloral hydrate administered intraperitoneally (0.4 ml for each 100 g body weight).

Presented (and awarded a thesis prize) at the 39th Brazilian Congress of Otolaryngology and Head and Neck Surgery, 31 March to 4 April 2008, Belo Horizonte, Brazil
Accepted for publication 14 June 2012 First published online 14 February 2013

Induction of inflammatory process

All rats underwent otomicroscopy with a surgical microscope (Model MC-31; DF Vasconcelos, Sao Paulo, Brazil) using cold light at 10-fold magnification. Twenty-four animals with normal appearance on bilateral otomicroscopy were included in this study.

Propylene glycol (molecular weight 76.09 u) was used to induce an inflammatory reaction and cholesteatoma. Mitomycin C (molecular weight, 334.33 u) was used to inhibit the development of these pathological processes, and gentamicin sulphate (molecular weight, 575.67 u) was used to decrease infection.

The rats underwent intratympanic injections on days 1, 8 and 15, in both their right ear (control group) and left ear (experimental group), via the tympanic membrane pars tensa, guided by a surgical microscope (×10). A total of three injections was received in each ear. The solution injected into the right ear contained 0.2 ml 50 per cent propylene glycol, 0.1 ml gentamicin sulphate (40 mg/ml) and 0.1 ml physiological saline (0.9 per cent). The solution injected into the left ear contained 0.2 ml 50 per cent propylene glycol, 0.1 ml gentamicin sulphate (40 mg/ml) and 0.1 ml mitomycin C (0.5 mg/ml).

Tympanic bulla collection and histological preparation

The animals were sacrificed 10 weeks after the last injection (i.e. on day 85) to enable otomicroscopic and light microscopic examination. In each animal, both tympanic bullas were fixed in buffered 10 per cent formol, decalcified in 10 per cent nitric acid and embedded in paraffin. Four-micrometre sections were cut from each paraffin block, in a transverse plane to the tympanic membrane, stained with haematoxylin and eosin, and examined.

Histological analysis

Each specimen's tympanic membrane and middle-ear mucosa were assessed for the presence of various predetermined histological features. The sections were then positioned in an Olympus BX50 (20x) microscope (Olympus, Tokyo, Japan) and photographed using a Samsung video camera (model SHC-410NAD; Samsung, Suwon, South Korea) coupled to AT/TV player version 6.3 software. A total of 152 photomicrographs underwent morphometric analysis, using measurements (in three different places) of the cross-sectional thickness of the tympanic membrane and middle-ear mucosa, utilising the computer graphics software program ScionImage for Windows (Beta 4.0.2).

Statistical analysis

We used the Student paired *t*-test, the Stuart–Maxwell test for marginal homogeneity and the McNemar exact test, applied using the Stata 9.2 statistical software program. A level of statistical significance of 5 per cent (i.e. $p < 0.05$) was used.

Results

Results were classified as either qualitative or quantitative.

Qualitative results

In the control group, eight ears showed microscopic features of tympanic bulla mucosal inflammation, with acute and chronic features of mild intensity. The acute inflammatory process was characterised by large numbers of neutrophils, but the predominant lineage was mononuclear, with lymphocytes and plasmocytes. The epithelium was of the ciliated columnar type. Cholesteatoma (defined as keratinised stratified squamous epithelium) were present in seven ears. Fibrosis with vascular congestion and neoformation was seen in eight ears. The tympanic membranes in this group showed clearly delimited layers, and only one perforation was detected.

In the experimental group, severe acute and chronic inflammation was seen in the tympanic bulla mucosa. In addition, severe fibrosis and vascular neoformation were present in nine ears. Cholesteatoma was present in nine ears (Figure 1). The tympanic membrane layers were also clearly delimited in this group, but perforations were found in five ears. In three ears, there was mild to moderate inflammation of the tympanic membrane.

Quantitative results

Tables I to IV present histological and otomicroscopic data for the experimental and control groups. The number of paired ears studied varied due to premature death and histological artefacts.

In the two animals used to establish normal histological parameters (which had not undergone any experimental manipulation), the mean cross-sectional thickness was 10.85 μm for tympanic membrane and 12.07 μm for middle-ear mucosa.

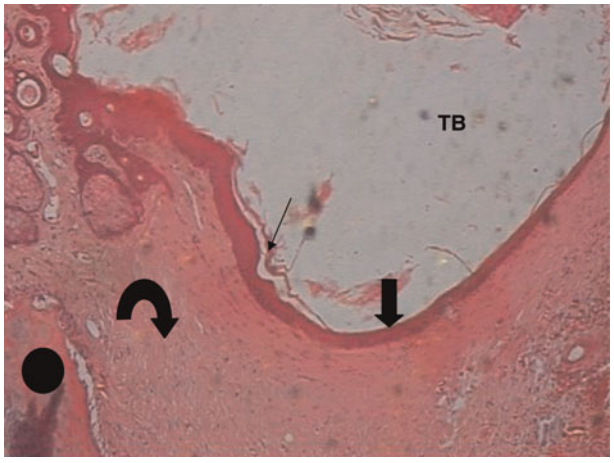

FIG. 1

Photomicrograph of tympanic bulla mucosa from an experimental group ear. TB = tympanic bulla cavity; thin arrow = desquamative keratin; thick arrow = keratinised stratified squamous epithelium (i.e. cholesteatoma); curved arrow = lamina propria; disc = tympanic bulla bony wall (H&E; ×100)

https://doi.org/10.1017/S002221511300011X Published online by Cambridge University Press

TABLE I
TYMPANIC BULLA MUCOSAL THICKNESS

Group	Ears (*n*)	Mucosal thickness (μm)				
		Mean	SD	Min	Max	Med
Exp	9	205.60*	153.30	29.50	564.90	152.30
Ctrl	9	27.24*	24.84	6.46	85.41	16.34

*Mean difference = 178.33 μm; $p = 0.004$ (Student's paired *t*-test); 95% confidence interval for mean difference = 76.83–279.82 μm. SD = standard deviation; Min = minimum; Max = maximum; Med = median; Exp = experimental; Ctrl = control

Discussion

As indicated in Table I, there was severe inflammatory activity in the experimental group, caused by mitomycin C and propylene glycol, compared with the control group ($p = 0.004$, comparison of mean tympanic bulla mucosal cross-sectional thickness). In the control group, mild inflammation was seen (eight ears), whereas in the experimental group inflammation was generally severe (seven ears).

Table II shows that there were no significant differences in tympanic membrane cross-sectional thickness between the two groups ($p = 0.371$). The highest values of tympanic membrane cross-sectional thickness corresponded to a variation of between 100 and 143 per cent, compared with values in normal rats undergoing no experimental manipulation. Larsson *et al.* observed a similar result in Mongolian gerbils.[7] In an experimental study on chinchillas, Masaki *et al.* found tympanic membrane alterations after two days, with total destruction of mucosal and epidermal layers, followed by fast re-epithelialisation and hyperplasia after three weeks.[8] Tympanic membranes were intact but structurally altered following 50 per cent propylene glycol application.[9] Tympanic membrane fibrosis was similar in the control and experimental groups, and was the main explanation for the observed hyperplasia.

As shown in Table III, we observed no statistically significant differences between the two groups at otomicroscopy ($p = 0.262$). This finding indicates a tendency towards spontaneous healing even after application of mitomycin C (a drug used in some studies mainly to keep tympanic membrane perforations patent).[10] Santos *et al.* found a reliable relationship between otomicroscopic appearance and histological findings in rats with induced myringosclerosis.[11]

Table IV indicates that there was no significant difference in the presence of exudate ($p = 0.125$), with both groups showing similar appearances, despite a statistically significant difference in the degree of middle-ear mucosal inflammation (see Table I). There was a similar incidence of tympanic bulla mucosal fibrosis in both groups ($p = 1.000$) (Table IV). This finding was unexpected; theoretically, mitomycin C should decrease fibroblast proliferation and reduce fibrosis. It may be that the severe inflammatory reaction induced in the experiment overcame the inhibitory effects of mitomycin C on fibroblasts and fibrosis development.

Cholesteatoma can be induced by injecting drugs into the tympanic bulla.[1,12–16] In the current study, cholesteatoma was induced by intratympanic injection via the tympanic membrane, in a model in which down-regulation of fibroblast action was expected due to mitomycin C. The cholesteatoma frequency in the control group was similar to previously published figures, i.e. 33–90 per cent.[12,13,17,18] In the current

TABLE III
OTOMICROSCOPIC FEATURES

Feature	Ears (*n*)		
	Ctrl	Exp	Total
Normal	2	0	2
Opacification	6	3	9
Perforation	1	5	6
Hyperaemia	0	1	1
Total	9	9	18

$p = 0.262$, control (Ctrl) *vs* experimental (Exp) groups (Stuart–Maxwell marginal homogeneity test).

TABLE II
TYMPANIC MEMBRANE THICKNESS

Group	Ears (*n*)	Tympanic membrane thickness (μm)				
		Mean	SD	Min	Max	Med
Exp	6	14.99*	8.12	7.00	29.38	13.89
Ctrl	6	12.05*	8.43	5.07	24.14	7.92

*Mean difference = 2.95 μm; $p = 0.371$ (Student's paired *t*-test); 95% confidence interval for mean difference = −4.76 to 10.66 μm. SD = standard deviation; Min = minimum; Max = maximum; Med = median; Exp = experimental; Ctrl = control

TABLE IV
TYMPANIC BULLA EXUDATE, FIBROSIS AND CHOLESTEATOMA

Status	Exdt (ears; *n*)		Fibr (ears; *n*)		Chol (ears; *n*)	
	Ctrl	Exp	Ctrl	Exp	Ctrl	Exp
Present	3	7	8	9	7	9
Absent	8	4	3	2	4	2
*p**	0.125		1.000		0.687	

*McNemar exact test. Exdt = exudate; Fibr = fibrosis; Chol = cholesteatoma; ctrl = control group; Exp = experimental group

https://doi.org/10.1017/S002221511300011X Published online by Cambridge University Press

study, there was no significant difference in cholesteatoma development between the two groups ($p = 0.687$) (Table IV). Some authors have reported no success in slowing cholesteatoma development using drugs such as cyclophosphamide, isotretinoin and 1.5 per cent hyaluronic acid.[15,19,20] However, other studies have reported success in arresting cholesteatoma induction using trans-retinoic acid, prednisolone and 5-fluorouracil.[12–14]

Mitomycin C is an alkylating agent. In this respect it is similar to 5-fluorouracil, which inhibits mitosis and protein synthesis and is active against all cells regardless of cell cycle phase. Thus, one could expect a similar effect of cholesteatoma inhibition with mitomycin C.[14] The concentration of mitomycin C used in the current study was based on previous research which detected middle-ear mucosa toxicity in a guinea pig model at 2.0 mg/ml but not at 0.4 mg/ml.

- **This study assessed mitomycin C as an inhibitor of otitis media and middle-ear cholesteatoma in a rat model**
- **Otitis media was created by intratympanic propylene glycol**
- **The experimental group had severe tympanic bulla mucosa inflammation despite mitomycin C use, compared with controls**
- **There were no other histological or otomicroscopic tympanic membrane differences**

In the current study's animal model, propylene glycol was used to create an inflammatory reaction in which skin cells from the hyperplasic epidermal layer of the tympanic membrane migrated through the basal lamina gaps into the thick connective tissue of the lamina propria and formed papillary projections of cells that completely penetrated the tympanic membrane to reach its medial surface, giving rise to cholesteatoma.[16] Palva and Johnson have found epithelial papillary projections invading the lamina propria of the tympanic membrane pars tensa and flaccida, in chronic otitis media in humans.[21] In the present study, histopathological analysis also showed a pattern of cholesteatoma distribution near the tympanic membrane; thus, epidermal invasion of the tympanic bulla mucosa may have arisen via the tympanic membrane. In two experimental group ears, cholesteatomas were found near the tympanic membrane, indicating a possible origin from external auditory canal epithelial migration. This finding brings to mind previous studies suggesting that basal cell proliferation may be responsible for cholesteatoma development.[17,18] In the current study, it is possible that cholesteatoma may have arisen through structurally altered or perforated tympanic membranes.

In the present study, no effect of mitomycin C on cholesteatoma induction was observed. Mitomycin C has a well-established inhibitory effect on connective tissue proliferation. However, in the current study this inhibitory effect appeared to have been overridden by a general inflammatory response induced by the combined effect of mitomycin C and propylene glycol, which favoured cholesteatoma formation.[10]

Conclusion

In this study, intratympanic mitomycin C was ineffective in preventing middle-ear otitis media and cholesteatoma development in a rat model.

Based on these findings, the clinical use of mitomycin C is inadvisable, as it has a potential deleterious effect on the middle-ear mucosa and tympanic membrane, which increases the risk of toxicity and favours the development of cholesteatoma.

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Dr A A Melo takes responsibility for the integrity of the content of the paper
Competing interests: None declared

https://doi.org/10.1017/S002221511300011X Published online by Cambridge University Press