The Journal of Laryngology & Otology (2009), ***123***, *482–487.*

doi:10.1017/S0022215108003848

Main Article

Platelet-rich plasma improves healing of tympanic membrane perforations: experimental study

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Abstract

Objective: The aim of this study was to investigate the effect of local application of platelet-rich plasma to perforated rat tympanic membranes, in terms of healing time and histopathological outcome.

Methods: Eighty-eight tympanic membranes of 44 rats were given a standard 3 mm perforation, and platelet-rich plasma was applied to the right tympanic membrane perforations. The left tympanic membranes were left to heal spontaneously, as controls. The 44 rats were divided into two groups. In group one, comprising 20 rats, daily otomicroscopic examination of the tympanic membrane perforations was performed. The 24 rats in group two were subdivided into four subgroups of six rats each; these subgroups were sacrificed sequentially on days three, seven, 14 and 28 for histopathological examination, regardless of tympanic membrane healing stage.

Results: In group one, the mean tympanic membrane healing times for tympanic membrane perforations receiving platelet-rich plasma and controls were respectively 10.2 ± 2.1 and 13.0 ± 2.9 days (mean $\pm$ standard deviation). This difference was statistically significant ($p < 0.001$). In group two, histopathological evaluation of tympanic membrane perforation healing at days three, seven, 14 and 28 did not reveal any statistically significant difference, individually or within the four groups as a whole.

Conclusion: These findings suggest that earlier healing of tympanic membrane perforations occurred in the platelet-rich plasma group compared with the control group. These findings suggest that platelet-rich plasma is effective in accelerating tympanic membrane perforation healing, and that it may be effective in human subjects, particularly as it is an autologous material.

Key words: Tympanic Membrane Perforations; Platelet Rich Plasma; Rat; Otologic Surgical Procedures

Introduction

Tympanic membrane perforation is one of the leading causes of conductive hearing loss; in such cases, tympanic membrane integrity must be re-established quickly in order to restore hearing and to protect the middle- and inner-ear structures from external insults.[1–4]

Surgical treatment may not always be an easy option because of associated morbidity or lack of health facilities. Cheaper, simpler and less invasive treatment methods for tympanic membrane perforation are needed.[1,4]

Many different materials have previously been used to treat tympanic membrane perforations, including hyaluronan, heparin, epidermal growth factor, fibroblast growth factor, platelet-derived growth factor and transforming growth factor.[1,3,5,6]

Platelets play an important role in haemostasis and wound healing. Alpha granules within platelets contain several growth factors that have potent effects upon wound healing. Platelet-rich plasma is defined as a small volume of plasma having a platelet concentration above baseline levels. Mixture of platelet-rich plasma and calcium results in degranulation of alpha granules and release of growth factors, forming a gel structure.

The rationale behind use of a platelet-rich plasma preparation for treatment of tympanic membrane perforation is to increase the platelet concentration and thereby increase the local concentration of growth factors.[7–12] The aim of this study was to investigate the effect of local application of platelet-rich plasma to perforated rat tympanic membranes, in terms of healing time and histopathological outcome.

Materials and methods

This experimental study was approved by the animal use committee of the Ondokuz Mayis University Medical Center.

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Accepted for publication: 29 July 2008. First published online 28 October 2008.

Forty-four healthy Sprague–Dawley rats with intact tympanic membranes, weighing 250 to 300 g, were used. All animals were anaesthetised with ketamine (50 mg/kg) and xylazine (10 mg/kg) administered intramuscularly. Two millilitres of intracardiac blood from each rat was collected in test tubes containing ethylene diamine triacetic acid. Anticoagulated blood was centrifuged at 5600 rpm. Three layers formed: a bottom layer consisting of red blood cells; a middle layer consisting of platelets and white blood cells; and a top, platelet-poor plasma layer. The top plasma layer was removed and the remaining material centrifuged again at 2400 rpm. After the second centrifugation, red blood cells were separated out and platelet-rich plasma was obtained.

Using an otomicroscope, bilateral, 3 mm perforations were created in the posterior quadrant of the rat tympanic membranes, using a pick. Platelet-rich plasma was activated with 10 per cent calcium glubionate (Ca Sandoz 10 per cent; Novartis, Stein, Switzerland) and a viscous gel obtained (Figure 1). This gel was applied to the right tympanic membrane perforations with a pick (Figure 2). The left tympanic membranes were left to heal spontaneously as the control group, based on the assumption that there were no systemic differences in healing between the left and right ears.

The rats were randomised into two groups in order to investigate clinical and histopathological tympanic membrane healing. Group one comprised 20 rats; these underwent daily otomicroscopic examination and the tympanic membrane healing times were recorded. Group two comprised 24 rats subdivided into four subgroups containing six rats each; these subgroups were sacrificed sequentially on days three, seven, 14 and 28 for histopathological examination, regardless of tympanic membrane healing stage.

After receiving pentobarbital 80 mg/kg intraperitoneally, the six rats in each subgroup were decapitated and the tympanic bullae opened. The specimens were stored in 10 per cent formaldehyde solution. To enable histopathological examination, the specimens were decalcified in a solution of formic acid and sodium citrate and the tympanic bullae were bisected, creating a transverse section of the tympanic membrane and bisecting through the short process of the malleus. Both pieces were embedded in paraffin blocks, which were cut into 5-μm thick sections, stained with haematoxylin and eosin and examined under light microscopy (at a magnification of ×40).

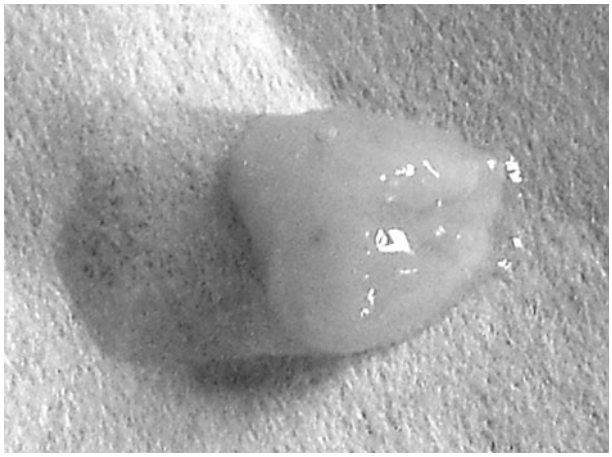

FIG. 1
Platelet-rich plasma in gel form.

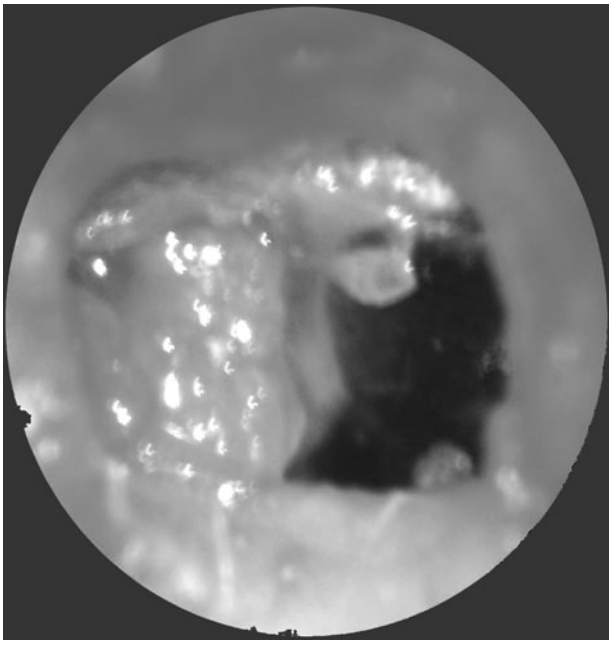

FIG. 2
Otoscopic view showing local application of platelet-rich plasma to tympanic membrane perforation.

Histopathologically, the presence of lamina propria oedema, neovascularisation, fibroblastic reaction and inflammatory cells was scored subjectively as −, +, ++ or +++. The tympanic membrane thickness was quantitatively measured in micrometres.

Statistical analysis was performed. The paired *t*-test was used for perforation healing times, the Wilcoxon signed ranks test for same day histopathology scores within each subgroup, and the Kruskal–Wallis test followed by the Mann–Whitney U test with Bonferoni correction for histopathology scores (comparing subgroups sacrificed on four different days).

Results

The 20 rats in group one underwent daily otomicroscopic examination; their tympanic membrane perforation healing times are shown in Table I. The mean healing times of the tympanic membrane perforations on the right (i.e. platelet-rich plasma treatment group) and the left (i.e. control group) were respectively 10.2 ± 2.1 and 13.0 ± 2.9 days (mean ± standard deviation). This difference was statistically significant ($p < 0.001$; paired *t*-test), with a mean difference of 2.8 days (95 per cent confidence interval 1.6 to 4.0 days). Persistent tympanic membrane perforation was not observed in any rat.

https://doi.org/10.1017/S0022215108003848 Published online by Cambridge University Press

TABLE I

GROUP 1 RATS: TYMPANIC MEMBRANE PERFORATION HEALING TIMES

Rat no	TM healing time (days)	
	Study ear	Control ear
1	12	17
2	10	8
3	9	10
4	8	15
5	7	9
6	13	11
7	10	12
8	12	15
9	8	11
10	10	12
11	7	9
12	13	16
13	14	17
14	9	12
15	13	16
16	9	14
17	9	16
18	8	13
19	12	10
20	11	17
Mean ± SD*	10.2 ± 2.1	13.0 ± 2.9

*$p < 0.001$, paired *t*-test (mean difference, 2.8 days; 95 per cent confidence interval, 1.6 to 4.0 days). No = number; TM = tympanic membrane; SD = standard deviation

The 24 rats in group two were sacrificed sequentially on days three, seven, 14 and 28 to enable histopathological examination, regardless of tympanic membrane healing stage. The Wilcoxon signed ranks test was used to compare data within each subgroup and within the whole of group two.

The rats sacrificed on days three, seven, 14 and 28 revealed no significant differences between the study and control ears (Tables II to V) with regards to lamina propria oedema, inflammatory cells, neovascularisation and fibroblastic reaction ($p > 0.05$). Although neovascularisation and fibroblastic reaction seemed to be more prominent on day seven in the study ears (Figure 3) compared with the control ears, this difference was not statistically significant ($p > 0.05$).

When all 24 rats in group two were considered together, regardless of the sacrifice day, no significant histopathological differences were found (Table VI).

The Kruskal–Wallis test was used to compare the four subgroups of group two. Although a significant difference was observed regarding neovascularisation in the study ears among the subgroups ($p = 0.031$), further individual comparison of subgroups using the Mann–Whitney U test with Bonferoni correction did not show any significance ($p > 0.05$). No statistically significant difference was found between the platelet-rich plasma and control groups regarding tympanic membrane thicknesses.

Discussion

Surgical treatment of tympanic membrane perforations has improved greatly since the 1950s. The success rate of surgical treatment is reported to be between 85 to 95 per cent. However, the major

TABLE II

GROUP 2 RATS SACRIFICED DAY 3: TM HISTOPATHOLOGICAL EVALUATION

Feature	Study ears*		Control ears†		p‡
	n	%	*n*	%	
L propria oedema					
–	1	16.6	1	16.6	
+	4	66.6	2	33.3	
++	–	–	2	33.3	
+++	1	16.6	1	16.6	
					0.492
Neovascularisation					
–	5	83.3	6	100.0	
+	1	16.6	–	–	
++	–	–	–	–	
+++	–	–	–	–	
					0.317
Fibroblastic reaction					
–	3	50.0	5	83.3	
+	2	33.3	1	16.6	
++	1	16.6	–	–	
+++	–	–	–	–	
					0.257
Inflammatory cells					
–	3	50.0	3	50.0	
+	2	33.3	2	33.3	
++	1	16.6	1	16.6	
+++	–	–	–	–	
					1.00

*$n = 6$; †$n = 6$; ‡Wilcoxon signed ranks test. TM = tympanic membrane; L = lamina

TABLE III

GROUP 2 RATS SACRIFICED DAY 7: TM HISTOPATHOLOGICAL EVALUATION

Feature	Study ears*		Control ears†		p‡
	n	%	*n*	%	
L propria oedema					
–	2	33.3	1	16.6	
+	1	16.6	4	66.6	
++	3	50.0	1	16.6	
+++	–	–	–	–	
					0.739
Neovascularisation					
–	2	33.3	5	83.3	
+	2	33.3	1	16.6	
++	2	33.3	–	–	
+++	–	–	–	–	
					0.129
Fibroblastic reaction					
–	2	33.3	3	50.0	
+	2	33.3	2	33.3	
++	1	16.6	1	16.6	
+++	1	16.6	–	–	
					0.461
Inflammatory cells					
–	2	33.3	2	33.3	
+	3	50.0	3	50.0	
++	–	–	1	16.6	
+++	1	16.6	–	–	
					1.00

*$n = 6$; †$n = 6$; ‡Wilcoxon signed ranks test. TM = tympanic membrane; L = lamina

https://doi.org/10.1017/S0022215108003848 Published online by Cambridge University Press

TABLE IV

GROUP 2 RATS SACRIFICED DAY 14: TM HISTOPATHOLOGICAL EVALUATION

Feature	Study ears*		Control ears†		p‡
	n	%	*n*	%	
L propria oedema					
–	3	50.0	2	33.3	
+	1	16.6	3	50.0	
++	2	33.3	–	–	
+++	–	–	1	16.6	
					0.655
Neovascularisation					
–	2	33.3	4	66.6	
+	4	66.6	1	16.6	
++	–	–	1	16.6	
+++	–	–	–	–	
					0.564
Fibroblastic reaction					
–	1	16.6	2	33.3	
+	4	66.6	3	50.0	
++	1	16.6	1	16.6	
+++	–	–	–	–	
					0.317
Inflammatory cells					
–	3	50.0	3	50.0	
+	2	33.3	2	33.3	
++	–	–	1	16.6	
+++	1	16.6	–	–	
					0.564

*$n = 6$; †$n = 6$; ‡Wilcoxon signed ranks test. TM = tympanic membrane; L = lamina

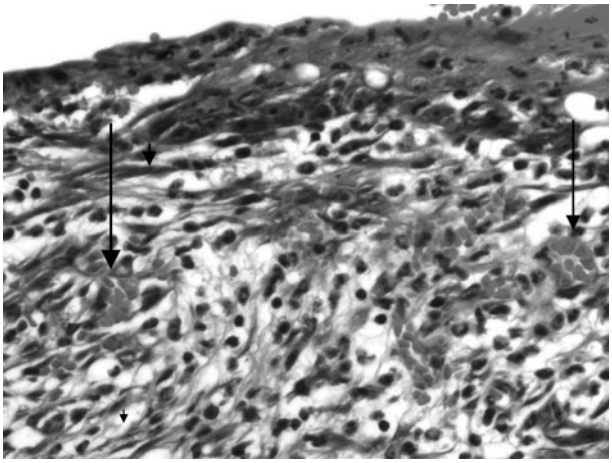

FIG. 3

Photomicrograph of tympanic membrane of a group two rat treated with platelet-rich plasma and sacrificed on day seven, showing neovascularisation (arrows) and prominent fibroblastic reaction (arrowhead) (H&E; ×40).

disadvantages of surgery include associated morbidity, expense and the need for specialist equipment. Thus, there is a need for cheaper, simpler and less invasive treatment methods for tympanic membrane perforation.[1,4,13]

Tympanic membrane healing is a complex process which includes epithelial migration, increased fibroblastic reaction, vascular proliferation and tissue remodelling. During typical wound healing, granulation tissue formation provides a scaffold for re-epithelialisation. In contrast, tympanic membrane

TABLE V

GROUP 2 RATS SACRIFICED DAY 28: TM HISTOPATHOLOGICAL EVALUATION

Feature	Study ears*		Control ears†		p‡
	n	%	*n*	%	
L propria oedema					
–	4	66.6	5	83.3	
+	2	33.3	–	–	
++	–	–	1	16.6	
+++	–	–	–	–	
					1.00
Neovascularisation					
–	6	100.0	6	100.0	
+	–	–	–	–	
++	–	–	–	–	
+++	–	–	–	–	
					1.00
Fibroblastic reaction					
–	–	–	2	33.3	
+	4	66.6	3	50.0	
++	1	16.6	1	16.6	
+++	1	16.6	–	–	
					0.157
Inflammatory cells					
–	6	100.0	6	100.0	
+	–	–	–	–	
++	–	–	–	–	
+++	–	–	–	–	
					1.00

*$n = 6$; †$n = 6$; ‡Wilcoxon signed ranks test. TM = tympanic membrane; L = lamina

TABLE VI

ALL GROUP 2 RATS: TM HISTOPATHOLOGICAL EVALUATION

Feature	Study ears*		Control ears†		p‡
	n	%	*n*	%	
L propria oedema					
–	10	41.6	9	37.5	
+	8	33.3	9	37.5	
++	5	20.8	4	16.6	
+++	1	4.1	2	8.3	
					0.638
Neovascularisation					
–	15	62.5	21	87.5	
+	7	29.1	2	8.3	
++	2	8.3	1	4.1	
+++	–	–	–	–	
					0.07
Fibroblastic reaction					
–	6	25.0	12	50.0	
+	12	50.0	9	37.5	
++	4	16.6	3	12.5	
+++	2	8.3	–	–	
					0.053
Inflammatory cells					
–	14	58.3	14	58.3	
+	7	29.1	7	29.1	
++	1	4.1	3	12.5	
+++	2	8.3	–	–	
					0.776

*$n = 24$; †$n = 24$; ‡Wilcoxon signed ranks test. TM = tympanic membrane; L = lamina

https://doi.org/10.1017/S0022215108003848 Published online by Cambridge University Press

healing begins with bridging of the squamous epithelial layer, followed by regeneration of the fibrous layer. Tympanic membrane perforations may heal spontaneously and completely, heal with a thin membrane, or persist unhealed. Maintaining normal thickness is one of the main goals of tympanic membrane perforation healing.[1,6,14–17]

Efforts to accelerate tympanic membrane perforation healing have followed two main strategies: (1) stromal support to guide the regenerating tissue; or (2) cellular regeneration and mobilisation. Paper-patch myringoplasty is a well known example of the former strategy, and is an effective technique for small, clean perforations. Mechanical debridement of perforation edges or cauterisation with silver nitrate and trichloroacetic acid have had limited success.

Growth factors have been widely studied as promoters of cellular regeneration and mobilisation,[18,19] and have been used to accelerate tympanic membrane perforation healing. Growth factors are signal proteins that control wound healing, tissue regeneration and normal body growth via paracrine and autocrine pathways. Following secretion from blood products or local tissues, growth factors activate specific target cells to multiply and migrate. Growth factors have been shown to accelerate normal and delayed wound healing.[19]

Mondain and Ryan[20] used immunohistochemical methods to demonstrate epidermal growth factor localisation on guinea pig tympanic membranes after traumatic tympanic membrane perforation. Epidermal growth factor levels peaked on day three and began to decrease on day five, supporting a role for epidermal growth factor in tympanic membrane healing. O'Daniel *et al.*[21] studied the binding of ^{125}I-labelled epidermal growth factor in porcine tympanic membrane; they reported the presence of specific epidermal growth factor receptors within three layers of the tympanic membrane, most numerously within the squamous epithelial layer.

Yeo *et al.*[4] applied a single, 2 μg dose of platelet-derived growth factor to experimental tympanic membrane perforations in rats. On day five, all tympanic membranes thus treated were completely healed; in contrast, two of the control ears still had not completely healed by day 15.

Platelet-rich plasma is a biological product created by condensing platelets *in vitro* and activating the alpha granules in order to stimulate secretion of growth factors. Since it is prepared from autologous blood, platelet-rich plasma is cheaper than growth factors and carries no risk of infection transmission. Use of platelet-rich plasma in the closure of tissue defects, particularly bone, has been reported.[11] To our knowledge, no previous study has investigated the role of platelet-rich plasma in tympanic membrane perforation healing.

Marx *et al.*[22] compared the results of bone grafting alone and bone grafting plus platelet-rich plasma in the treatment of mandibular defects following tumour resection; they found that platelet-rich plasma increased the speed and quantity of bone regeneration. Following tooth extraction, Anitua[23] filled the resultant tissue defect with plasma rich in growth factors, and reported an increase in epithelialisation and bone density. Kim *et al.*[24] found that platelet-rich plasma improved the healing of bone defects around titanium dental implants.

Dere *et al.*[25] investigated the use of basic fibroblast growth factor in an experimental guinea pig tympanic membrane perforation model. They found thicker tympanic membranes and hyperplasia of the epithelial layer. They also reported hyperplasia of the fibrous layer, with prominent neovascularisation and fibroblastic reaction. Prominent neovascularisation and fibroblastic reaction was observed in the study group on day seven, but the difference was not significant. Tympanic membrane thickness did not differ significantly between the study and control group ears ($p > 0.05$).

Mondain *et al.*[26] reported shorter tympanic membrane perforation healing time following fibroblast growth factor treatment in an experimental rat model.

- **This study aimed to investigate the effect of local application of platelet-rich plasma to rat tympanic membrane perforations, in terms of healing time and histopathological outcome**
- **Perforations receiving platelet-rich plasma had a significantly quicker healing time**
- **Platelet-rich plasma appears to be effective in improving tympanic membrane perforation healing in this rat experimental model; thus, its use should be investigated in humans, as it is an autologous material**

Fina *et al.*[27] used basic fibroblast growth factor in an experimental guinea pig tympanic membrane perforation model. They applied basic fibroblast growth factor, either directly to the perforation or via a gelfoam pledget, and found a faster healing response when no scaffolding material was interposed. In the present study, the mean tympanic membrane healing time was 10.2 ± 2.1 days in the platelet-rich plasma treated group and 13.0 ± 2.9 days in the control group. Healing times were consistent with the literature, and platelet-rich plasma application significantly shortened the tympanic membrane perforation healing time ($p < 0.001$).

Conclusion

The histopathological and clinical findings of this study support the theory that platelet-rich plasma accelerates wound healing and shortens tympanic membrane perforation closure time. Platelet-rich plasma treatment may represent a reasonable alternative to surgery, since its preparation (from autologous blood) and topical application are simple. This is the first study to assess the effects of platelet-rich plasma on tympanic membrane perforation healing. We suggest the possible effectiveness of platelet-rich plasma as a topical application to

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tympanic membrane perforations in human patients in the out-patient clinic setting.

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Competing interests: None declared