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ORIGINAL ARTICLE

Effects of Autologous Serum on Experimental Traumatic Tympanic Membrane Perforation

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Hypothesis: Autologous serum has positive effects on the healing of acute experimental traumatic perforations of the tympanic membrane. And also autologous serum is one of the treatment options of acute tympanic membrane perforations.

Background: The majority of acute perforations of the tympanic membrane heal spontaneously. However, some surgical treatment is needed for persisted perforations. The closure occurs by squamous epithelial migration. Drugs that stimulate this regenerative process may aid in the closure of the perforation, obviating the need for more extensive treatments. There is no previously reported case about effects of autologous serum on perforated tympanic membrane in the literature. This study will be the first one to determine the effects of autologous serum on the healing process of the acutely formed tympanic membrane perforations.

Materials and Methods: Based on power analysis of presenting study, we used 20 rats (p1: 0.99 and p2: 0.77 and power: 0.80, Systat 12 for Windows). Twenty rats with bilateral normal tympanic membranes were included in the study by obtaining Institutional Animal Care and Use Committee (IACUC) approval. The posterior quadrant of the tympanic membranes in both ears of the rats was perforated with a 20-gauge needle. Perforations within the left ears of the rats were treated with autologous serum and the right ears were left untreated as controls. Two weeks later the animals were decapitated and their external ears were separated at the osteocartilaginous junctions bilaterally. 40 surgical specimens (20 right ear and 20 left ear) were fixed in formaldehyde, decalcified in formic acid and then prepared for histological evaluation. The pieces were embedded in paraffin, and the tissue blocks were cut into slides 5 µm thick, treated with hematoxylin and eosin, and examined under light microscopy. The parameters weretympanic membrane thickness, fibroblastic reaction, neovascularization and inflammation

Mann-Whitney Test , Chi-Square Tests and student's t-test (SPSS for Win. Ver. 11.5) were used to compare the parameters in the control (right) and treated (left) ears of the rats.

Results: There were no significant differences in the histologic parameters, tympanic membrane thickness, fibroblastic reaction, neovascularization and inflammation, between the treated groups and control ears.

Conclusion: The study has showed that autologous serum has still some positive effects on healing of tympanic membrane healing even it is not statistically significant.

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Tympanic membrane (TM) perforations can be caused by infection, penetrating trauma by foreign bodies or surgical instruments inserted into the external auditory canal or blunt or blast trauma. Following the injury, the TM thickens as a result of edema, inflammation, and neovascularization that is placed primarily in the fibrous layer. However, the principal changes that effect closure of the perforation are the keratin movement toward the center of the perforation, epithelial proliferation, and the eventual formation of a new fibrous layer [1]. The majority of acute perforations of the tympanic membrane, especially those involving less than a quarter of the TM area, heal spontaneously [2-4]. In some cases, surgical treatment may be necessary for nonhealing perforations. Several materials and techniques have been used to repair the perforations to avoid the standard myringoplasty technique. A wide variety of techniques have been presented to remove the preventive squamous epithelium and trigger a fibroblastic reparative reaction in the middle fibrous

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layer of the TM. Cauterizing the edges with chemicals (i.e., silver nitrate, trichloracetic acid) is a time-tested simple office procedure, which is suitable for small to moderate perforations [5]. Topical hyaluronic acid (HA) and epidermal growth factor (EGF) applications were recently reported to be successful in the treatment of traumatic TM perforations [6-11]. In this study tried the autologous serum to help on TM perforation closure. Autologous serum eye drops are useful in managing the corneal wounds and in mechanical corneal ulcers as an adjuvant therapy. It is believed that the serum component of blood also contains the metabolic rsubstances such as glucose, amino acids, vitamins, electrolytes, and growth and regulatory factors, required for the wound healing process,. When blood comes into contact with tissue, it instigates an immune reaction [12]. Therefore, with the isolation of cells from the blood, serum permits the supply of nutrients but not immunologic cells. We assumed the autologous serum has some benefits on perforated TM by this way.

The goal of this study was to evaluate the effects of autologous serum on the healing of acute experimental traumatic perforations of the tympanic membrane in rats by as examining TM thickness, fibroblastic reaction, neovascularization and closure.

Materials and Methods

Male albino rats reproduced in Istanbul University Cerrahpasa Medical Faculty Animal Research Laboratories, weighing approximately 300-350g each, were examined by using the operation microscope. Twenty animals with bilateral normal TMs were included in the study. Institutional Animal Care and Use Committee (IACUC) approval was obtained for this study. All animals were anesthetized with intraperitoneal ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg) injections. The posterior parts of the tympanic membranes in both ears were perforated by using a 20-gauge needle. The perforations in the left ears of the rats were treated with autologous serum. The perforations in the right ears were left as controls.

Autologous serum was prepared from the rats by obtaining blood from the tail veins. A total of 1 to 1.5 mL of blood was centrifuged for 5 minutes at 1500 rpm as described by Tsubota [13]. The serum was carefully separated in a sterile manner and put into a bottle with a coating that cuts out ultraviolet light. The bottles were kept in a refrigerator at 4°C until needed. Autologous serum was applied to the lateral surface of the perforations in the left ears once drop daily for 2 weeks.

Cervical dislocation was performed 2 weeks later from the time tympanic membranes in both ears of the subjects were perforated. The animals were decapitated and their external ears were separated at the osteo-cartilaginous junctions bilaterally. For histopathologic evaluation, 40 surgical specimens (20 right ears and 20 left ears) were fixed in formaldehyde and decalcified in formic acid. The specimen were embedded in paraffin, and the tissue blocks were cut into 5 μm thick sections treated with hematoxylin and eosin, and examined under light microscopy (Figures 1 and 2).

Figure 1. Persistent tympanic membrane perforation. Arrows point out perforation . There is no reparative activity around the perforation. EEC, external ear canal; TC, tympanic cavity, TM; tympanic membrane

Figure 2. Healed tympanic membrane perforation. EEC, external ear canal; TC, tympanic cavity, TM; tympanic membrane, IE; inner ear

Figure 3. The slide shows very thick TM and angiogenesis, fibroblastic activity and inflammation of TM. EEC, external ear canal; TC, tympanic cavity, TM; tympanic membrane, IE; inner ear, M; manubrium mallei

Histologically, the thickness of the TM was quantitatively measured, whereas the fibrous layer was evaluated semiquantitatively to assess the fibroblastic reaction as well as neovascularization and also inflammatory activity (Figure 3). The fibroblastic reaction was subjectively scored as negative,(+), (++), or (+++). A negative score indicated no fibroblastic reactions; (+), (++), and (+++) scores indicated mild, moderate, and marked fibroblastic reactions, respectively. Similarly, neovascularization was subjectively scored as negative, (+), (++), or (+++). A negative neovascularization score indicated no new vascular proliferative activity; (+), (++) and (+++) scores were used to determine mild, moderate and marked neovascularization, respectively. Inflammation was subjectively scored as negative, (+), (++), or (+++). A negative Inflammation score indicated no new inflammatory activity; (+), (++) and (+++) scores were used to determine mild, moderate and marked inflammation or abscess, respectively. TM closure rate indicated no closure, (+) was used to determine totally healing.

For statistical analysis of the data, the Mann-Whitney Test, Chi-Square Tests and Student's t test (SPSS for Win. Ver. 11.5) were used to compare the parameters in the control (right) and treated (left) ears of the rats.

Results

Tympanic membrane thickness

The mean TM thickness in the control group (the untreated right ears) was 23.78 µm (12.5-42 µm). The mean TM thickness in the treated group (the left ears) was 24.23µm (21-42 µm) When the TM thicknesses of the right and left ears were compared; no significant differences were observed between the autologous serum -treated and control ears in rats (Student's t test was used; p=0.853),

Fibroblastic reaction

The fibroblastic reactions were subjectively scored as negative (-), (+), (++), or (+++). The mean fibroblastic reaction score of the control group was 1.1 (+)(median: 1).The mean fibroblastic reaction score of the treated group was 1.2 (+) (median: 1). The fibroblastic reaction scores were also compared in each group; there were no significant differences between the autologous serum-treated and the control ears (p= 0.84) (Table 1).

Neovascularization

Neovascularization was subjectively scored as negative, (+), (++) or (+++). No significant differences in the neovascularization scores were observed between the two groups. The mean neovascularization score of the control group was 1.4 (+) (median: 1).The mean neovascularization score of the autologous serum -treated group was 1.6 (+) (median: 1).

The neovascularization scores were compared in each group; there were no significant differences between the autologous serum -treated and the control ears in rats (p= 0,82) (Table 1).

Inflammation

Inflammation was subjectively scored as negative, (+), (++), or (+++). No significant differences in the inflammation scores were observed between the two groups. The mean inflammation score of the control group was 1.1 (+) (median: 1) .The mean inflammation score of the autologous serum -treated was 1.35 (+) (median: 1).The inflammation scores were compared in each group; there were no significant differences between the autologous serum -treated and the control ears in rats (p =0.56) (Table 1).

Table 1.

Parameters	treated (left) ear / 20 ears				control (right) ear / 20 ears				results	
	0+	1+	2+	3+	0+	1+	2+	3+	p (Mann-Whitney U Test)	
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)		
Inflammation	8 (40,0)	5 (25,0)	4 (20,0)	3 (15,0)	7 (35,0)	5 (25,0)	2 (10,0)	6 (30,0)	**0,56**	NS
Neovasculation	3 (15,0)	9 (45,0)	5 (25,0)	3 (15,0)	1 (05,0)	12 (60,0)	3 (15,0)	4 (20,0)	**0,82**	NS
Fibroblastic reactions	7 (35,0)	6 (30,0)	5 (25,0)	2 (10,0)	8 (40,0)	3 (15,0)	6 (30,0)	3 (15,0)	**0,84**	NS

Tympanic Membrane Closure

Of the 20 TM in control group, 17 TM healed (85%). Of the 20 TM in treated group, 18 TM healed (90%). No significant differences in the rate of TM closure were observed between the two groups (we used chi-square test; p=0.5).

We have not seen any side effect or complication because of autologous serum treatment.

Discussion

Tympanic membrane perforations can be caused by many etiological factors including infection, penetrating trauma by foreign bodies or surgical instruments inserted into the external auditory canal or blunt or blast trauma. Traumatic perforations often occur in healthy members of the population. The spontaneous closure rate, with or without stenting, is approximately 90% in traumatic perforations of the tympanic membrane [14].

Topical hyaluronic acid (HA) and epidermal growth factor (EGF) applications were recently to treat traumatic TM perforations. Many studies carried out in animals and humans showed a significant increase in tympanic membrane healing when HA was used [15-17]. The effect of HA was believed to be through regulation of the healing pattern of the fibrous layer by preventing dehydration of the perforation margins [16, 18]. After acute tissue injury, EGF is normally supplied to the wound by the inflammatory reparative process. Enhanced VEGF, FGF, lymphocytes and collagen fibrils are important indicators of promoted wound healing [19, 20]. The factors we mention above are naturally found in serum. Serum component of blood also contains the necessary metabolic requirements for the wound healing process, such as glucose, amino acids, vitamins, electrolytes, and growth and regulatory factors. On the other it is easy to obtain the autologouse serum. It is cheaper than any other medication. Eye drops made from autologous serum are widely used as an adjuvant treatment in mechanical corneal ulcers. Recently Kakehata et al [21] proposed using autologous serum in chronically perforated TM [21]. The present study is focused on the effect of the autologous serum in treating the acute TM perforations in animal models.

In this study, the thickness of the TM was measured quantitatively, whereas the fibrous layer was evaluated semiquantitatively to assess the fibroblastic reaction and neovascularization and also the inflammatory activity. No significant difference was found in regard to the histologic parameters between the treated TM's and controls (Table 1). Even though the difference was not significant, all parameters in autologous serum treated group were in favor of better healing.

Conclusion

In conclusion, autologous serum has no significant effect on the healing process of TM perforations. The supportive effect of the autologous serum within the healing process of acutely developed TM perforations even not being significant should encourage further studies on this issue.

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