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The role of bone marrow stem cells in healing perforation of marmot tympanic membrane

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Abstract:

Introduction: The tympanic membrane plays an important role in the physiology of hearing. If perforation occurs, the hearing power will decrease. Role of perforation closure operation results are still not satisfactory. Latest knowledge about stem cells as the cells was able to differentiate into other cells to produce a new treatment expectation in handling perforation. **Method:** The aim of this research was to assess the role of bone marrow stem cells in the healing of marmot tympanic membrane perforation. The number of sample were totaling 22 marmots that were divided into three groups, group I amounted to 8 marmots, in group II were 7 marmots and in group III were 7 marmots. All marmots with chronic perforations made in the tympanic membrane perforator then injected with *Staphylococcus aureus* bacteria taken from microbiology section at tympanic cavum. When there has been a chronic perforation and declared dry, the group I taped marmot bone of marrow stem cells, amnion membrane attached to group II, group III as the control (without treatment). **Results:** There was no significant difference between the mean of age of the sample in the three groups ($p > 0.05$) and also no significant difference in mean body weight among the samples into three groups ($p > 0.05$). In the first observation of perforation, closure has not occurred among the three groups yet, on the second observation there were significant differences according to the percentage of the tympanic membrane closure group ($p < 0.05$) in which groups of stem cells, it closures on 3 samples (50%) than other groups. In the third observation there were significant differences in the percentage cover of amnion membrane according to group ($p < 0.05$). In the group of stem cell and amnion membrane, the closure occurs each 6 (100%) and 5 samples (100%), whereas in the control group the closure of the tympanic membrane occurred in 2 samples (40%). **Conclusion**: The role of bone marrow stem cells as the cells that are able to differentiate into other cell can be covered perforation of the tympanic membrane was faster than using amniotic membrane closure and control groups.

Key words: Bone marrow; tympanic membrane; marmots; stem cells; healing perforation.

Introduction

Tympanic membrane perforation is a common trace symptom of long inflammation and often repeated from middle ear infections. Healing perforation can occur spontaneously or require intervention depending on the pathology and the patient's condition. Perforations that occur can affect the function of hearing patients and eliminate the function of protection against invading germs that enter through the external acoustic meatus, also gives a fairly large psychological effect on patients when there is inflammation of the middle ear with the secretions that come out especially when it smells [1].

Since the 17th century efforts have made with various attempts to close the tympanic membrane perforation, especially the use of prosthetic material. Started using cauterization materials in 1876 to the use of vein grafts in 1960. In 1960-1970 it was began to use materials derived from cadaver homograft as the tympanic membrane, dura, and the pericardium with varying degrees of success. At the present time these materials have been abandoned because of the potential to transmit diseases such as HIV [2]. *Temporalis fascia* was first introduced by Heermann 1958 and to date is the selected material, and is often reported success rate of 93% -97%. However, the use of *temporalis fascia* require surgery are often rejected by most people for many reasons, which are not yet supporting facilities, the cost of expensive, require hospitalization with the old controls, the possibility of complications, the results are sometimes unsatisfactory so need to look for other possible methods easier and simpler to give good results [1].

Latest knowledge about stem cells or stem cells with the ability to regenerate into other cells can be used in this situation. For example, one type of multipotent stem cells that mesenhymal stem cells (MSCs) have the ability to differentiate into several cell types including fibroblasts, bone, cartilage, muscle (striated muscle and smooth muscle) and brain cells. One study Dennis, 2004 reported that adult MSCs, when injected into animals, will be settled on the injured area and improve the function of the network. MSC is relatively easy to bone marrow aspirated from the iliac crest as in, vertebrae [3,4], the tibia and femur [5,6] and is very easy to develop volume when cultured. Bone marrow stem cells can be transformed into a number of cell types and can produce "tissue repair factors'. Several reports on the successful use of these cells as therapeutic restoration stroke, myocardial infarction, ontogenesis imperfect [7-10] supports the use of MSCs in wound healing [11].

Research on tympanic membrane perforation using embryonic stem cells is done by Von Unge, Joris JJ, Petri O, [10] in Sweden in *mongolian gerbils*. The results showed that the perforation MT healing faster than perforation given normal saline. Anisur Rahman in Sweden tried to do the closing perforations in rats with chronic MT MSC. The results obtained with the healing of tympanic membrane perforation using stem cells as the control group who did not use stem cells that differentiate tympanic membrane is strength in the face of pressure on healing with stem cells greater than the control.

Materials and Methods:

This experimental study use design a post-test only control group design. Samples were male marmots and marmot strain originating from the Laboratory Research and Veteriner Development Center of Maros 8-12 weeks old, weighing 350-400 mg. Before used, the marmots first adapted in the laboratory atmosphere for 7 days, then they were fed standard and drinking enough. Marmots were used in this study refers to the research previously used to see the healing tympanic membrane perforation. Determination of the sample according to the provisions of the WHO with a minimum sample size of 5 marmots. In this study used 22 marmots divided by 3 such as the first treatment group consisted of 8 marmots, treatment group II consisted of 7 marmots and third treatment group (control) consisted of 7 marmots.

Experimenting marmots were randomly divided into 3 groups. Two treatment groups of the control group. Marmot in the MT group I pinned droplets are perforated bone marrow stem cells. Marmot group II in MT that

perforation of amniotic membranes attached. While the marmots group III (control) without giving something. Bone marrow stem cells taken from the bone marrow of marmot. *Otoskopi* examination and cleaning wax NaCl before treatment [12]. Eighteen guinea pig tails after put to sleep with *ketamine hydrochloride* 25 mg / kg IM will be injected bacteria using a microscope at MT left and right with no needle. 23. After the needles penetrate the tympanic membrane of bacteria sprayed with ½ ml syringe into the tympanic cavity. When it happens the infection process macroscopically visible MT hyperemia, otorrhoea, and has formed a small perforation to the MT, the marmots were given oral antibiotics enrofloksasin dose of 2.5 mg / kg for 7 days so that the perforation becomes dry. Spinal tap done when the perforation has occurred and dries without any signs of infection such as otorrhoea, hyperemia. The location of the tibia and femur. Furthermore, bone marrow processing performed in clinical pathology laboratories.

Group I: of those 8 marmots with former otitis media has been put to sleep with *ketamine*, cleaning done on the external acoustic meatus and disinfection of the external acoustic meatus to the earlobe by using alcohol 70%. Used microscope in action. Created injury in approximately perforation with a syringe needle perforator. After the injury, perforation MT droplet covered with bone marrow stem cells containing about 0.02 µ. Focus attention on the application in the area of perforation, but the droplets must also attach to the lateral MT. If you have been convinced that the perforation had been closed by the stem cells, the external acoustic meatus can be covered with a sterile dry ear tampons, try not to touch put stem cells (preferably put on the pars kartilagineus)

Group II: Seven marmots with former otitis media has been put to sleep with ketamine hydrochloride, then after cleaning and disinfection at the meatus. Subsequently injury marmots as the first group. Attachment on amniotic membrane perforation with a 0.9% NaCl fluid dripped.

Group III: Seven marmots with former otitis media is left as a control group. Experimental animals sample were controlled every 3 days. All data collected is presented in tables and bar charts. Data analysis was performed using SPSS version 17. Statistical test used the *Kruskal-Wallis test* aimed at assessing the homogeneity of age and body weight samples, and *Likelihood Ratio test* aimed to compare the closure of the tympanic membrane between the treatment groups (stem cells and amniotic membrane) with group control (no treatment). This study was approved by the Health Research Ethics committee of the Faculty of Medicine, Hasanuddin University, Makassar.

Results:

Data obtained from the study is presented in the following tables and figures, (Syamsihar, 2012) [13]. Based on the statistical test of Kruskall-Wallis test of sample age descriptive (weeks) base on group, it was not found significant differences in the mean age of the sample among the three groups, indicating that the three groups of samples are considered homogeneous in terms of age ($p = 0.347$), as can be seen on the following Table 1.

Table 1: Descriptive Statistics of Age (Weeks) Sample by Group

Group	n	Minimum	Maximum	Average	Standard Deviation
Stem Cells	6	10	12	11,3	0,8
Amnion Membrane	5	9	12	10,4	1,1
Control	5	9	12	10,6	1,3

Kruskal-Wallis (p=0,347)

In Table 2 below illustrates the descriptive statistical of weight (grams) of the sample obtained. According to the body weight group, there was no significant difference among the three groups. It also showed that the three groups of samples are considered homogeneous in terms of body weight (p = 0.416)

Table 2: Descriptive Statistics of Body Weight (g) Sample by Group

Group	n	Minimum	Maximum	Average	Standard Deviation
Stem Cells	6	350	400	379,2	19,1
Amnion Membrane	5	368	400	377,6	13,4
Control	5	375	400	387,0	10,4

Kruskal-Wallis (p=0,416)

Table 3: Result of Observations 1 of Timpani Membrane by Group

			Group			
			Stem Cells	Amnion Membrane	Control	Total
Observation 1	Closure	n	0	0	0	0
		%	0,0%	0,0%	0,0%	0,0%
	No	n	6	5	5	16
		%	100,0%	100,0%	100,0%	100,0%
Total		n	6	5	5	16
		%	100,0%	100,0%	100,0%	100,0%

Likelihood Ratio (p cannot be counted as a single line containing all 0)

The results of the first observation cannot be tested statistically because the closing has not occurred on the tympanic membrane three sample groups.

Table 4: Result of Observation II of Tympanic Membrane by Group

			Group			
			Stem Cells	Amnion Membrane	Control	Total
Observation II	Closure	n	3	0	0	3
		%	50,0%	0,0%	0,0%	18,8%
	No	n	3	5	5	13
		%	50,0%	100,0%	100,0%	81,3%
Total		n	6	5	5	16
		%	100,0%	100,0%	100,0%	100,0%

Likelihood Ratio (p=0,028)

In the observation II, there was a significant difference in the percentage of tympanic membrane closure according to group ($p < 0.05$). In the group of stem cells occurs closure on 3 samples (50%), whereas the other two groups tympanic membrane closure has not occurred.

Table 5: Result of Observation III of Tympanic Membrane by Group

			Group			
			Stem Cells	Amnion Membrane	Control	Total
Observation III	Closure	n	6	5	2	13
		%	100,0%	100,0%	40,0%	81,3%
	No	n	0	0	3	3
		%	0,0%	0,0%	60,0%	18,8%
Total		n	6	5	5	16
		%	100,0%	100,0%	100,0%	100,0%

Likelihood Ratio (p=0,013)

In the observation III, there was a significant difference in the percentage of tympanic membrane closure according to group ($p < 0.05$). In the group of Stem Cell and Amniotic Membrane closures on each of 6 (100%) and 5 samples (100%), whereas in the control group occurred closure of the tympanic membrane in 2 samples (40%)

Table 6: Result of observation IV of Tympanic Membrane by Group

			Group			
			Stem Cells	Amnion Membrane	Control	Total
Observation IV	Closure	n	6	5	3	14
		%	100,0%	100,0%	60,0%	87,5%
	No	n	0	0	2	2
		%	0,0%	0,0%	40,0%	12,5%
Total		n	6	5	5	16
		%	100,0%	100,0%	100,0%	100,0%

Likelihood Ratio (p=0,070)

In the observation IV, there was no significant difference in the percentage of tympanic membrane closure according to the groups (p> 0.05). Stem cells group and amniotic membrane closures on each of 6 (100%) and 5 samples (100%), whereas in the control group tympanic membrane closure occurred in 3 samples (60%). It is seen that at the end of last observation, there are still 2 samples (40%) in the control group who had not experienced the closure of the tympanic membrane.

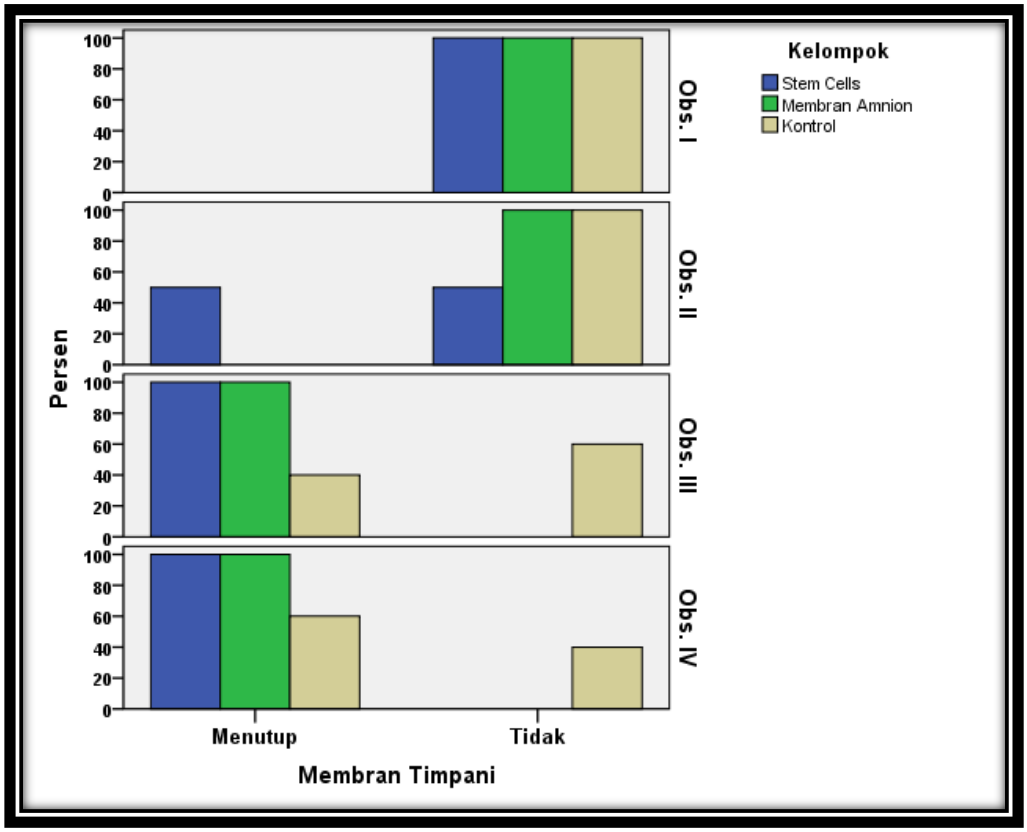

Figure 1: Tympanic Membrane Closure by Group of Samples

Specification:

- In the first observation: no sample experienced tympanic membrane closure
- In the second observation: 50% of samples in the group of stem cells has undergone closure of tympanic membrane
- In the third observation: all samples (100%) in the group of stem cell and amniotic membrane

has undergone closure of the tympanic membrane, whereas in the control group occurred in 40% of samples closure
- In the fourth observation: 60% of samples in the control group had experienced the closure of the tympanic membrane and the remaining 40% were not.

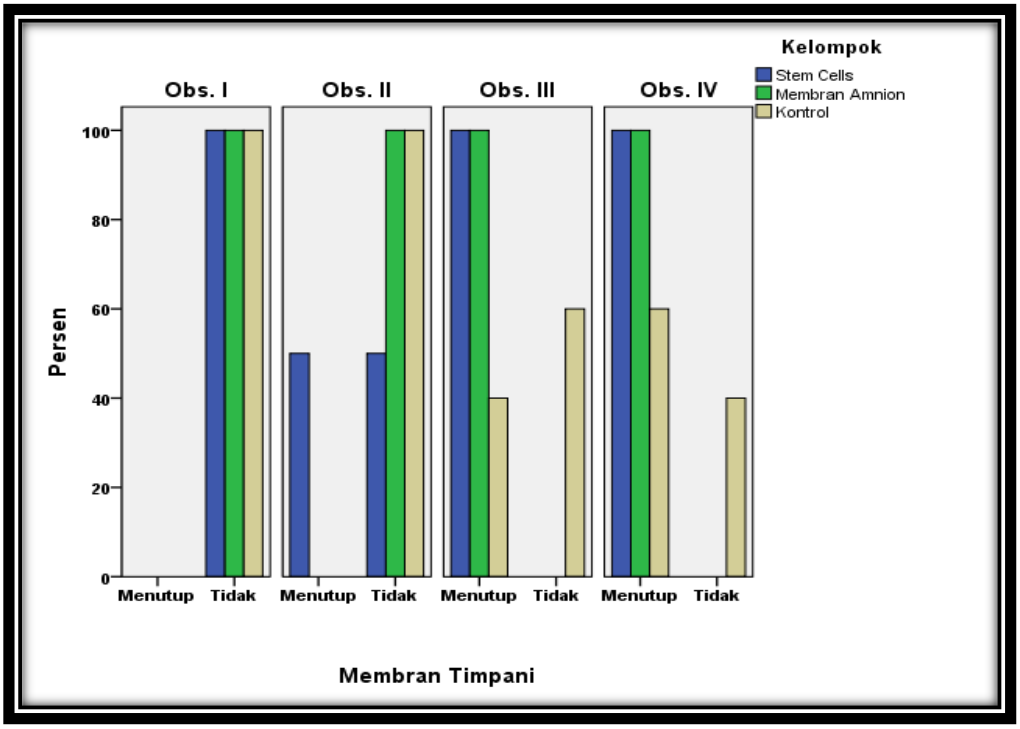

Figure 2. Membrane Closure Tympanic by Group of Samples

Specification:
- In the first observation: no sample experienced tympanic membrane closure
- In the second observation: 50% of samples in the group of stem cells has undergone closure of tympanic membrane
- In the third observation: all samples (100%) in the group of stem cell and amniotic membrane has undergone closure of the tympanic membrane, whereas in the control group occurred in 40% of samples closure
- In the fourth observation: 60% of samples in the control group had experienced the closure of the tympanic membrane and the remaining 40% were not.

Discussion

Trauma, acute or chronic otitis media and application of ventilation tubes can cause permanent perforation of the tympanic membrane. Chronic perforation is a major problem, especially in poor countries or developing countries. Millions of people have been affected by hearing loss. Many researches previously has been done used a *paper-patch test* and *fat plug* for example by Mehmet [14] in Turkey using rats. When 30 days after miringoplasti, the rat's tympanic membrane plastered with paper patch-test examined and obtained completely closed, the membrane becomes a normal color, no signs of infection. However, acute perforation closes spontaneously in both humans and experimental animals with different chronic perforations are many situations experienced by humans [14,15]. Only a few experiments with use animals with chronic perforation have been reported. The experiment is

performed on laser *myringotomy* then attached some substances that can widen the perforation as hydrocortisone, mitomicin C [15-17]. In the experiments we tried to do with the tympanic membrane perforation by injecting Staphylococcus aurous bacteria of 900 bacteria / 0.5 ml into the tympanic cavity. If there have been any signs of infection occurs usually on day 5 to -7 MT that characterized by hyperemia, otorrhoea. Subsequently Enrofloksasin type antibiotics for 7-14 days until dry MT. Average perforation occurs 1-2 mm.

Latest discoveries in the fields of biology and tissue development makes possible the test cell supplied from external sources and move the original endogenous cells to repair tissue damage or loss. Bone marrow stem cells led to the closure of perforation of the tympanic membrane of marmots because it has the ability to differentiate into other cell in this case becomes the tympanic membrane *epithelium*. When compared with the closing of the amniotic membrane, the closure with stem cells was more quickly. Observations carried out on average every 3 -4 days due to the use of *ketamine anesthesia* in marmots. In the closed stem cells, stem cells already occurred on day III-VI (Observation II), although not completely closed. In amniotic membrane closure occurred on day IX (Observation II) late for 2 days. The control group had started closing on the day of XIII (Observation III). All perforations occurred in the marmots ear was chronic perforation with an average of between 1-2 mm perforation. Research by Anisur Rahman in Sweden assesses chronic tympanic membrane perforation closure of marmot using human bone marrow stem cells on average had a chronic perforation closure between 9-14 days. While Magnus von Unge [10] using stem cells derived from rat embryonic stem cells experienced a perforation closure about 5 days.

The use of amniotic membrane perforation resulted in the closure of amniotic membrane on the observation III. Amnion is known as a biological bandage in stimulating wound healing. Last results of the control group experienced a slow healing though.

This is probably due to perforation which occurs is a small perforation.

Conclusion

This research concluded that the administration of bone marrow stem cells can accelerate the healing of tympanic membrane perforation compared to the use of amniotic membrane and controls. Further research is needed to compare the use of other types of stem cells such as embryonic or derived from umbilical cord blood.

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Authors guarantee that there is no conflict of interest for the publication of this article.

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