Efficacy of Amniotic Membrane-Mesenchymal Stem Cell Therapy for Burn wounds: Metaanalysis study

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ARTICLE INFO	ABSTRACT

Background : Burn injury is a trauma with high morbidity and mortality that requires special management from the start. stem cells have generated great hopes for the treatment of numerous conditions including burns. The Metaanalysis was performed for these preclinical studies to assess the efficacy and possible mechanisms of Amniotic Membrane Mesenchymal Stem Cell (AM-MSC) in treating burn wounds.

Methods: . Seven studies identified by searching PubMed, MEDLINE, databases from inception to October 2020. In addition, a manual search of references of studies was performed to obtain potential studies. Stata 16 was used for all data analysis.

Results : The overall meta-analysis showed that AM-MSC therapy significantly improved burn healing rate (SMD 3.34, 95% CI 1.82 to 4.86), irrespective of transplant type, burn area, and treatment method in the control group

Conclusions: Metaanalysis showed that AM-MSC therapy exerts appears to exert a positive effect in burn wound healing, mainly through angiogenesis and anti-inflammatory actions. There is, therefore, justification for continued efforts to evaluate variations in future clinical studies using stem cells to treat a burn wound in order to maximize the effectiveness. the use of stem cells as an adjunct to first-line therapies in burns.

INTRODUCTION

The prolonged inflammatory process in burns causes tissue fragility, leading to disfiguration of the tissue structure that ends with deformity of shape and dysfunction. If the patient survives, the healing process becomes the next problem. The prolonged inflammatory process in burns causes tissue fragility, leading to disfiguration of the tissue structure that ends with deformity of shape and dysfunction. [1]

To avoid this, several attempts were made, such as early excision and skin grafting, however this was limited due to the limited availability of donors. Another effort undertaken is wound care which aims to increase the epithelialization process. Treatment of burns that have been carried out is using silver sulfadiazine. Apart from acting as a topical antibiotic and lysing the eskars, this preparation is also pro-inflammatory, produces exudate, and is toxic to fibroblasts so that the healing process is slow. [1]

Stem cells are undifferentiated cells characterized by their capacity for self-renewal and differentiation into various cell types. The use of stem cells to regenerate damaged tissues and organs has given rise to great hopes in the treatment of a variety of conditions, for which current therapeutic options are ineffective. The use of stem cells has attracted considerable interest also in the field of wound healing, and burns in particular, as a means to promote skin regeneration. However, such stem cell therapies remain experimental have been reported most of the studies on stem cell

mediated repair of burn wounds have been conducted in animal models.

Animal experiment has its special approach in increasing the understanding of the physiological and pathological processes of a disease, which lays a foundation for future clinical trials. In addition, preclinical reviews can more systematically evaluate the mechanisms of stem cell efficacy and provide vital evidence for stem cell research. Thus, we aimed to perform a Meta analysis for these preclinical studies to assess the efficacy and possible mechanisms of stem cells in treating burn wounds.[2]

METHODS

This is a meta-analysis study which aims to assess the efficacy of amniotic membrane mesenchymal stem cells (AM-MSC) on the rate of epithelialization in burns. The type of research included in this study is the one that analyzes and compares the efficacy of giving amniotic membrane mesenchymal stem cell (AM-MSC) on the healing of burns on the skin of experimental animals.

Literatur Reseasch

We conducted a thorough search to assess the association between stem cell therapy and burns. PubMed, MEDLINE , from their inception to oktober 2020. Thesearch phrases used in PubMed are as follows: Amniotic Membran OR Mesenchymal Stromal Cells OR mesenchymal stem cell OR Stem Cells OR progenitor cells OR cell treatment OR cell therapy Burns OR burn wound healing OR wound Regeneration The search was limited to animal trial studies and be published English. In addition, we performed a manual search of references of studies to obtain potential studies.

Study Selection

Innclusion Criteria were prespecified as follows (1) Research assessing the impact of giving Amniotic membrane mesenchymal stem cell (AM-MSC) on burn healing in experimental animals (2) Research that has parameters for assessing Burn Healing based on clinical, laboratory, histological and immunohistochemical basis (3) Research that has sufficient data to be analyzed (has mean and SD values) (4)Research in the form of full text (5) Research in English (6) Research obtained from the PubMed and MEDLINE databases for the period 2010-2020. The Exclusion Criteria were prespectified as follows: (1) Research that has different operational definitions (2) Research with mortality outcomes due to the application of amniotic membrane mesenchymal stem cells (AM-MSC)(3) Anonymous research (4)Duplicated research or previously published research.

Data Extraction

Two authors independently extracted detailed information from the included studies, and the disagreement was resolved by a third author. The following data were collected: (1) the first author and publication year; (2) countries of the studies; (3) animal characteristics (including species, number, burn degree, and area); (4) administration methods of treatment group and control group; (5) stem cell information (including cell type, number, origin, and transplant type); and (6) primary and secondary outcomes.

Analisis Data

Data analysis using a fixed effect model or random effect model. In the fixed effects model it is assumed that the variability between various studies is based solely on the opportunity factor; meaning that if the research is carried out indefinitely, eventually the same results will be obtained. In the random effects model, in addition to intra-study variability, inter-study variability is also taken into account. With this technique, a wider confidence interval will be obtained than in the fixed effects model. The software used to perform the meta-analysis is stata 16. The results of data processing are presented in a forest plot graph and a funnel plot to describe the combined effect size of each variable studied.

Publication Bias

The technique used to identify the existence of publication bias in this study is to use a funnel plot. The funnel plot is used

to see the distribution of articles that are combined in the meta-analysis. If the distribution of the articles is not symmetrical, there is publication bias on the relationship of the variables being studied. If the intercept value is equal to zero (0), it is concluded that publication bias does not affect the relationship of the variable being tested. The trim and fill technique is used to estimate the relevant studies that are missing in order to eliminate publication bias on the relationship of the variables under study. The publication bias test was performed using the Comprehensive Metaanalysis (CMA) version 3 software.

RESULT

Study selection

The literature search was carried out comprehensively according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) method. The search was carried out on the PubMed and MEDLINE databases in October 2020. The PRISMA flow diagram depicting the literature search and selection process is shown in Figure 5.1. In the primary literature search process, which was carried out by entering a keyword combination, it was found in 97 articles in the PubMed and MEDLINE databases and the evaluation results of duplicated articles by title showed 40 articles with similar titles and were subsequently excluded from this study.

The next evaluation was carried out by examining the titles of each literature that had been searched based on previously agreed keywords, there were 32 literatures excluding 25 literature that had no relation to the title. Furthermore, an evaluation of the literature based on abstracts and quality assessment is carried out so that the final results of 7 literature are obtained which will then be analyzed in this study. (figure 1)
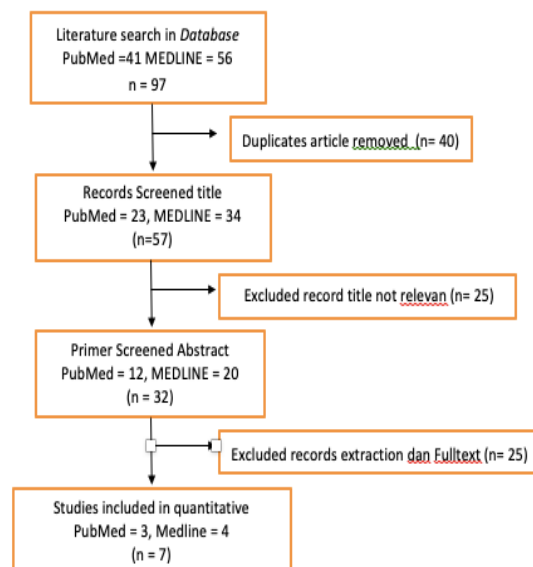
figure 1. Flowchart of the details of study selection

Caracteristics of studies

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Table 1 Characteristics of the included studies

Authors (Study Year)	Country	Animal	Study design	Burn area (cm2)	Burn degree	Cell Type	Application Methode	Intervention group	Control Group
Shi *et al.*, 2019	China	rats C57BL/6	Invivo	2,25	3	Amniotic Membrane Mesenchymal stem cell	Subcutan injection	Mesenchymal stem cell	Hydrogel
Zhang *et al.*, 2015	China	rats Sprague Dawley	Invivo dan Invitro	2	3	Amniotic Membrane Mesenchymal stem cell	Subcutan injection	Mesenchymal stem cell	Phosphat Buffer Salin
Mahmood *et al.*, 2019	Pakistan	rats Sprague Dawley	Invivo dan Invitro	4	3	Amniotic Membrane Mesenchymal stem cell	Subcutan injection	Mesenchymal stem cell	Phosphat Buffer Salin
Navas *et al.*, 2018	Mexico	rats Wistar	In Vivo	No Mention	No mention	Amniotic Membrane Mesenchymal stem cell	Subcutan injection	Mesenchymal stem cell	Phosphat Buffer Salin
Li *et al.*, 2019	China	rabbit	Invivo dan Invitro	1	2	Amniotic Membrane Mesenchymal stem cell	Subcutan injection	Mesenchymal stem cell	Phosphat Buffer Salin
Hashemi *et al.*, 2020	Iran	rats Albino	In Vivo	1,3	3	Amniotic Membrane Mesenchymal stem cell	Subcutan injection and applied	Mesenchymal stem cell	Phosphat Buffer Salin
Gholipour-Kanani, 2014	Iran	Rats Sprague Dawley	In vivo	1,1 cm	3	Amniotic Membrane Mesenchymal stem cell	applied	Mesenchymal stem cell	scalfold

Study outcome

The outcome of each study analyzed in this meta-analysis study is shown in Table 2. In this study, an analysis of the timing of the outcome assessment, parameters, the scoring system and the significance of each parameter to the burn rate was carried out in each search result literature. In this study, it appears that the evaluation time of each literature varies from day 0 to the longest, namely the 28th day after the intervention, namely in the form of clinical evaluation of parameters in the form of wound healing speed or burn coverage size compared to initial conditions. Studies in the analyzed literature also saw the outcome of the laboratory results in the form of pro-inflammatory cytokine levels taken from the blood of experimental animals as well as histological assessment by evaluating the epithelialization of wounds accompanied by the presence of inflammatory cells [3,4].

Primary outcome

This meta-analysis had the primary outcome of how fast the burn epithelialization was seen from the time of wound closure and the size of the wound after treatment during the defined outcome period. A meta-analysis of 7 literature shows that the Amniotic Membrane Mesenchymal Stem Cell significantly accelerates the healing of burns in rats when compared to controls (figure 2).

Tabel 2. Study Outcome

Authors	Time to outcome	Outcome parameter	Parameter scoring	Burn degree	Baseline signification	Group signification	Other outcome
Zhang et al., 2015	Day 0,1,2,3,5, and 7th of blood analysis Day 14,21 and 28 Histopathological examination	Burn epithelialization rate, burn size WBC, CRP, IL 6, IL 10TNF alpha laboratory	Wound size Duration of wound closure	3	24 hours after the burn, there was an increase in pro-inflammatory cytokines p <0.05	proinflammatory cytokines in the intervention group decreased p <0.05 The rate of burn reepitelialization in the mesenchymal group was very high compared to the control group .	No
Mahmood et al., 2019	day 5, 10, 15 and 20	Burn reepithelialization rate, burn coverage area Histology, collagen PCR	Laboratory Histopathology immunohistochemistry	3	P<0,05	Reepitelialization in the intervention group was superior compared to the control group Collagen in the intervention group was higher than the control	No
Navas et al, 2018	Day 12	Burn reepithelialization rate, burn coverage area	Wound size Duration of wound closure	No Mention	P<0,01	Wound vascularization using the intervention group was better than the control group on day 12 with p value <0.05	No
Li et al, 2019	Hari ke 0, 7, 14 dan 21	Burn reepithelialization rate, burn coverage area	Wound size Duration of wound closure	2	No Mention	The wound closure in the intervention group was better on days 14 and 21 when compared to days 0 and 7	Vascularization
Hashemi et al., 2020	Hari ke 7 dan 14	re-epithelialization of burns rate	Reepitelisasi	3	P<0,05	Epithelialization rate in all groups occurred in all groups except for the AM-MSC group which was applied on day 7	inflamation
Gholipour-Kanani, 2014	day 5, 10 dan 15	re-epithelialization of burns rate	Wound size Duration of wound closure	3	No mention	Wound closure in the intervention group was seen on day 15	No
Shi et al., 2019	Hari 1, 3 dan 7 dam 14	Burn reepithelialization rate, wound size, laboratory Histology and histochemistry	Wound size Duration of wound closure Hystology and Histology and histochemistry	3	P<0,05	Day 1 there was no significant difference in the 2 study groups Day 3 there was a granulation tissue in the mesenchymal group Day 14 Pidemal thickness was significant in the mesenchymal group and reepithelialization was achieved	No
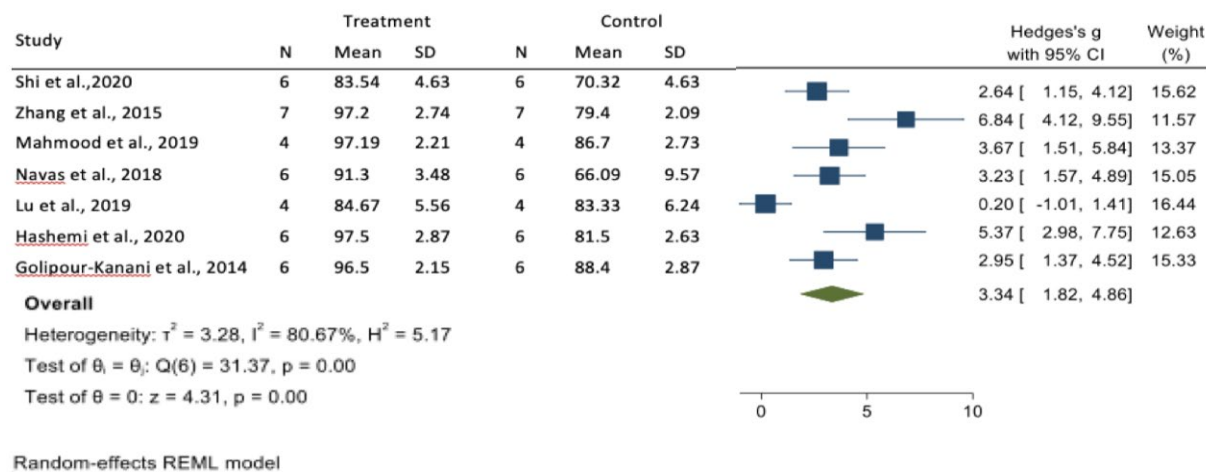
Figure 2. The forest plot: the effects of AM-MSC therapy for increasing healing rate of burn wounds compared with controls

Statistical analysis of 7 research articles using meta-analysis with the help of stata 16 software using random effects analysis model. Heterogeneity (I2) was used to determine the discrepancy for the effect of each surgery. Fixed effect method is used if heterogeneity is low, meanwhile random effect method is when heterogeneity is high. The results of the analysis showed that the variation in the study was heterogeneous, with a smaller p value of 0.05 in the heterogeneity test, namely p <0.001 and the value of variation between studies (I2) of 80.67%. High heterogeneity is indicated by the value of I2 which has a value above 75%. so in this study high heterogeneity and the model used was random efffect.

The results of data analysis displayed on the forest plot in Figure 5.2 show the efficacy value of the amniotic membrane mesenchymal stem cell on the speed of burn epithelialization in experimental animals with a p value <0.05 and a pooled odds ratio value of 3.34 with a 95% CI of 1.82. up to 4.86, because the value does not exceed the number 0, it can be concluded that the treatment group receiving amniotic membrane stem cell therapy has a significantly higher efficacy when compared to the control group.

Publication bias

Based on the Funnel Plot, it can be seen that 7 studies are asymmetrically distributed where the research distribution is less balanced on the left and right of the center line boundary. This means that there is potential for publication bias related to the conclusion. The impact of this publication bias is that the results or information produced will be less accurate, because the published literature may not represent the research that has been done on a topic.

To overcome publication bias, the trim and fill technique was used. The trim and fill technique is a technique used to estimate the number of relevant studies that are missing so that the funnel plot becomes symmetrical and is not affected by publication bias. The results of the trim and fill show that the data is symmetrical, there is no advice and funnel plot from the study is final, so that publication bias can be overcome.
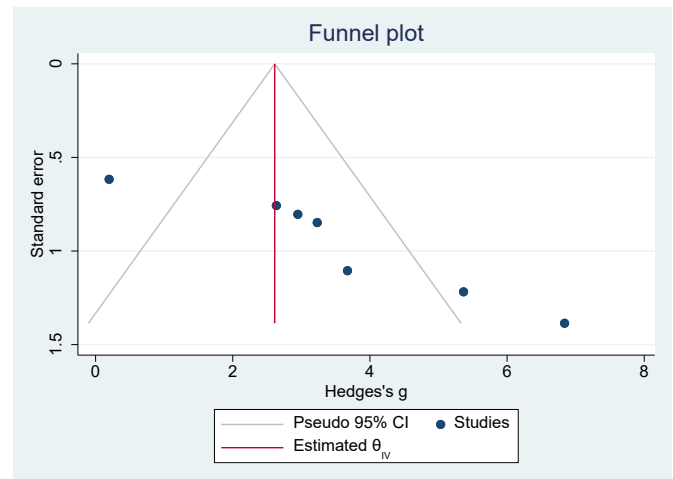

Figure 3. Funnel plot for wound healing.

DISCUSSION

Dermal wound repair is a complex and dynamic process involving the interaction between cells and molecules, including regulation of inflammation, the formation ofextracellular matrix (ECM), the release of growth factors, and angiogenesis . Previous experience has shown that in order for burn wounds to heal, some of the abovementioned key steps are necessary . Stem cells are known for their capacities of self-renewal and multilineage differentiation that have been regarded as a novel treatment strategy to overcome the aforementioned complications . Thus, the present review aimed toprovide the preclinical evidence available in the literature to elucidate the efficacy and mechanisms of stem cells for burn wounds.[5]

Stem cell therapy is an emerging method based on the proliferation or differentiation of stem cell transplants to repair or even replace damaged tissue or organs, which essentially offers new possibilities for regenerative medicine. Stem cells can be isolated from placenta, adipose tissue, umbilical, embryonic, bone, gingival and other tissues. It is reported that stem cell transplants have been applied to treat a variety of disease models and significantly improve their prognosis, including burns, digestive diseases, kidney disease, and autoimmune diseases. In recent years, stem cell therapy has been shown to have good potential as a potential treatment for burns,

because stem cells can influence many burn healing processes, including accelerating extracellular matrix (ECM) synthesis, alleviating the inflammatory response, and increasing angiogenesis. Through clinical trials that have been reported, most studies on stem cells mediate the acceleration of burn healing that has been carried out in experimental animals. Animal experiments have a special approach in enhancing understanding of the physiological and pathological processes of a disease, namely laying the groundwork for future clinical trials[5]

A study shows that the effect of stem cell therapy in second degree burns is much higher than that of third degree burns. This observation can be attributed to imperfect tissue damage in second degree burns, which can provide a microenvironment and nutrients for stem cells to have a therapeutic effect. It could be that in the future, stem cell therapy will be combined with other therapies that provide these environmental or nutritional benefits, which could be more conducive to the repair of severe burns. The wound healing process is severely impaired in patients with severe burns. The wound itself acts as a mechanical barrier, preventing migration of cytokines and growth factors. In addition, a compensatory anti-inflammatory response develops after an injury triggers systemic immunosuppression, which affects the inflammatory phase, when this phase is very important in the wound healing process [6,7,8]

The majority of the literature analyzed in this meta-analysis study used the Amniotic Membrane Mesenchymal stem cell (AM-MSC). AM-MSC itself is Mesenchymal stem cell (MSC) where MSC can come from fat tissue and adult bone marrow, but can also come from the placenta, tendons and umbilicals. MSCs modulate immune responses through the release of growth factors and cytokines. MSC is pro-angiogenic, anti-apotosis and anti-fibrotic in certain cells. This combination of effects can stimulate cell populations in target organs such as the healing process of wounds on the skin [8,9]

CONCLUSION

The preclinical evidences from this meta-analysis demonstrated that stem cell therapy exerts healing function for burn wounds, mainly through angiogenesis and anti-inflammatory action. We also found that there were efficacy variations, across stem cell type, burn degree, and burn area. These findings demonstrate the need for considering variations in future clinical studies using stem cells to treat a burn wound in order to maximize the effectiveness. In general, stem cells can potentially become a novel therapy candidate for burn wounds.

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