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Antibacterial efficacy of mesenchymal stem cell administration in diabetic rats infected with MRSA: An experimental study

Mezenkimal kök hücre uygulamasının MRSA ile enfekte olmuş diyabetik ratlardaki antibakteriyel etkinliği: Deneysel çalışma

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Abstract

Aim: The antibacterial effects of mesenchymal stem cell (MSC) administration and vancomycin, tigecycline and daptomycin treatment were compared in a diabetic rat model with wound infection due to methicillin-resistant *Staphylococcus aureus* (MRSA).

Methods: Experiment, negative and positive control groups were created using 60 rats. The antibacterial efficacy of MSC, vancomycin, tigecycline and daptomycin were compared. All rats had diabetes induced with streptozotocin. They were shown to be hyperglycemic with fasting blood glucose monitoring. During surgery, subdermal pouches were created. Group 0 (negative control group) was not infected or treated. All other groups were infected with MRSA. Group 1 (positive control group) was infected but not treated. The other 4 groups were determined as treatment groups: Group MSC was treated with MSC, Group Van treated with vancomycin, Group Tig treated with tigecycline and Group Dap treated with daptomycin. After one week of treatment, samples were collected following euthanasia. Tissue samples were evaluated after histopathologic staining with hematoxylin/eosin. The presence of MSC in the wound region was shown by immunofluorescent staining. Bacterial colony counts were identified quantitatively. TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF and Caspase-3 levels in blood samples were measured with the ELISA method.

Results: While bacterial colonization was not observed in Group 0, a clear colonization was identified in Group 1. Full eradication was achieved in Group Tig and Group Dap. Eradication could not be achieved for 1 rat in Group Van and 4 rats in Group MSC. The uncontaminated negative control group rats (Group 0) had minimal inflammation, while the most severe inflammation was observed in infected and untreated rats (Group 1) (P<0.001). Group-MSC, Group-Van, Group-Dap and Group-Tig had moderate levels of inflammation and edema. The MSC group showed significant increase in vascularity (P=0.001). Adhesion and fibrosis were observed significantly less in the negative control group and MSC groups, similarly (P<0.001, P<0.001).

Conclusion: MSC may exert antibacterial-like effects for MRSA-induced wound infection treatment in diabetic rats, and limit the inflammation in and around the wound. Further clinical studies researching the synergistic effects of MSC with antibiotherapy for treatment of diabetic wound infections are needed.

Keywords: Mesenchymal stem cell, Vancomycin, Tigecycline, Daptomycin, Methicillin-resistant Staphylococcus aureus, Diabetes mellitus

Öz

Amaç: Mezenkimal kök hücre (MSC) uygulamasının Metisiline dirençli *Staphylococcus aureus* (MRSA) kaynaklı yara enfeksiyonu üzerindeki antibakteriyel etkileri, diyabetik rat modelinde, vankomisin, tigesiklin ve daptomisin tedavisi ile karşılaştırıldı.

Yöntemler: 60 rat ile oluşturulan deney ve negatif-pozitif kontrol grupları oluşturuldu. MSC, vankomisin, tigesiklinin ve daptomisinin antibakteriyel etkinlikleri karşılaştırıldı. Ratların tümü streptozotosin ile diyabetik hale getirildi. Açlık kan glukozu takibiyle hiperglisemik oldukları gösterildi. Cerrahi olarak cilt altına keseler oluşturuldu. Grup-0 (negatif kontrol grubu) enfekte ve tedavi edilmedi. Diğer tüm gruplar MRSA ile enfekte edildi. Grup-1 (pozitif kontrol grubu) enfekte edildi fakat tedavi edilmedi. Diğer 4 grup tedavi grupları olarak belirlendi. Grup-MSC; MSC ile, Grup-Van; vankomisin ile, Grup-Tig; tigesiklin ile, Grup-Dap; daptomisin ile tedavi edildi. Bir haftalık tedavi sonrası ötenazi ile numuneler toplandı. Doku örnekleri histopatolojik olarak hematoksilen/eosin ile boyanarak değerlendirildi. Yara bölgesinde MSC varlığı immun floresan boyama ile kanıtlandı. Kantitatif olarak bakteri koloni sayıları tespit edildi. Alınan kan numunelerinden TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF ve Kaspaz-3 seviyeleri ELISA yöntemiyle ölçüldü.

Bulgular: Grup-0'da bakteri kolonizasyonu gözlenmez iken, Grup-1'de belirgin kolonizasyon saptandı. Grup-Tig ve Grup-Dap'ta tam eradikasyon sağlandı ve Grup-Van'da 1, Grup-MSC'de 4 ratta eradikasyon sağlanamadı. Kontamine edilmeyen negatif kontrol grubunda (Grup-0) minimal inflamasyon düzeyi izlenirken, en şiddetli inflamasyon enfekte edilmiş ve tedavi verilmemiş ratlarda (Grup-1) gözlendi (P<0,001). MSC, Vankomisin, Daptomisin ve Tigesiklin gruplarında orta düzeyde inflamasyon ve ödem gözlendi. MSC grubunda önemli vaskülarite artışı görüldü (P=0,001). Negatif kontrol grubunda ve MSC grubunda benzer oranda anlamlı olarak daha az adezyon artışı ve fibrozis görüldü (P<0,001, P<0,001).

Sonuç: Bu çalışma, diyabetik ratlarda MRSA kaynaklı yara enfeksiyonu tedavisinde MSC'nin antibakteriyel etkinliği sağlayabileceğini düşündürmektedir. Buna ilaveten MSC sayesinde yara bölgesinde sınırlandırılmış bir inflamasyon sağlanabileceği düşünülmüştür. Diyabetik yara enfeksiyonlarının tedavisinde antibiyoterapiyle birlikte MSC kullanımının sinerjistik etkisinin araştırılacağı klinik çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Mezenkimal kök hücre, Vankomisin, Tigesiklin, Daptomisin, Metisiline dirençli Staphylococcus aureus, Diabetes mellitus

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Introduction

Mesenchymal stem cells (MSC) are obtained from non-hematopoietic bone marrow cells which can theoretically renew themselves and differentiate into a variety of cells [1,2]. According to their source, they may release characteristic active mediators and antimicrobial peptides [3]. It is reported in a variety of studies that these secretions strengthen the natural immune response against bacterial infection [4]. 3 stages defined in injured tissue repair begin with hemostasis after the inflammatory period ends in 24-48 hours and continues with proliferative and maturation stages. During the inflammatory period, the infection shield is strengthened by neutrophil and macrophage migration, which may be equivalent to the rate of angiogenesis, for the foundations of tissue repair laid in this stage [5]. In addition to fibroblastic proliferation and differentiation, macrophages stimulate angiogenesis by collagen production and secretion of transforming growth factor beta (TGF-β), platelet derived growth factor (PDGF), interleukin 1 (IL-1), platelet activated growth factor (PAGF), transforming growth factor alpha (TGF-α), tumor necrosis factor (TNF-α), fibroblast growth factor (FGF), and epidermal growth factor (EGF) [6].

Wound infections are a significant problem for many types of surgery, especially vascular surgery, in the short and medium term. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens and is resistant to many antibiotics [7,8]. Methicillin-resistant *Staphylococcus* strains which are resistant to glycopeptides have been reported [9]. Additionally, wound infections that don't respond to antibiotherapy cause high morbidity and mortality [10]. New generation broad-spectrum tetracyclines such as vancomycin and daptomycin-tigecycline are the strongest treatment choices for *Staphylococcus* infections with excessive resistance [11].

In this study, the antibacterial efficacy of MSC obtained from rat fatty tissue was compared with strong antibiotics with known efficacy for MRSA treatment, namely, daptomycin, vancomycin and tigecycline.

Materials and methods

Groups: Sixty rats were divided into a total of 6 groups of 4 treatment groups and negative and positive control groups (age>6 months, weight: 300-350 g).

Diabetic rats: Rats were given streptozotocin-induced diabetes (STZ; Sigma-Aldrich, St Louis, MO), (IP, 60 mg/kg, single dose). One week later, fasting blood sugar obtained from the tail vein was shown to be higher than 200 mg/dL [12].

MRSA: MRSA strains were isolated from the tissue culture of a patient treated due to surgical wound site infection in Kırıkkale University Faculty of Medicine Hospital. A sterilized colony was obtained from a single wound infection with gram staining, catalase reaction, tube coagulation test and API-staph test (BioMérieux, Lyon, France). Methicillin resistance was analyzed using the Kirby-Bauer disk diffusion method.

Surgery and contamination: All rats were anesthetized with ketamine hydrochloride (Pfizer, Lüleburgaz, Turkey) and xylazine hydrochloride (Bayer AG, Leverkusen, Germany). They were shaven and cleaned with povidone-iodine. Under sterile conditions, all rats, except the negative control group, had bacterial seeding of MRSA $2x10^7$cfu/ml using a tuberculin injection in created pouches [10].

MSC: Commercially sold rat adipose tissue-derived GFP-labeled mesenchymal stem cells (MLP laboratory, Istanbul, Turkey) were brought to the laboratory in accordance with cold chain rules and centrifuged after a rapid defrost technique with 37 °C water bath. The supernatant was removed, and the pellet was resuspended. Cell counts and viability were measured (Countess®, Invitrogen, San Diego, CA, USA). For each rat, $1x10^6$ cells per injection were prepared [13].

Treatment: Group MSC was treated with mesenchymal stem cells (administered locally to the wound site, $1x10^6$ MSC, single dose); Group Van was treated with vancomycin (IP, 15 mg/kg, 2 times per day) [14]; Group Dap was treated with daptomycin (IP, 3 mg/kg, 2 times per day) [15]; and Group Tig was treated with tigecycline (IP, 10 mg/kg, 2 times per day) [16]. The negative and positive control groups received no treatment.

During the experiments, rats were housed in fives per cage. They were kept under standard environmental conditions (12 hours light/dark cycle, temperature ~ 21 ºC) and fed with standard rat feed and water ad libitum.

Euthanasia: One week after surgery, rats were euthanized with high-dose anesthesia and tissue and blood samples were taken.

Histopathological Evaluation: Perigraft and skin subdermal samples were collected and fixated in formalin for 2 days, dipped in ethanol and xylene bath, submerged in paraffin, divided and stained with hematoxylin/eosin. Inflammation severity was graded histopathologically: Grade 0 showed no neutrophils, Grade 1 showed some neutrophils, Grade 2 showed moderate amount of neutrophils, and neutrophils were commonly observed in Grade 3 [17].

Immunofluorescent Antibody (IFA) imaging: The presence of GFP-labeled MSCs in the wound region was shown with a fluorescent antibody microscope.

Infection Evaluation: Tissue samples were fragmented and cultured with agar, and colonies were quantitatively counted.

ELISA: TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF and Caspase-3 levels of blood samples were measured with ELISA and recorded.

Statistical analysis

SPSS 20.0 (SPSS; Chicago, IL, USA) software was used for statistical analysis. Continuous data were presented as mean (standard deviation) and non-normal categoric data were presented as median with 25-75% interval. The chi-square test was used to compare categoric variables. The Mann-Whitney U (MWU) and Kruskal Wallis tests (two-way comparisons of groups) were used to compare continuous data with non-normal distribution. $P<0.05$ was considered statistically significant.

Results

All groups were examined macroscopically first. MRSA colonization was observed in all rats in the positive control group (10/10; 4.7 $x10^9$CFU/mL), whereas no colonization was observed in the negative control group. In the VAN group, one (1/10) rat was colonized. Full eradication was achieved in DAP and TIG groups, and not achieved in 4 rats (4/10) in the MSC

treatment group. During the study, no animal showed clinical symptoms due to antibiotherapy or MSC.

Histopathological evaluation: Histopathological evaluation was performed semiquantitatively using a conventional microscope (Nikon Eclipse E600, Nikon AG Instruments, Switzerland). Cases were grouped as absent, mild, moderate and prominent, and scored with 0, 1, 2, and 3, respectively [17]. The final score was obtained by summing the obtained values.

First, sections were examined to see the distribution differences of inflammation using a x10 objective (4.9 mm^2). One section, where inflammation was observed homogenously, was chosen in each rat and evaluated for areas bordered with inflammatory cells, which showed intense inflammation. Following H&E staining of these intensely inflamed areas, all specimen were examined semiquantitatively by an experienced pathologist under 20x magnification (0.785 mm^2). All rats were then classified according to the above-mentioned scoring system.

H&E evaluation: No contamination was noted histopathologically and minimal inflammation was observed in Group 0. Group 1 and Group DAP showed significantly more severe inflammation compared to other groups. The histiocytic response and fibrosis parameters, used to measure the level of inflammation, were the highest among all groups. The findings observed in MSC, tigecycline and vancomycin groups were similar to each other: Histopathologic findings showed mild-to-moderate inflammation and edema. Significantly increased vascularity was observed in the MSC group. The increase in fibrosis was minimal in Group 0 and MSC groups.

IFA evaluation: GFP-labeled MSCs were localized at the site of surgery injected with IFA. Especially in the MSC group, there was no significant difference in the intensity of inflammation as noted above, but a marked increase in vascularity was observed where stem cells had concentrated.

Biochemical evaluation: TNF-α, TGF-β, IL-1, PDGF, FGF, VEGF and Caspase-3 levels, measured with ELISA, were markedly increased in Group 1 and Group DAP, consistently with histopathological evaluation. The increases in the other groups, particularly the MSC group, were less compared to these two. There was no increase in the negative control group.

ELISA: Along with histopathological assessment, level of inflammation markers were researched in blood and similar increases at moderate levels were observed in all antibiotherapy groups. There was no increase in Group 0. In terms of TGF-β, Group 0 and Group 1 were similar.

FGF, IL-1, TNFα, and PDGF levels of Group MSC were similar to all treatment groups and lower levels of Caspase-3 were detected. Comparisons between the groups for inflammation parameters measured with ELISA are comparatively shown in table 1. During the study, no side effects linked with antibiotherapy or MSC administration were observed in any of the animals. The multiple comparison of inflammation parameters and pathological evaluations of the groups is presented in table 2 and table 3, respectively.

Table 1: Comparison of inflammation parameters among the groups

Variables		n	Median	Minimum	Maximum	*P*-value
FGF (pg/mL)	Group-0	10	165.00	105.47	199.23	<0.001
	Group-1	10	330.68	249.52	602.46	
	Group-Van	10	374.34	218.58	532.51	
	Group-Dap	10	176.33	145.44	258.75	
	Group-Tig	10	205.50	133.50	778.30	
	Group-MSC	10	173.24	30.05	281.52	
TGF-β (pg/mL)	Group-0	10	49.08	18.59	70.42	0.010
	Group-1	10	87.19	40.44	177.14	
	Group-Van	10	107.26	49.21	137.50	
	Group-Dap	10	71.48	41.33	92.60	
	Group-Tig	10	67.37	51.33	91.78	
	Group-MSC	10	49.08	16.11	92.62	
IL-1 (pg/mL)	Group-0	10	22.66	12.84	32.48	<0.001
	Group-1	10	155.22	107.76	251.77	
	Group-Van	10	124.99	109.07	146.49	
	Group-Dap	10	61.44	25.94	73.22	
	Group-Tig	10	69.35	22.66	141.49	
	Group-MSC	10	71.90	24.90	237.30	
VEGF (pg/mL)	Group-0	10	204.64	79.91	259.14	<0.001
	Group-1	10	558.42	248.31	676.62	
	Group-Van	10	412.73	101.53	648.59	
	Group-Dap	10	353.75	268.95	518.98	
	Group-Tig	10	294.29	184.21	544.54	
	Group-MSC	10	254.69	41.71	418.81	
TNF-α (pg/mL)	Group-0	10	67.70	46.98	96.31	0.001
	Group-1	10	244.49	120.10	576.87	
	Group-Van	10	258.93	81.97	730.95	
	Group-Dap	10	190.66	37.02	318.43	
	Group-Tig	10	96.90	71.98	334.30	
	Group-MSC	10	204.97	120.10	929.27	
PDGF (pg/mL)	Group-0	10	4.22	2.15	6.14	<0.001
	Group-1	10	6.66	6.24	7.89	
	Group-Van	10	6.39	5.05	6.89	
	Group-Dap	10	6.54	5.40	7.09	
	Group-Tig	10	5.83	3.89	8.51	
	Group-MSC	10	5.90	4.33	6.99	
Caspase-3 (pg/mL)	Group-0	10	6.75	4.08	8.86	<0.001
	Group-1	10	12.63	11.24	13.74	
	Group-Van	10	12.32	10.66	18.54	
	Group-Dap	10	13.00	10.84	20.41	
	Group-Tig	10	11.92	10.56	14.64	
	Group-MSC	10	8.63	6.72	12.16	

P-values were determined by Kruskal-Wallis H test and $P<0.05$ was considered statistically significant.

Table 2: Multiple comparison of inflammation parameters (*P*-values)

FGF (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.096	0.028	0.650
Group-1		0.364	0.001*	0.059	0.001*
Group-Van			0.001*	0.049	0.001*
Group-Dap				0.256	0.545
Group-Tig					0.131
TGF-β (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.021	0.008	0.068	0.067	0.894
Group-1		0.880	0.174	0.111	0.023
Group-Van			0.070	0.072	0.008*
Group-Dap				0.935	0.307
Group-Tig					0.178
IL-1 (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.001*	0.003*	0.001*
Group-1		0.005*	0.001*	0.001*	0.023
Group-Van			0.001*	0.002*	0.104
Group-Dap				0.496	0.174
Group-Tig					0.406
VEGF (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.076	0.001*	0.010	0.248
Group-1		0.019	0.010	0.004*	0.001*
Group-Van			0.705	0.450	0.151
Group-Dap				0.257	0.049
Group-Tig					0.290
TNF-α (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.033	0.033	0.003*	0.001*
Group-1		0.791	0.186	0.003*	0.762
Group-Van			0.364	0.070	0.940
Group-Dap				0.130	0.427
Group-Tig					0.005
PDGF (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.001*	0.018	0.008*
Group-1		0.016	0.162	0.096	0.002*
Group-Van			0.520	0.545	0.151
Group-Dap				0.406	0.112
Group-Tig					0.940
Caspase-3 (pg/mL)	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.001*	0.001*	0.001*	0.021
Group-1		0.762	0.406	0.762	0.001*
Group-Van			0.705	0.406	0.001*
Group-Dap				0.001*	0.345
Group-Tig					0.002*

P-values were determined with the Mann-Whitney U test.* $P<0.010$, For post hoc multiple comparisons, statistical significance was assessed at $P<0.010$ levels for 6 groups.

Table 3: Pathological evaluations and comparison of groups (*P*-values)

Fibrosis	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.146	0.001*	0.146	0.146
Group-1		0.001*	0.010	0.001*	0.001*
Group-Van			0.001*	1.000	1.000
Group-Dap				0.001*	0.001*
Group-Tig					1.000
Histiocytic response	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.542	0.001*	0.342	0.615
Group-1		0.001*	0.055	0.003*	0.002*
Group-Van			0.001*	0.131	0.276
Group-Dap				0.001*	0.001*
Group-Tig					0.648
Vascularization	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.374	0.001*	0.615	0.648
Group-1		0.001*	0.021	0.001*	0.001*
Group-Van			0.001*	0.170	0.661
Group-Dap				0.001*	0.001*
Group-Tig					0.342
Granulocytic response	Group-1	Group-Van	Group-Dap	Group-Tig	Group-MSC
Group-0	0.001*	0.029	0.001*	0.004*	0.317
Group-1		0.001*	0.028	0.001*	0.001*
Group-Van			0.001*	0.383	0.131
Group-Dap				0.001*	0.001*
Group-Tig					0.022

P-values were determined with the Mann-Whitney U test.* $P<0.010$, For post hoc multiple comparisons, statistical significance was assessed at $P<0.010$ levels for 6 groups.

Discussion

We did not investigate whether MSC obtained from rat fatty tissue had any antibacterial effects on MRSA. Group 0 had no colonization and Group 1 had proliferation shown with CFU counts. The presence of GFP-labeled MSC in the administration area was shown with fluorescent microscopy. A variety of in vivo studies showed that MSC prevented bacterial sepsis and supported bacterial scavenging [3]. However, we unfortunately did not have the same level of success in our study of MRSA treatment in diabetic rats. During evaluation of this outcome, it should be kept in mind that DM makes wound healing particularly difficult [18]. Eradication was not achieved in 4 rats in the MSC group and 1 rat in the vancomycin group. In a study of Meisnel et al. [19], human MSC and MSC-released IFN-α and TNF-α were shown to successfully inhibit *S. Epidermis* proliferation. Contrary to this study, Guerra et al. [20] found that bone marrow-derived MSC ($1x10^6$ cells) did not inhibit the colony-forming capability of biofilm-related Staphylococcus. A mouse study by Qian et al. [21] showed that MSC exerted strong antibacterial and anti-inflammatory effects on S. aureus infection. Although the species of the subjects was different, the basic difference from our study is the diabetic condition of the rats, which further reduces the success rates for resistant strains. In our study, the more pronounced vascular structure formation in the MSC group shows that MSCs were effective at a cellular level, albeit insufficiently compared to antibiotherapy. One may conclude that MSC and antibiotherapy combination may achieve more effective infection control. Alcayaga-Miranda et al. [3] researched the synergistic interaction of antibiotherapy and MSC in an in vivo mouse model and reported that survival rates may increase while inflammation reduces. They strongly recommended combined treatment to prevent sepsis. In this study, diabetic rats were not used, which may have rendered more realistic results. Future studies should be planned to measure the success of combined treatment in a diabetic rat study. TGF-β was the only parameter which was similar in negative and positive control groups. It may be concluded that comparison of this parameter will not contribute to evaluation, at least in this setup. Apart from this parameter, the levels of all parameters measured with ELISA were similar among the treatment groups. MSC caused less histopathological fibrosis compared to all treatment groups, and we believe that similarity of results between Group MSC and antibiotherapy groups should be noted. All treatment groups were significantly different from the untreated positive control group (Group 1) in terms of inflammatory parameters and this is not associated with eradication only. Prevention of inflammation is thought to stem from the immunomodulatory effects of MSC. Additionally, while daptomycin is the strongest known antibiotic against MRSA, it increased all inflammation parameters, especially fibrosis. This may be due to a possible side effect apart from the healing process. The significantly lower fibrosis in the MSC group may be considered an additional positive effect achieved by limiting inflammation. In our current study, Caspase-3 levels were negative among all MSC groups with no similarity to the treatment groups. The systemic effects of MSC may have contributed to this result. In the literature, a variety of studies mention the anti-inflammatory or inflammation-limiting properties of MSC, whose response to pathogens is akin to that of the natural immune cells [22]. MSC migrate to the injury site and exert paracrine effects by secreting a range of soluble mediators, which simulate angiogenesis, remodeling and immune cell activation [23]. At the same time, they actively contribute to bactericidal activity [24]. This reaction initially begins with a series of reactions like receptor identification, signal conduction and specific inflammation. The immune process involves suppression of T cells, macrophage activation, neutrophil aggregation, collagen synthesis, fibroblast proliferation, platelet activation, fibrinolysis and angiogenesis regulation [25]. All these processes speed up infection healing and improve clinical status accompanying the wound healing process. In the literature, there are various studies similar to ours. Kong et al. [26] added MSC administration to linezolid treatment in a rabbit model of MRSA-induced pneumonia and showed that MSC-linezolid combination treatment was superior to linezolid treatment only. In addition, inflammatory markers such as IL-8, IL-6, TNF, CRP, and IL-10 were found to decrease dramatically in MSC-linezolid treated group of animals compared to the linezolid group. Extensive in vitro studies showed that MSCs can suppress proliferation of T and B cells by inhibiting cell division [27]. This immunomodulatory potential of MSCs is associated with cell-to-cell interactions and a number of soluble factors, such as NO in T cells (28). Although the systemic immunosuppressive characteristics of MSCs in humans and animals was reported in various disease models, these immunomodulatory effects have not been observed in vivo. Therefore, further studies in animals and humans are needed to evaluate the use of MSC as immunotherapy. Although this study was not as effective as the others suggesting MSCs may be an alternative to antibiotherapy, this is one of the first studies providing data on wound infection and antibacterial efficacy of MSC, especially in diabetic rats. The increased vascularity and tissue repair, more limited inflammation, and 60% (6/10 rats) eradication success in MSC-treated rats compared to other treatment options should not be overlooked. The results are associated with many variables, such as infection control, wound healing, inflammation regulation, and different processes in diabetic tissue. Data obtained in our study are considered to positively contribute to the final outcome about the use of MSC.

Limitations

This is an experimental preliminary study and was carried out in the laboratory. It is not a clinical trial. New clinical advanced phase studies are needed. We investigated the efficacy of stem cell administration for MRSA in diabetic rats only. The effect of mesenchymal stem cell administration on non-diabetic rats can be investigated. In addition, research on the effectiveness of mesenchymal stem cell administration on other pathogenic bacteria are needed.

Conclusion

Even though we did not achieve the same level of success with the other, similar studies investigating MSC treatment as a new alternative to antibiotherapy, it should be kept in mind that this study involved wound infection in diabetic rats. Inflammation was notably limited. This in vivo study of a rat infection model is a preliminary study and further, more comprehensive phase 1 and 2 studies to determine dosing and administration methods are needed.

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