EXPERIMENTAL STUDY

Effects of adipose tissue-derived mesenchymal stem cells and/or sildenafil citrate in experimental colon anastomosis model

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ABSTRACT

BACKGROUND: This study aimed to evaluate the healing effects of adipose tissue-derived mesenchymal stem cells (AT-MSC) and sildenafil citrate alone or in combination of colon anastomosis experimental model.

METHODS: A total of 40 female Wistar rats were randomly distributed to four groups: Control (without any intervention post-anastomosis), stem cell (AT-MSC injection on the anastomosis site), SIL (oral gavage of 10 mg/kg sildenafil citrate), and stem cell + SIL (AT-MSC injection and oral administration of sildenafil citrate) groups. Rats were euthanized 5 days post-anastomosis. Intra-abdominal adhesion status and anastomotic burst pressure were measured to assess anastomotic healing. Hydroxyproline and TNF-α level, neutrophil leukocyte infiltration, epithelial regeneration, and necrosis in the anastomosis tissue were examined.

RESULTS: Anastomosis leakage and anastomosis burst pressure were not different among the groups. Treatment with sildenafil, stem cell, and stem cell + SIL reduced the degree of perianastomotic adhesions compared to control ($p<0.05$). A significant increase was noted in hydroxyproline in the stem cell and stem cell + SIL groups ($p=0.001$). AT-MSC injection alone or in combination with sildenafil citrate reduced the TNF-α concentration at the anastomosis site ($p=0.001$). Histopathological examination revealed that all treatments enhanced the clearance of the necrotic debris, reduced leukocytes infiltration, and accelerated the retraction of anastomosed ends except control ($p=0.001$). Epithelial regeneration was more pronounced in the stem cell group than other groups ($p=0.001$). Macrophage density was lower in groups treated with the SIL or stem cell groups than the control and stem cell + SIL groups ($p=0.001$).

CONCLUSION: Sildenafil citrate and/or AT-MSC in the anastomosed rats promoted the anastomosis healing that was more pronounced in groups receiving stem cell injections.

Keywords: Anastomosis burst pressure; colon anastomosis; hydroxyproline; sildenafil citrate; stem cell; wound healing.

INTRODUCTION

Colorectal surgical interventions may be indicated for several reasons such as cancer, diverticulitis, or inflammatory bowel diseases.[1] As the surgical experience and the use of staplers increase, more surgeries end up with anastomoses instead of diverting stomas. However, anastomotic leaks (ALs) are still troublesome situations that could cause hazardous consequences.[2] All problems caused by AL results in impaired colon functionality and longer hospital stay that could even extend to death.[3–5] The incidence of AL ranges between 3 and 20%.[1] Those force clinicians to invest new probable techniques for reducing occurrence of AL.[6]

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Stem cell therapy promotes wound healing through immunomodulative, secretory, and trophic functions.[7,8] Past studies have shown promising efficacy of adipose tissue-derived mesenchymal stem cells (AT-MSCs) on healing various tissues, such as cardiac muscle, skin, and esophagus.[9–11] In colorectal anastomosis models, transplantation of AT-MSC through biosuture,[12] serosal injection,[13] or cell sheets[14,15] has shown complete failure in wound healing. Therefore, further studies are warranted to ascertain the precise outcomes of AT-MSC therapy in colorectal anastomosis models.

Anastomosis healing is affected by several factors including blood supply and tissue oxygenation.[16] Poor blood supply or inadequate vascularization may aggravate the risk of complications including AL.[17] Sildenafil promotes wound healing through increasing the tissue perfusion.[18] Many studies were done to investigate the sildenafil effect on wound healing process for colorectal anastomosis models; however, all of them were inconclusive.[19–22]

Because of this uncertainty in the literature, we conducted this study in rat models to evaluate definite effects of AT-MSC and sildenafil citrate either single or combination therapy on colorectal anastomosis.

MATERIALS AND METHODS

Primary End Point

There are several studies that advocate the preventive effects of AT-MSC injection or sildenafil citrate monotherapies. Hence, there is a possible cumulative effect on healing for combination of these two modularities. In this study, we want to evaluate whether the cumulative effect of AT-MSC and sildenafil citrate combination is better than monotherapies on anastomosis healing.

Animals and Experimental Design

All interventions, procedures, and practices were approved by the local ethical committee before the study through letter no. 2018/18. A total of 44 female Wistar albino rats (240–330 g) were maintained under conventional rearing conditions. Ad libitum feed and water were provided throughout the experiment. Four rats were used for claiming AT-MSC samples and excluded for group stratification.

Rats were randomly distributed into four groups (n=10). All rats in each group underwent end-to-end anastomosis on the descending colon following a full-thickness incision. One group left untreated after anastomosis and served as the control group. The second group (SIL group) received 10 mg/kg/day sildenafil citrate by oral gavage starting 2 days before colon anastomosis until the end of experiment. AT-MSC was injected at the proximal and distal ends of anastomosed colon immediately after the anastomosis before suturing the laparotomy site for the third group (stem cell group). The fourth group (stem cell + SIL group) received combined therapy. Sildenafil and AT-MSC in the stem cell + SIL group were administered the same way as in the SIL and stem cell groups. The duration of the experiment was 5 days. At the end of experiment, all the rats were euthanized by cervical dislocation under general anesthesia.

AT-MSC Culture Preparation and Characterization

Stem cells were prepared from the adipose tissue of four rats (exclusive of the study groups) according to the method outlined by Zhu et al.[23] followed by identification using flow cytometer (BD FACSCalibur, Becton Dickinson, NJ, US) and marking with 1,1′-dioctadecyl-3,3,3′, 3′-tetramethylindocarbocyanine perchlorate.

End-to-end Anastomosis of Descending Colon

The rats were subjected to 12 h fasting before the surgical intervention. Following a midline incision under general anesthesia, end-to-end anastomosis of descending colon was conducted. Each rat in the stem cell and stem cell + SIL groups received 0.1 ml AT-MSC (4×10^5 AT-MSC per 0.1 ml) injections into the proximal and distal ends of anastomosed colon. The laparotomy incisions were closed with 3/0 silk.

Macroscopic Observations

Following euthanasia of the rats, colon segments were divided in 2 cm length from each proximal and distal ends of anastomosis. Intra-abdominal adhesions were evaluated by visual scoring according to Houston and Rotstein.[24] Anastomosis burst pressure was measured according to the method described by Pehlivanlı et al.[25] The excised colon was longitudinally cut into two parts. One portion was fixed in 10% neutral buffered formalin for 24–48 h; other portion was stored at −80°C for further biochemical analysis.

Biochemical Analysis

Colon tissues were homogenized; hydroxyproline and TNF-α concentrations were measured in each sample through the method of Reddy and Enwemeka[26] and ELISA (Cat No: E-EK-R0019, Elabscience, TX, US), respectively.

Histopathology

Fixed colon tissues were passed through graded alcohols, cleared in xylene, embedded in paraffin, sectioned in 5 µm thickness, and stained with hematoxylin and eosin (H&E). The sections were evaluated under a light microscope (B × 51, Olympus, Japan). Histopathological changes at the anastomosis site were scored in terms of neutrophil leukocyte infiltration, epithelial regeneration, and necrosis of mucosal layer according to the method described by Irkorucu et al.[21] with minor modification for epithelial regeneration. Necrosis of mucosal layer and neutrophil leukocyte infiltration was scored as (-) absence, (+) mild, (++) moderate, and (+++) severe. Epithelial regeneration was scored as 0 = no

epithelization; 1 = coverage of granulation tissue by a single layer of epithelial cells at wound edges; 2 = nearly complete to complete coverage of granulation tissue by a single layer of epithelial cells; and 3 = complete epithelial regeneration with glandular epithelium. The gap between the mucosal edges of the anastomosis site was also measured. The H&E sections were examined under the 40× objective. The measurement was performed for three sections and the average was calculated. Identification of macrophages was ensured by staining the colon sections with macrophage-specific Iba1 antibodies using the immunohistochemical method to avoid confusion between fibroblast and endothelial cells. The details are given in the immunohistochemical staining section. Collagen accumulation at the anastomosis site was evaluated using a more specific hydroxyproline test instead of H&E staining.

Immunohistochemistry

The colon sections were stained with macrophage-specific Iba1 primary antibody using a polymer-based detection method. Following initial preparation, sections were incubated with the primary antibody against Iba1 (Wako, 013-27691, Japan) at a dilution of 1:2000 for 60 min at room temperature. Subsequently, sections were incubated with Rabbit-on-Rodent HRP-Polymer (Catalog #RC542, Biocare, USA) for 60 min at room temperature. The peroxidase reaction was carried out with 3,3-diaminobenzidine tetrahydrochloride chromogen (Thermo Fisher Scientific, US) for 5 min. All sections were systematically evaluated under 20× objective. The Iba1-positive cells were counted at the anastomotic site (five fields analyzed per sample) and average was calculated.

Detection of Stem Cells Labeled with DIL

Localization of stem cells in the anastomosed site was evaluated in the stem cell and stem cell + SIL groups by sectioning (5 µm thickness) the colon samples using a cryomicrotome. Sections were washed with Tris buffer saline-Tween 20, treated with DAPI solution for nuclear staining, mounted with Immuno-Mount, and examined under an immunofluorescence microscope (Nikon Eclipse E600) at 565 nm wavelength.

Statistical Analysis

All statistical data were analyzed with SPSS software (Statistical Product and Service Solutions; version 25.0, IBM Corp., Armonk, NY, US). Normality of data and homogeneity of variance was tested using Kolmogorov-Smirnov test and Levene's test, respectively. One-way ANOVA with Tukey's HSD post hoc test was applied to analyze the effect of treatments. Categorical data were analyzed with Fisher's exact test and Chi-square test. In cases where the expected frequencies were less than 20%, Monte Carlo simulation method was adopted to include these frequencies in the analysis. Differences among means and frequencies were assumed significant at 95% probability. Results were presented as mean±standard deviation and frequencies in case of categorical data.

RESULTS

Anastomosis Leakage, Perianastomotic Adhesions, and Anastomosis Burst Pressure

Table 1 shows the AL, perianastomotic adhesions, and anastomosis burst pressure in rats. The prevalence of ALs was not significantly different between groups. Severity of perianastomotic adhesions decreased in all groups in comparison with the control group (p=0.043). Sildenafil citrate and stem cell alone or in combination had no effect on the anastomosis burst pressure in rats.

Biochemical Findings

Hydroxyproline and TNF-α levels of anastomosed colon tissues are shown in Table 2. Tissue hydroxyproline concentration was greater in stem cell administered groups (stem cell and stem cell + SIL) than other groups (p=0.001). TNF-α concentrations were lower in groups injected with AT-MSC (stem cell and stem cell + SIL) (p=0.001).

Assessment of Therapeutic Benefit of Treatments on Colonic Healing Using Histological Criteria and Immunohistochemistry

Effects of treatments on colonic anastomosis healing are pre-

Table 1. Frequency of anastomosis leakage and perianastomotic adhesions, and anastomosis burst pressure (mm/Hg) in anastomosed colon tissues of rats treated with sildenafil, adipose tissue derived stem cells alone or in combination

Item		Control	SIL	Stem cell	Stem cell + SIL	p-value
Anastomosis leakage	Absent	8	9	10	10	0.60
	Present	2	1	0	0	
Perianastomotic adhesions	Mild	0[a]	4[b]	6[b]	4[b]	0.043
	Moderate	4	2	2	5	
	Severe	6[b]	4[a,b]	2[a,b]	1[a]	
Anastomosis burst pressure		144.2±50.9	125.6±34.7	139.8±38.5	153.9±40	0.52

[a, b]Frequencies with different superscripts within the same row are significantly different. SIL: Sildenafil citrate.

Table 2. Hydroxyproline (μg/mg wet tissue) and TNF-α (pg/g wet tissue) levels in anastomosed colon tissues of rats treated with sildenafil, adipose tissue derived stem cells alone or in combination

Item	Control	SIL	Stem cell	Stem cell + SIL	p-value
Hydroxyproline	0.54 (0.42–0.56)[a]	0.54 (0.50–0.56)[a]	0.69 (0.61–1.10)[b]	0.69 (0.63–0.76)[b]	0.001
TNF-α	487±55.25[b]	479.33±68.73[b]	320.67±56.24[a]	357.67±68.6[a]	0.001

[a, b]Means with different superscripts within the same row are significantly different. SIL: Sildenafil citrate.

Table 3. Histopathological findings in anastomosed colon tissues of rats treated with sildenafil, adipose tissue derived stem cells alone or in combination

Item		Control	SIL	Stem cell	Stem cell + SIL	p-value
Epithelial regeneration	Absent	3	0	0	0	0.001
	Mild	7[a,b,c]	10[c]	4[b]	10[a,c]	
	Moderate	0[a]	0[a]	6[b]	0[a]	
Neutrophil leukocyte infiltration	Mild	0[a]	0[a]	6[b]	3[a,b]	0.001
	Moderate	0[a]	4[b]	4[b]	7[b]	
	Severe	10[a]	6[b]	0[c]	0[c]	
Necrosis	Mild	0[a]	0[a]	6[b]	2[a,b]	0.001
	Moderate	0[a]	6[b]	4[b]	8[b]	
	Severe	10[a]	4[b]	0[c]	0[c]	

[a,b,c]Frequencies with different superscripts within the same row are significantly different. SIL: Sildenafil citrate.

Table 4. Histomorphometry and immunohistochemistry of Iba1 of anastomosed colon tissues of rats treated with sildenafil, adipose tissue derived stem cells alone or in combination

Item	Control	SIL	Stem cell	Stem cell + SIL	p-value
Macrophage infiltration[1]	414.9±8.62[a]	385.5±8.09[b]	343.5±6.35[b]	425±9.19[a]	0.001
Gap between anastomosed ends (μm)	1092.9±91.64[a]	433±40.14[b]	159.7±38.65[c]	382.5±18.4[b]	0.001

[a,b,c]Means with different superscripts within the same row are significantly different. SIL: Sildenafil citrate. [1]All sections were systematically evaluated under 20X objective. The Iba1-positive cells were counted at the anastomotic site (5 fields analyzed per sample) and average was calculated.

sented in Table 3, and representative photomicrographs of HE-stained colon sections are shown in Figure 1.

Mucosal necrosis was seen more in the control group than in the other groups. The removal of the necrotic debris was significantly accelerated in the stem cell and stem cell + SIL groups. Intense neutrophil leukocyte infiltration in the anastomosis region was noted in the control group. Application of SIL, stem cell, or stem cell + SIL caused reduction in leukocytic infiltration. We notice a single layer of regenerated epithelium evolving from the intact epithelium extending toward the granulation tissue surface. Only stem cell treatment expedited the epithelial regeneration evident from the nearly complete coverage of the granulation tissue by a single layer of epithelium in six rats.

Macrophage infiltration and gap between anastomosed ends are presented in Table 4. The width between anastomotic ends was higher in the control group than any other group, whereas the stem cell group had narrower ends than the SIL and stem cell + SIL groups (p=0.001). A significant decrease in macrophage infiltration occurred in the stem cell and SIL groups compared with the control and stem cell + SIL groups (p=0.001). However, there was no significant difference for macrophage infiltration which was not different between the control and stem cell + SIL groups (Fig. 2).

DISCUSSION

As ALs cause many difficulties, clinicians are forced to discover alternative solutions to reduce the risk of leak occurrence. That is probably the reason for many clinical trials about this topic. However, at the time we plan for our study, this is the unique report about application of AT-MSC and sildenafil citrate in combination to evaluate the healing process of anastomosis. There are some other studies about positive effects

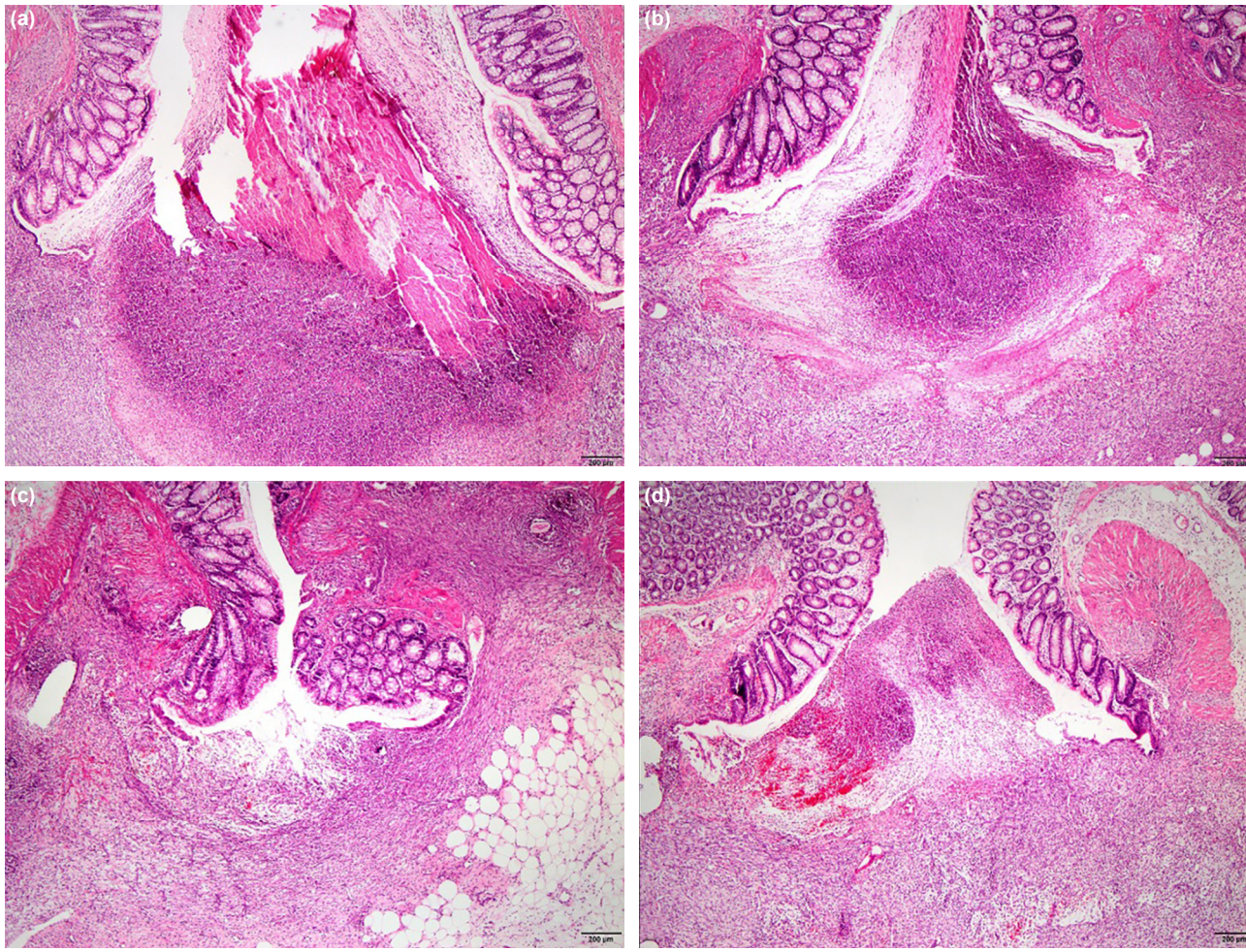

Figure 1. Effect of adipose tissue-derived mesenchymal stem cells and sildenafil (SIL) alone or in combination on the colorectal anastomosis healing in rats, H&E staining. **(a)** Control group; complete loss of mucosa replaced by a coagulum of eosinophilic debris in addition to a thick zone of inflammatory infiltrate primarily of neutrophils along the base of necrotic debris as well as a thick layer of early granulation tissue nearby the inflammatory zone. **(b)** SIL group; histological features similar to that of the control group, however, with lesser necrotic debris and inflammatory infiltrates, and a single layer of epithelium extending towards the surface of granulation tissue. **(c)** Stem cell group; anastomosis area almost cleared from the necrotic debris along with a nearly complete covering of granulation tissue by a single layer of epithelium, and diminution of gap between the wound edges. **(d)** Stem cell + SIL group; necrotic debris and neutrophil leukocyte infiltration seemingly lesser than the SIL group.

of monotherapies. As we present the combination of AT-MSC and sildenafil citrate reduced the severity of perianastomotic adhesions, Pascual et al.[12] and Morgan et al.[14] reported a decrease in perianastomotic adhesions with the application of AT-MSC therapy, while Ayten et al.[19] advocated the administration of sildenafil reduced perianastomotic adhesion after colon anastomosis in case of intra-abdominal infection. In contrast, some other studies had showed that failure of stem cell injection at the anastomosis site had no effect on prevention of perianastomotic adhesions.[13–15] Similarly, sildenafil at a dosage of 10 or 20 mg/kg failed to reduce the perianastomotic adhesions in rat models of ischemic colon anastomosis.[21] Moreover, no effect of stem cell and sildenafil citrate application alone or in combination was noted on the occurrence prevention of AL occurrence. Similar results were also reported by the researchers who administered stem cells[13] and sildenafil.[21] However, some other studies reported a decrease in AL due to the application of stem cell therapy in anastomosis models.[14,15] As we consider anastomosis burst pressure; despite some discrete findings in the literature,[13,20] we found no significant difference between groups. In fact, the anastomosis burst pressure remained unaffected in the models receiving stem cell therapy or sildenafil.[12,14,15,20,21] Reasons that cause these differences could be counted as follows; differences in the animal-related parameters, the models used (such as simple end-to-end anastomosis, ischemic, or infectious models), anastomosis procedure (such as 4, 5, or 8 primary sutures), the application method of stem cells (such as biosutures, cell sheet, or serosal injection), as well as the route and dosage of sildenafil citrate. Despite elevated hydroxyproline levels in the stem cell groups (stem cell and stem cell + SIL groups), the anastomosis burst pressure remained unaffected. The hydroxyproline level indicated that the amount of total collagen may not contribute significantly

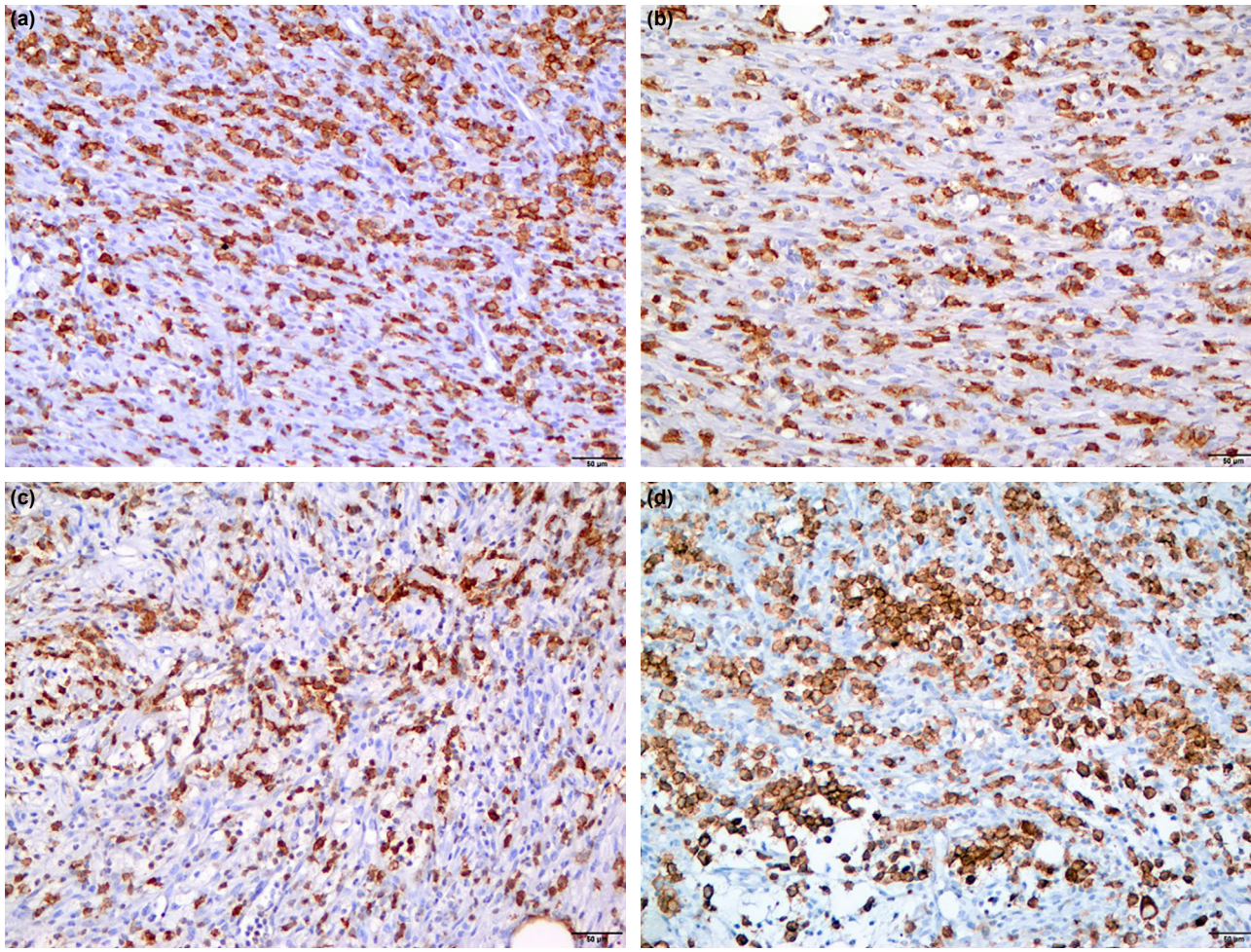

Figure 2. A comparison of macrophage density at the anastomosis site in rats receiving adipose tissue-derived mesenchymal stem cells injection and sildenafil (SIL) alone or in combination. Representative photomicrographs of polymer-based immunohistochemical staining using pan-macrophage marker Iba1. **(a)** Control group, **(b)** SIL group, **(c)** stem cell group, and **(d)** stem cell + SIL group.

to the tensile strength at the anastomosis site in comparison with that to the construction and arrangement of collagen matrix.[27–29]

In the present study, the administration of sildenafil citrate or stem alone or in combination decreased the degree of neutrophil infiltration when compared with the control group. The decrease was more obvious in the stem cell and stem cell + SIL groups. Similar results were also reported in response to the AT-MSC therapy[13] and with treatment with 20 mg/kg sildenafil.[21] Similarly, Alvarenga et al.[30] reported that AT-MSC therapy decreased the neutrophil infiltration in high-risk anastomosis model, which was evident from the lowered myeloperoxidase activity at the anastomosed site. In contrast, sildenafil showed no effect on neutrophil infiltration in incomplete anastomosis model (4-primary sutures), although the neutrophil infiltration was diminished in the complete anastomosis model (8-primary suture).[20] This difference in the results may be attributed to the difference in the anastomosis models. TNF-α levels, a pro-inflammatory cytokine, were lower in the stem cell and stem cell + SIL groups. Likewise, Fu et al.[31] described that AT-MSC injection reduced the TNF-α levels in rat models with colitis and Morgan et al.[14] reported that AT-MSC cell sheet application downregulated the TNF-α mRNA expression induced by the carrier (absorbable gelatin sponge) at the anastomosed site. Similar results were also reported by Alvarenga et al.[30] in a high-risk anastomosis model. These results thus confirmed the anti-inflammatory effects of stem cells. Tissue injury attracts the inflammatory cells that accomplish the infiltration of neutrophils as first line of defense. These cells initiate the removal of foreign particles, bacteria, and necrotic tissues through the production of oxy-radicals and proteases during phagocytosis. The presence of foreign particles, bacteria, or necrotic tissues for a prolonged duration may result in the persistence of inflammatory response that may damage the adjacent tissues. Sildenafil possesses wound-healing properties that are contributed by limiting the inflammatory process, which was also evident from the lower degree of neutrophil leukocyte infiltration in our study. However, no difference in the TNF-α level was noted between the control and SIL groups in this study. Raposo et al.[32] demonstrated that sildenafil citrate downregulated the

mRNA expression of IL-1β and IFN-γ; however, it did not affect the TNF-α mRNA expression. Thus, sildenafil citrate possibly causes an indirect anti-inflammatory effect through lowering the pro-inflammatory cytokine levels at the anastomosis site, which, in turn, expedited the process of wound healing. The effectiveness of sildenafil citrate application was further increased after combined with stem cells.

In an inflammatory response, neutrophil infiltration is followed by macrophage infiltration, which has pro-inflammatory profile, namely, M1 macrophage. These macrophages conduct phagocytotic activities and transform into macrophages with anti-inflammatory (M2) characteristics during the late inflammatory phase. In addition, M2 macrophages activate fibroblast proliferation, angiogenesis, and re-epithelization.[5] In this present study, macrophage infiltration was recorded to be lower in groups administered with stem cells or sildenafil citrate when compared with the control and stem cell + SIL groups. However, macrophage infiltration was found to be numerically greater in the stem cell + SIL group than in the control group. According to some previous studies, AT-MSC injection increases the infiltration of anti-inflammatory M2 macrophages in experimental anastomosis model.[15,33,34] The lowering of macrophage infiltration in the stem cell group may be attributed to the indistinct identification of M1 and M2 macrophages due to the pan-macrophage marker Iba1. Similarly, sildenafil citrate was reported to promote the polarization of macrophages from M1 to M2.[35] It can thus be speculated that sildenafil may have increased the polarization of M1 macrophages to M2 macrophages in the present study, which was reflected in the declining macrophage infiltration in the SIL group. Moreover, an increased macrophage infiltration in the stem cell + SIL group can be attributed to the synergism of the polarization effect of stem cells and sildenafil citrate.

Following inflammation, the proliferative phase of wound healing process is initiated. Fibroblasts relocate to the wound site and synthesize the collagen during this phase so as to restore the tissue defect.[5] In this study, the concentration of hydroxyproline, which is an integral part of the collagen protein,[36] was assessed in the anastomosed colorectal tissues. Our results revealed that the AT-MSC therapy (stem and stem cell + SIL groups) had greater hydroxyproline concentration than the control and SIL groups. Similar results were also reported previously using sildenafil[20,21] and AT-MSC therapy[13,14,37] in anastomosis models. Our results thus confirmed the effects of sildenafil and stem cells. AT-MSC possesses the ability to promote the release of FGF2, which stimulates the fibroblasts and, in turn, increases the collagen synthesis.[37] Epithelial regeneration is another event of the proliferative phase of the wound-healing process. In our study, a mild degree of epithelial regeneration was recorded in sildenafil-administered rats (SIL and stem cell + SIL groups). However, the rats receiving stem cells only showed relatively greater degree of epithelization. In addition, epithelization was completely absent in three of the 10 rats in the control group. Cakir et al.[20] reported that the administration of sildenafil increased the epithelial regeneration at the anastomosis site in incomplete anastomosed rats. Sildenafil stimulates the nitric oxide (NO) activity, which is the regulator of angiogenesis, inflammatory response, and epithelial regeneration. NO, in turn, promotes the release of various cytokines and growth factors that regulate the epithelial regeneration.[38] Therefore, in the present study, sildenafil citrate was recorded to initiate the epithelization, resulting in a mild degree of epithelization at the anastomosis site. In this study, negligible or a minute quantity of DIL-labeled stem cells was seen in AT-MSC injected groups. Therefore, we believe that AT-MSC may have stimulated the epithelial regeneration due to the increased secretory growth factors from the endogenous cells or by themselves.[34] In this study, the administration of sildenafil alone or in combination with AT-MSC injection decreased the gap between the anastomosed ends, which decreased even further in rats injected with AT-MSC alone. The reduction in the gap between the anastomosed ends may be attributed to the increased number of myofibroblasts that facilitated wound contraction by bridging the wound edges.[39]

Conclusion

The study revealed that AT-MSC therapy may promote the anastomosis wound healing by increasing the resolution of inflammatory response, stimulating the collagen synthesis, enhancing the re-epithelization, and by reducing the post-operative adhesions with the adjoining tissues. Although, the administration of sildenafil resolved the inflammatory response at a faster pace than the control group, the effectiveness was lower in comparison with the stem cell groups. Moreover, a combined use of AT-MSC injection and sildenafil did not enhance the effectiveness of each other to a distinct extent.

Ethics Committee Approval: This study was approved by the Adnan Menderes University Animal Experiment Ethics Committee (Date: 30.01.2018, Decision No: 2018/018).

Peer-review: Internally peer-reviewed.

Authorship Contributions: Concept: M.D., E.M.Y., M.H.Ç.; Design: E.M.Y., R.T., Ç.Y., A.E.D.; Supervision: M.H.Ç., A.E.D.; Resource: Ç.Y., E.M.Y., R.T.; Materials: Ç.Y., R.T., A.E.D.; Data: M.D., E.İ.; Analysis: M.H.Ç., A.E.D.; Literature search: M.D.; Writing: M.D., E.İ.; Critical revision: M.D., E.İ., E.M.Y.

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DENEYSEL ÇALIŞMA - *ÖZ*

Deneysel kolon anastomozu modelinde adipoz kökenli mezenkimal kök hücrelerin ve/veya sildenafil sitratın etkileri

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AMAÇ: Bu çalışmada, adipoz kökenli mezenkimal kök hücrelerin (AT-MSC) ve sildenafil sitratın tek başına veya kombine edilerek deneysel kolon anastomozu modeline iyileştirici etkilerinin değerlendirilmesi amaçlandı.

GEREÇ VE YÖNTEM: Toplam 40 dişi Wistar cinsi sıçan rastgele dört gruba dağıtıldı: Kontrol grubu (anastomoz esnasında ve sonrasında herhangi bir müdahale olmayan), kök hücre (anastomoz bölgesine AT-MSC enjeksiyonu yapılan), sildenafil sitrat (10 mg/kg sildenafil sitratın oral gavajı uygulanan) ve kök hücre + sildenafil sitrat (AT-MSC enjeksiyonu ve sildenafil sitratın oral uygulaması) grubu. Tüm sıçanlar anastomoz sonrası beşinci gün sakrifiye edildi. Anastomoz iyileşmesini değerlendirmek için, karın içi yapışıklık durumu ve anastomoz patlama basıncı ölçüldü. Doku örneklerinde hidroksiprolin ve TNF-α düzeyi, nötrofil lökosit infiltrasyonu, epitel rejenerasyonu ve nekroz miktarı incelendi.

BULGULAR: Gruplar arasında anastomoz kaçağı ve anastomoz patlama basıncı ölçümlerinde anlamlı fark yoktu. Sildenafil, kök hücre ve kök hücre + sildenafil ile tedavi, kontrol ile karşılaştırıldığında perianastomotik adezyonların derecesini azaltmıştır ($p<0.05$). Kök hücre ve kök hücre + sildenafil gruplarında hidroksiprolin düzeyinde önemli bir artış kaydedildi ($p=0.001$). AT-MSC enjeksiyonu tek başına veya sildenafil sitrat ile kombinasyon halinde anastomoz bölgesinde TNF-α konsantrasyonunu düşürmüştür ($p=0.001$). Histopatolojik inceleme, kontrol grubu ile karşılaştırıldığında sildenafil ve kök hücre uygulamalarının nekrotik dokuların temizlenmesini arttırdığını, lökosit infiltrasyonunu azalttığını ve anastomoz uçlarının birleşimini hızlandırdığını ortaya koydu ($p=0.001$). Epitel rejenerasyonu, kök hücre grubunda diğer gruplara göre daha belirgindi ($p=0.001$). Makrofaj yoğunluğu, sildenafil veya kök hücre grupları ile tedavi edilen gruplarda kontrol ve kök hücre + sildenafil gruplarına göre daha düşüktü ($p=0.001$).

TARTIŞMA: Kolon anastomozu yapılan sıçanlarda sildenafil sitrat ve/veya AT-MSC uygulamaları, özellikle kök hücre uygulanan gruplarda daha belirgin olacak şekilde anastomoz iyileşmesine katkı sağladı.

Anahtar sözcükler: Anastomoz patlama basıncı; hidroksiprolin; kolon anastomozu; kök hücre, sildenafil sitrat, yara iyileşmesi.

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