

THE EFFECTS OF MESENCHYMAL STEM CELL THERAPY ON COLON ANASTOMOSIS IN RATS SUBJECTED TO RADIOTHERAPY

Radyoterapi Almış Ratlarda Mezenkimal Kök Hücre Tedavisinin Kolon Anastomozuna Etkileri

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ABSTRACT

ÖZ

Objective: Colorectal cancer is the third most common malignancy worldwide. Although neoadjuvant radiotherapy (nRT) improves local control in rectal cancer, it increases the risk of anastomotic leakage. Mesenchymal stem cells (MSCs) have been shown to support wound healing. This study aimed to evaluate the effects of MSC therapy on anastomotic healing after nRT in a rat model.

Material and Methods: Fifty-five Wistar albino rats were allocated into four groups: Control, nRT, nRT + local MSCs, and nRT + local + intraperitoneal MSCs. A single 12 Gy dose of radiotherapy was administered preoperatively to the nRT groups. Each rat received a total of approximately 1×10^6 adipose-derived MSCs in 0.1 mL; in the local MSC group, the entire dose was administered subserosally to the anastomotic site, whereas in the combined group, half of the dose was administered locally and the remaining half intraperitoneally. Anastomotic healing was assessed using burst pressure (BP), histopathological evaluation, and biochemical analyses.

Results: Rats receiving nRT demonstrated significant weight loss and lower BP values. In the MSC-treated groups, BP values were higher compared with the nRT-only group, although these differences did not reach statistical significance. Histopathological and biochemical parameters did not differ significantly among the groups. Notably, gross anastomotic separation was observed only in the nRT-only group, whereas no such separation was detected in the MSC-treated groups. Fluorescent imaging confirmed the presence of differentiated MSCs at the anastomotic site.

Conclusion: Although MSC therapy did not result in statistically significant improvements in biochemical or histopathological parameters, the absence of gross anastomotic separation and the tendency toward higher burst pressure values suggest that MSCs may support local anastomotic healing after neoadjuvant radiotherapy. These findings indicate a potential role for MSC therapy as an adjunctive strategy, warranting further experimental and clinical investigation.

Amaç: Kolorektal kanserler dünya genelinde en sık görülen üçüncü malignitedir. Rektum kanserinde neoadjuvan radyoterapi (nRT) lokal kontrolü artırmakla birlikte anastomoz kaçığı riskini yükseltmektedir. Mezenkimal kök hücrelerin (MKH) yara iyileşmesini destekleyici etkileri olduğu bilinmektedir. Bu çalışmada, nRT sonrası sıçanlarda kolon anastomoz iyileşmesi üzerine MKH tedavisinin etkilerinin değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Elli beş Wistar albino sıçan dört gruba ayrıldı: kontrol, nRT, nRT + lokal MKH ve nRT + lokal + intraperitoneal MKH. nRT gruplarına ameliyat öncesinde tek doz 12 Gy radyoterapi uygulandı. Her bir sıçana anastomoz hattına lokal olarak yaklaşık 1×10^6 adipoz doku kökenli mezenkimal kök hücre 0,1 mL hacimde uygulandı; nRT + lokal MKH grubunda dozun tamamı anastomoz hattına lokal olarak verilirken, nRT + lokal + intraperitoneal MKH grubunda dozun yarısı lokal, kalan yarısı intraperitoneal olarak uygulandı. Anastomoz iyileşmesi barsak patlama basıncı (BPB), histopatolojik ve biyokimyasal incelemelerle değerlendirildi.

Bulgular: Radyoterapi uygulanan gruplarda anlamlı kilo kaybı ve daha düşük BPB değerleri izlendi. Kök hücre uygulanan gruplarda BPB değerleri nRT grubuna kıyasla daha yüksek olmakla birlikte bu fark istatistiksel olarak anlamlı değildi. Histopatolojik ve biyokimyasal parametreler açısından gruplar arasında anlamlı fark saptanmadı. Ancak yalnızca nRT grubunda anastomoz hattında makroskopik ayrışma gözlenirken, kök hücre uygulanan gruplarda makroskopik ayrışma izlenmedi. İmmunofloresan incelemede, anastomoz hattında farklılaşmış kök hücrelerin varlığı gösterildi.

Sonuç: Mezenkimal kök hücre tedavisi, biyokimyasal ve histopatolojik parametrelerde istatistiksel olarak anlamlı bir iyileşme sağlamamakla birlikte, makroskopik anastomoz ayrışmasının görülmemesi ve daha yüksek patlama basıncı eğilimi göstermesi nedeniyle, neoadjuvan radyoterapi sonrası lokal anastomoz iyileşmesini destekleyici potansiyel bir etkiye sahip olabilir. Bu bulgular, mezenkimal kök hücrelerin adjuvan bir tedavi yaklaşımı olarak ileri deneysel ve klinik çalışmalarla değerlendirilmesi gerektiğini düşündürmektedir.

Keywords: Colorectal neoplasms, radiotherapy, anastomotic leak, mesenchymal stem cells, wound healing

Anahtar Kelimeler: Kolorektal neoplaziler, radyoterapi, anastomoz kaçığı, mezenkimal kök hücreler, yara iyileşmesi



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INTRODUCTION

Colorectal cancer is the third most common malignancy worldwide, and according to the 2020 data of the Turkish Statistical Institute, it represents a similar incidence in Türkiye, accounting for approximately 9.1% of all cancers. The biological behavior of colorectal tumors may vary according to their anatomical localization within the colon and rectum. Compared with colon tumors, rectal cancers exhibit a higher incidence of lymph node metastasis and are associated with increased postoperative complications, including anastomotic leakage. Therefore, colon and rectal cancers are often evaluated as distinct clinical entities.¹⁻⁴

Neoadjuvant radiotherapy (nRT) administered before rectal cancer surgery has been shown to improve local control and contribute to disease-free survival.⁵ However, the risk of anastomotic leakage after colorectal surgery is influenced not only by patient-related factors such as age, sex, obesity, hypertension, and diabetes mellitus, but also by tumor stage, neoadjuvant treatment, surgical technique, and the type of reconstruction.⁶ Reported anastomotic leakage rates range from 2.4% to 20.6%, and this rate may increase to as high as 69% following neoadjuvant chemoradiotherapy.⁶ Anastomotic leakage is associated with substantial morbidity (20-30%) and mortality (7-12%), highlighting its clinical significance.⁷

Short-course radiotherapy may induce acute toxicity and excessive fibrosis, thereby impairing wound healing.^{8,9} The release of transforming growth factor- β (TGF- β), vascular endothelial growth factor (VEGF), tumor necrosis factor- α (TNF- α), and proinflammatory cytokines such as interleukin-1 (IL-1) and interleukin-8 (IL-8) plays a critical role in radiation-induced tissue injury and delayed healing.^{10,11}

First described in the 1960s, stem cells are undifferentiated progenitor cells capable of self-renewal and differentiation. Adult-derived stem cells, which can be isolated from various tissues, have demonstrated the ability to enhance wound healing through paracrine effects, immunomodulation, and differentiation into tissue-specific cell types.¹¹⁻¹³ These properties suggest a potential role for stem cell therapy in improving anastomotic healing under compromised conditions such as radiotherapy.

In this study, we aimed to investigate the effects of mesenchymal stem cell therapy on anastomotic safety and to evaluate its potential to mitigate the adverse effects of neoadjuvant radiotherapy on colonic anastomotic healing in a rat model.

MATERIALS AND METHODS

A total of 55 male Wistar albino rats, weighing 350-500 g, were used. Seven rats were reserved for stem cell

harvesting, and the remaining 48 rats were randomly allocated into four equal groups (control, radiotherapy, radiotherapy + local stem cells, and radiotherapy + local and systemic stem cells). Each group (n=12) was equally subdivided for sacrifice on postoperative day 3 (n=6) and day 7 (n=6). Throughout the experiment, all rats were maintained under standard laboratory conditions and fed identically.

In each group, following a midline laparotomy, a colon segment was resected at the rectosigmoid junction and end-to-end anastomosis was performed. Radiotherapy was administered to groups 2, 3, and 4 as a single 12 Gy fraction, and stem cell injection was applied to the anastomotic site in groups 3 and 4 (Figure 1). In group 4, systemic stem cell therapy was additionally administered via intraperitoneal injection. In groups 3 and 4, a total of 1×10^6 mesenchymal stem cells were administered per rat. In group 3, the entire dose was injected locally into the proximal and distal edges of the anastomotic line, whereas in group 4, half of the dose was administered locally and the remaining half was delivered intraperitoneally. Rats from each group were sacrificed on postoperative days 3 and 7, and tissue samples were collected for histopathological and biochemical analyses.

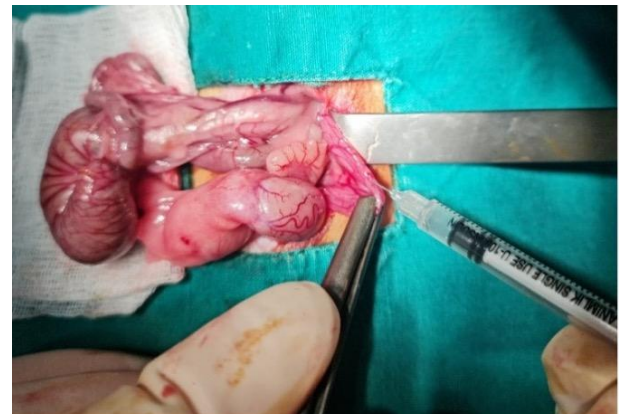


Figure 1: Local stem cell injection to the proximal and distal edges of the anastomotic line via subserosal administration.

Radiotherapy was performed at Başkent University Hospital using a 6 MV linear accelerator (Elekta Versa HD, Elekta AB, Stockholm, Sweden) (Figure 2). During radiotherapy, rats were placed in the supine position and immobilized to minimize movement. The irradiation field was carefully planned to target the pelvic region, including the rectosigmoid colon, while minimizing exposure to surrounding abdominal organs. The radiation field was collimated, and the remaining body parts were shielded using lead blocks to reduce unintended radiation exposure. After anastomosis, colonic burst pressure (BP) was measured, while hydroxyproline (Hyp) and matrix metalloproteinase-2

(MMP-2) levels were determined using the enzyme-linked immunosorbent assay (ELISA) method. Histopathological examinations were conducted in a blinded manner, assessing acute and chronic enteritis parameters.

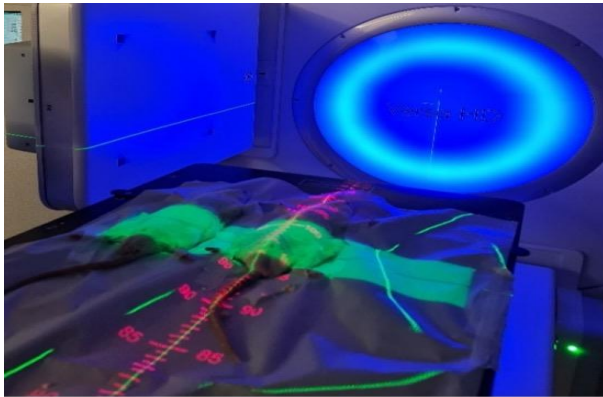


Figure 2: Rats undergoing radiotherapy.

Stem cells were obtained from rat adipose tissue, digested with collagenase, and centrifuged. The isolated cells were labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) fluorescent dye. Burst pressure measurements and biochemical analyses were performed using standard procedures (Figure 3).

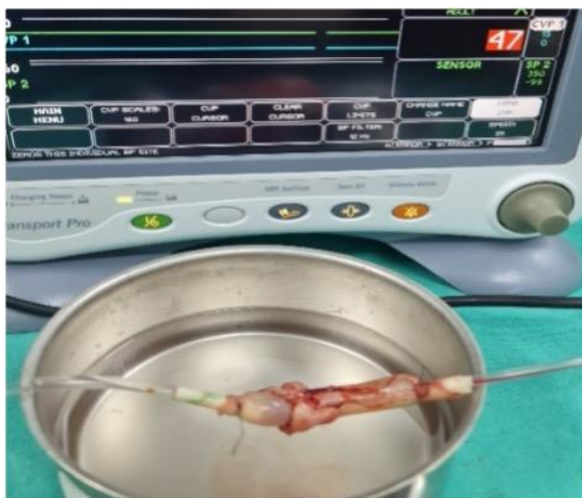


Figure 3: Measurement of colonic burst pressure (BP) after anastomosis.

Statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Normality was assessed prior to analysis. Non-normally distributed data were analyzed using the Kruskal-Wallis test, followed by Bonferroni-corrected *post-hoc* pairwise comparisons when appropriate. Paired comparisons were conducted using the Wilcoxon test. A p value of < 0.05 was considered statistically significant. This experimental study was conducted with the approval of the Başkent University Animal Experiments Local Ethics Committee (DA22/01) and was supported by the Başkent University Research Fund.

RESULTS

Physical Assessment

Body weights of the rats were recorded from baseline until postoperative day 7. In Group 1 (no radiotherapy), no significant difference was observed between baseline body weight and the day of surgery; however, significant weight loss was detected on postoperative days 3 and 7. Rats in the radiotherapy-treated groups (groups 2, 3, and 4) experienced significant weight loss during both preoperative and postoperative periods. No statistically significant difference in postoperative weight loss was observed between the stem cell-treated groups (groups 3 and 4) and the other groups (Table 1).

Intestinal Burst Pressure

Intestinal burst pressure (BP) was measured on postoperative days 3 and 7, and in all animals, rupture occurred at the anastomotic line (Figure 4). Across all groups, BP values on postoperative day 3 were significantly lower than those measured on postoperative day 7. Although median BP values on both postoperative days were numerically higher in the stem cell-treated groups (groups 3 and 4) compared with the radiotherapy-only group (group 2) and the control group (group 1), these differences did not reach statistical significance. Post-hoc pairwise comparisons with Bonferroni correction revealed no statistically significant differences between group 2 and group 3 or between group 2 and group 4 on postoperative day 3 or day 7.

Table 1: Changes in body weight of rats over time

		Baseline weight(g)	Weight on operation day (g)	Postoperative day 3 (g)	Postoperative day 7 (g)	p value
Group 1	Mean±SD	388.75±44.01	388.92±43.20	370.75±37.88	355.67±30.13	0.002
	Median	366.5 (340-475) ^a	365.5 (342-474) ^a	357 (323-432) ^b	347.5 (333-414) ^b	
Group 2	Mean±SD	434.67±30.62	374.83±36.39	371.50±34.27	369.17±31.12	0.009
	Median	430 (397-500) ^a	366.5 (329-452) ^b	365.5 (326-425) ^b	363 (332-414) ^b	
Group 3	Mean±SD	428.78±18.19	368.33±17.33	354.44±22.95	363.20±22.83	0.005
	Median	425 (403-455) ^a	363 (347-395) ^b	355 (320-380) ^c	361 (339-387) ^{b,c}	
Group 4	Mean±SD	417.44±47.64	355.67±51.92	340.22±46.67	360.80±53.43	0.003
	Median	407 (330-500) ^a	355 (273-445) ^b	344 (269-421) ^c	380 (281-421) ^c	

Note: Friedman Test, Kruskal-Wallis Test. Superscript letters (a, b, c) indicate significant differences between time points within the same row

Notably, wide variability in BP measurements was observed in group 2 on postoperative day 7, which was associated with poor anastomotic healing and gross separation at the anastomotic line in three rats (50%). In contrast, no gross anastomotic separation was observed in any of the stem cell-treated groups.

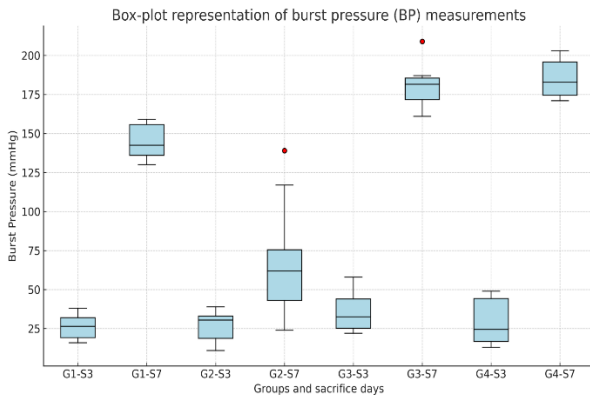


Figure 4: Box-plot representation of burst pressure (BP) measurements for each group and sacrifice day.

Biochemical Assessment

Hydroxyproline (Hyp) and matrix metalloproteinase-2 (MMP-2) levels were evaluated on postoperative days 3 and 7. According to Kruskal-Wallis analysis, no statistically significant differences in Hyp levels were observed among the four groups at either time point. *Post-hoc* pairwise comparisons with Bonferroni correction confirmed the absence of significant differences between the radiotherapy-only group (group 2) and the stem cell-treated groups (groups 3 and 4). Within-group analysis demonstrated that median Hyp levels in group 4 were numerically higher on postoperative day 3 than on day 7; however, this difference did not reach statistical significance.

Similarly, no statistically significant differences in MMP-2 levels were detected among the groups on postoperative day 3 or day 7. Although a numerical decrease in MMP-2 levels from postoperative day 3 to day 7 was observed in the stem cell-treated groups, this change was not statistically significant (Table 2).

Table 2: Comparison of hydroxyproline and matrix metalloproteinase-2 levels across groups

		Hyp (µg/g tissue)	MMP-2 (ng/ml)
G1 S3	Mean±SD	132.38±54.70	85.65±24.97
	Median	132.1 (61.2-199.1)	82.74 (59.87-123.29)
G1 S7	Mean±SD	237.28±94.48	86.01±20.27
	Median	248.95 (123.4-341.7)	80.22 (65.52-118)
G2 S3	Mean±SD	179.93±96.28	63.53±19.09
	Median	164.8 (66-312.4)	67.44 (35.27-83.62)
G2 S7	Mean±SD	197.13±85.34	64.12±18.83
	Median	194.2 (64.8-308.6)	70.81 (26.43-76.44)
G3 S3	Mean±SD	174.8±114.49	66.78±12.97
	Median	152.4 (70.1-357.7)	67.62 (53.78-87.19)
G3 S7	Mean±SD	250.65±41.15	84.6±31.90
	Median	239.8 (218-305)	89.67 (41.56-117.51)
G4 S3	Mean±SD	277.65±76.78	52.79±13.41
	Median	266.4 (200-377.8)	55.3 (35.04-65.52)
G4 S7	Mean±SD	203.32±102.86	72.93±23.66
	Median	148.2 (117.8-327.8)	66.36 (46.1-106.63)
p value		.215	.180

Hyp: Hydroxyproline, MMP-2: Matrix metalloproteinase-2, G: Group, S3:Postoperative day 3 sacrifice, S7:Postoperative day 7 sacrifice, Kruskal-Wallis test.

Histopathological Assessment

Histopathological evaluation was performed by scoring acute and chronic tissue changes on postoperative days 3 and 7. In all groups, median acute histopathological scores were numerically higher on postoperative day 3

compared with day 7; however, these differences were not statistically significant. Comparison of acute histopathological scores among the four groups revealed no statistically significant differences at either time point (Figure 5).

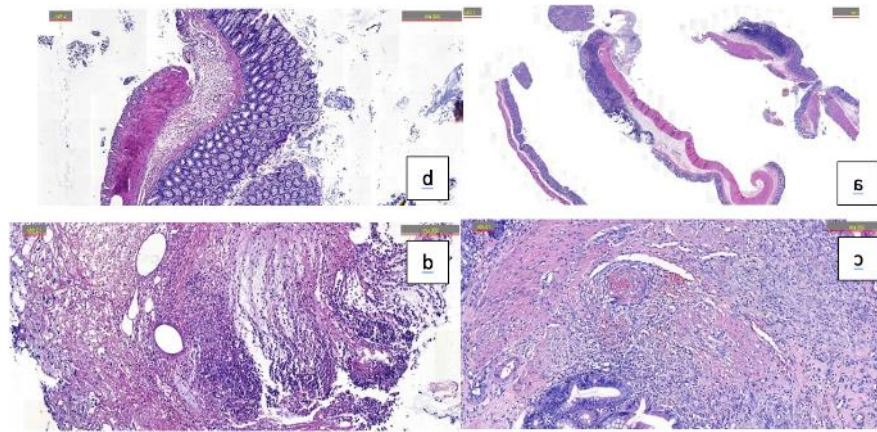


Figure 5: Histopathological findings: (a) Dense lymphocytic infiltration in the lamina propria; (b) Hyaline necrosis in arterioles; (c) Ectatic vascular structures in the lamina propria; (d) Superficial ulceration focus

Similarly, no statistically significant differences were observed in chronic or total histopathological scores among the groups on postoperative day 3 or day 7. *Post-hoc* pairwise comparisons with Bonferroni correction confirmed the absence of significant differences between the radiotherapy-only group (Group 2) and the stem cell-treated groups (Groups 3 and 4) for acute, chronic, or total histopathological scores at both time points (Table 3).

Table 3: Comparison of histopathological findings in the groups sacrificed on postoperative days 3 and 7

		Acute changes	Chronic changes	Histopathological total score
G1	Mean±SD	4.5±3.51	4.5±1.87	9±5.22
S3	Median	3.5 (2-11)	4 (3-8)	8 (5-19)
G1	Mean±SD	3.33±2.07	4.83±2.23	8.17±3.97
S7	Median	3 (0-6)	5 (2-8)	9 (2-14)
G2	Mean±SD	5.5±3.51	3.83±2.40	9.33±2.88
S3	Median	4.5 (2-11)	4 (1-6)	9 (5-13)
G2	Mean±SD	4.5±1.97	6.67±2.34	11.17±3.97
S7	Median	4 (2-8)	6.5 (3-10)	10.5 (5-16)
G3	Mean±SD	4.6±1.52	5.6±1.52	10.2±2.17
S3	Median	4 (3-7)	5 (4-8)	11 (7-12)
G3	Mean±SD	2.5±1.73	5±1.63	7.5±2.38
S7	Median	2.5 (1-4)	5 (3-7)	6.5 (6-11)
G4	Mean±SD	5.5±4.12	6.75±2.36	12.25±5.56
S3	Median	6 (1-9)	6 (5-10)	12 (6-19)
G4	Mean±SD	3.8±2.28	5.4±2.30	9.2±4.55
S7	Median	3 (1-7)	5 (2-8)	8 (3-15)
P value		.792	.441	.584

G: Group., S3:Postoperative day 3 sacrifice, S7:Postoperative day 7 sacrifice. Kruskal-Wallis test

Stem Cell Imaging

Dil-labeled stem cells were identified at the anastomotic line using immunofluorescence microscopy, demonstrating localization of transplanted cells at the target site (Figure 6).

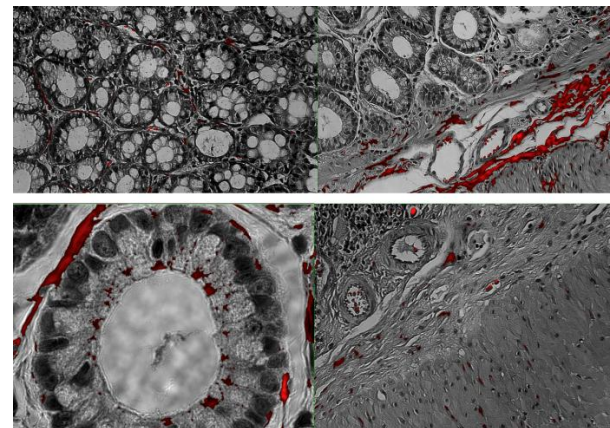


Figure 6: Dil-labeled stem cells observed in endothelial and arteriolar walls and stromal tissue

DISCUSSION

In this experimental study, we evaluated the adverse effects of radiotherapy on colonic anastomotic healing and explored the potential role of mesenchymal stem cell therapy in attenuating these effects. The findings of the present study suggest that radiotherapy is associated with increased weight loss, reduced anastomotic burst pressure (BP), and impaired early wound healing. Although stem cell therapy did not result in statistically significant improvements in measured parameters, several observations indicate a possible supportive effect on anastomotic integrity.

Significant preoperative weight loss was observed in all radiotherapy-treated groups. Postoperative weight loss occurred across all groups, and no statistically significant differences were detected between the stem cell-treated and non-treated groups. These findings indicate that stem cell therapy did not influence systemic weight changes, which is consistent with previous reports suggesting that the combined metabolic stress of radiotherapy and surgery leads to unavoidable weight loss.¹⁴

Anastomotic burst pressure is a widely accepted mechanical indicator of anastomotic integrity and reflects collagen deposition and tissue strength during

healing. Consistent with the established temporal pattern of anastomotic repair, BP values were significantly lower on postoperative day 3 than on day 7 in all groups.¹⁵ Although BP values were numerically lower in the radiotherapy-only group and higher in the stem cell-treated groups, these differences did not reach statistical significance. Importantly, gross anastomotic separation and marked variability in BP measurements were observed exclusively in the radiotherapy-only group, whereas no gross separation occurred in the stem cell-treated groups. While these findings do not provide statistical evidence of superiority, they suggest that stem cell therapy may contribute to more stable anastomotic healing following radiotherapy, in line with previous experimental observations.¹⁵

Hydroxyproline (Hyp) content is an indirect marker of collagen deposition and anastomotic healing. In the present study, Hyp levels did not differ significantly among the groups on postoperative days 3 or 7. Although numerically higher Hyp values were observed in the stem cell-treated groups, this difference was not statistically significant. This finding may be explained by the early inflammatory and hypoperfusive effects of radiotherapy, which are known to impair collagen synthesis during the initial phases of wound healing.¹⁶

Matrix metalloproteinase-2 (MMP-2) plays a critical role in extracellular matrix remodeling during anastomotic repair. While transient increases in MMP activity are considered part of normal healing, sustained elevations have been associated with anastomotic failure.^{17,18} In this study, MMP-2 levels did not differ significantly among the groups at either postoperative time point, indicating that stem cell therapy did not exert a measurable modulatory effect on MMP-2 activity under the conditions tested.

Histopathological evaluation revealed no statistically significant differences in acute or chronic tissue changes among the groups. Nevertheless, the absence of gross anastomotic separation in the stem cell-treated groups represents a clinically relevant observation. Although histopathological superiority could not be demonstrated, this macroscopic finding may indicate a supportive local effect of stem cell therapy on anastomotic healing, as previously suggested in experimental models.¹⁹

Several limitations of this study should be acknowledged. The use of an animal model limits direct extrapolation of the findings to clinical practice. In addition, the relatively small sample size and short follow-up period may have reduced the ability to detect subtle histopathological or biochemical differences. Further studies with larger cohorts, extended observation periods, and alternative dosing or delivery strategies are warranted to clarify the potential role of

stem cell therapy in enhancing anastomotic healing following radiotherapy.

In this experimental study, the effects of mesenchymal stem cell therapy on colonic anastomotic healing were evaluated in rats subjected to neoadjuvant radiotherapy. Radiotherapy was associated with weight loss and reduced anastomotic burst pressure values, particularly in the early postoperative period. Although the stem cell-treated groups demonstrated numerically higher burst pressure values and no gross anastomotic separation, these findings did not reach statistical significance.

Biochemical parameters, including hydroxyproline and matrix metalloproteinase-2 levels, as well as histopathological assessments, showed no statistically significant differences among the groups. Accordingly, the present study did not demonstrate definitive mechanical, biochemical, or histopathological evidence of a beneficial effect of mesenchymal stem cell therapy on anastomotic healing following radiotherapy.

Nevertheless, the absence of gross anastomotic separation and the more homogeneous distribution of burst pressure values observed in the stem cell-treated groups represent clinically relevant findings. These observations suggest a potential supportive role of mesenchymal stem cell therapy in anastomotic healing after radiotherapy and warrant further investigation. Future studies with larger sample sizes and adequately powered experimental designs are required to clarify the therapeutic potential of mesenchymal stem cells in this setting.

Conflict of Interest: The authors declare that they have no conflict of interest.

Researchers' Contribution Rate Statement: Concept/Design: HOA, SY, GY, KT, AŞ, AÇU, PB, Cİ; Analysis/Interpretation: KT, Cİ, MYÇ, SY, GY; Data Collection: KT, AŞ; Writer: KT; Critical Review: SY, MYÇ, EST; Approver: KT, AŞ.

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