Evaluation of prophylactic and therapeutic effects of sildenafil on acute radiation proctitis in rats[1]

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Abstract

Purpose: To investigate the prophylactic and therapeutical effects of sildenafil in a model of acute radiation proctitis (ARP).

Methods: All experimental procedures of this study was examined by histopathological, immunohistochemical and transmission electron microscopic analysis.

Results: Our histopathological evaluations indicated significant increases in lesion severity, cryptic apsis, cryptitis, cryptic distortion, reactive atypia and infiltration depth of the control (proctitis) group. While the prophylaxis group and the treatment group had significantly lower scores. High-dose group showed similar results as prophylaxis group. Histopathological findings of the prophylaxis group was more significant than the treatment group. Immunoreactivities of IL-1β, FGF-2, TNF- α and HIF-1α increased in the control group especially in the epithelial and cryptic regions. On the contrary, sildenafil application caused significant decreases of inflammatory markers in all treatment groups, specifically better results in the prophylaxis group.

Conclusion: The sildenafil has anti-inflammatory effects on ARP, as well as protective effects against ARP and the protective effect of sildenafil surpasses its therapeutic effect histopathologically.

Key words: Sildenafil Citrate. Proctitis. Inflammation. Ultrastructure. Rats.

■ Introduction

Clinical radiation to the pelvic region has been performed to treat the rectum cancer, gynecological malignities, prostate cancer and lymphoma. Acute Radiation Proctitis (ARP) is inflammation and damage to the rectum, appeared as a major clinical complication of ionizing radiation[1,2]. More than 75% of the patients received radiation therapy have the symptoms of ARP and only 20% of them have symptoms related to the chronic radiation proctitis (CRP)[3-4].

The pathogenesis of radiation proctitis has not been clarified totally yet. However, it is known that firstly mucosal damage is observed due to the radiation, subsequently connective tissue is enlarged and remodeled, lastly fibrosis and ischemia are observed[5]. These kinds of pathological damage are varied in ARP and CRP. Cryptic apsis and cryptitis are generally observed in acute radiation proctitis. Moreover, T lymphocytes, macrophages and neutrophils are designated in the process[5,6].

There are many therapeutical and protective methods against the radiation proctitis whereas none of them are gold standards. Many previous reports focus on the various methods used for treating ongoing inflammation but fail to address its pathophysiology. There is therefore the need for a better understanding of the precise mechanisms underlying radiation-induced proctitis to determine best methods of protection and treatment[7].

Sildenafil, which was developed for the treatment of erectile dysfunction, inhibits cyclic guanosine monophosphate-specific phosphodiesterase-5, an enzyme that is abundantly present in the pulmonary vasculature, and thereby promotes nitric oxide-mediated vasodilatation, which in turn decreases pulmonary vascular resistance[8,9]. Recently several studies have reported the beneficial effects of sildenafil to treat inflammatory diseases, to alleviate the endothelial cell dysfunction that can occur under conditions of ischemia reperfusion injury[10-12], chronic heart failure[13], and coronary artery disease[14,15]. However, the mechanism of how sildenafil affect inflammatory processes in ARP or CRP is not known yet.

In this study, our purpose is to investigate the prophylactic and therapeutical effects of sildenafil in a model of ARP by histopathological, immunohistochemical and transmission electron microscopical analysis.

■ Methods

All experimental procedures of this study was approved by the Animal Care and Use Committee of The University of Health Sciences Bagcilar Training and Research Hospital approved (Protocol No:2015-17).

Forty female Sprague- Dawley rats (250± 30g, with ages 4-8 weeks), supplied by the Bagcilar Training and Research Hospital Animal Center (BADABEM), Istanbul, Turkey, were kept under the laboratory conditions of the same center, housed in a controlled room with 12-hour lighte-dark cycles at 22°C, and fed with standard pellet chow including 21% protein and daily fresh water. All rats were captured in separate cages including 8 rats in each one and were divided into five random groups (n=8) as;

Group 1.; Sham group received equal amount of isotonic solution,group 2; Control (Radiation) group received Radiation (RT) + equal amount of isotonic solution,group 3; Prophylaxis group received 10 mg/kg/day Sildenafil for 7 days,group 4; Treatment group received first RT and then 10 mg/kg/day Sildenafil for 15 days,group 5;High dose group received first RT and then 50 mg/kg/day Sildenafil for 15 days

Establishment of radiation proctitis

All rats of RT applied groups, except sham group, were fixed on an acrylic plate on back position by strapping the tails and legs under anesthesia by 5mg/kg xylazine (Rompun®-Bayer-Germany) and 30mg/kg ketamine (Ketalar®-Pfizer-Turkey) (Figure 1). All parts of the body and extremities were excluded from the radiation area except the target lower pelvic region in 3x4 cm dimension including 2 cm long rectum. The dimensions of radiation area (Elekta, Model Precise Treatment System, 6mev electron energy, 0.5 bolus material) were 20x20 cm^2 and a single fraction of 2500 cGy was given[16].
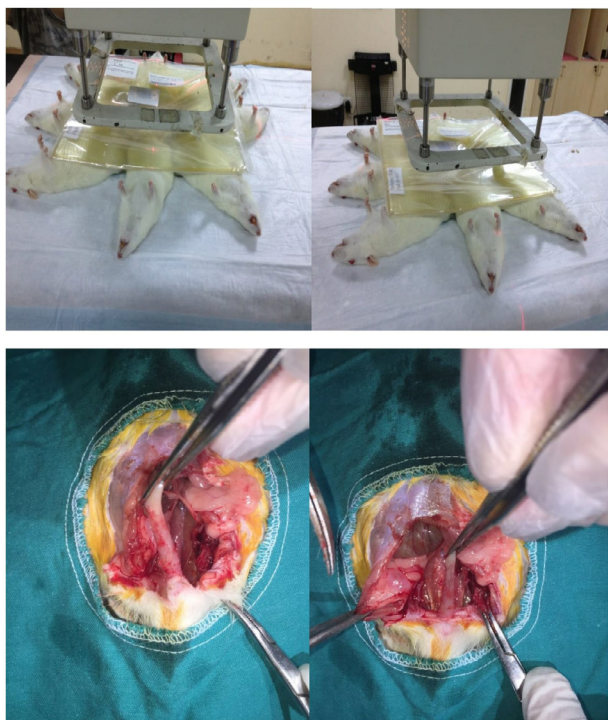

Figure 1 - Application of radiation (*above*) and dissection of rectums (*below*) after the sacrification of rats.

A single 10 mg/kg/day dose of Sildenafil (INDEA 165459/N) was injected daily by oral gavage to Group 3 for 7 days before radiation. Group 4 was only injected for 15 days after radiation. Group 1 was injected by daily single dose of isotonic solution at the same volume by oral gavage for 15 days without performing radiation. Group 2 was injected by daily single dose of isotonic solution at same volume by oral gavage for 15 days after radiation. None of the rats died during the experiment.

Laparotomy was performed on every animals at 15th days of radiotherapy under ketamine anesthesia (10 mg/kg xyilazine and 60 mg/kg ketamine) (Figure 1); the rats were sacrificied by intracardiac puncture.

Rectums were dissected and washed with isotonic solution for histopathological, immunohistochemical and ultrastructural evaluations. Under observation of a pathologist, the tissues were cut into 3 equal horizontal pieces and put into relevant fixatives[17].

Histopathological evaluation

All relevant pieces of rectal segments were cut into 1cm^3 size, fixed by 4% neutral formalin, applied routine procedure for tissue preparation and embedded into the paraffin blocks. 5µm paraffin sections were stained by hematoxylin and eosin and evaluated by the pathologist as blind for twice under a light microscope and photographed (Nikon Eclipse E600).

Lamina propria (inflammation), invasion of leucocytes through the cryptic epithelium (cryptitis), deterioration of crypts (cryptic distortion), regenerative / restorative atypia of epithelial cells (reactive atypia) and inflammatory infiltration were investigated. Severity of the lesion was evaluated as scores between 0-3; 0: None, 1: mild lesion, 2: moderate lesion and 3: severe lesion[16].

Cryptit apsis was noted as positive or negative for each slide. Infiltration depth was

measured by an image analysis system of a video attached microscope (Zeiss Axioplan 2).

Immunohistochemical analysis

All relevant pieces of rectal segments were fixed by 4% neutral formalin, applied routine procedure for tissue preparation and embedded into the paraffin blocks. 5μm-thick sections were stained immunohistochemically by using Streptavidin-Biotin-Peroxidase method with monoclonal and polyclonal antibodies tagged to indicate cell and tissue antigens, according to previously described method[17]. Primary antibodies against inflammatory markers for tumor necrosis factor α (TNF-α), hypoxia-inducible factor 1α (HIF-1α), interleukin 1β (IL-1β) and fibroblast growth factor 2 (FGF-2) and angiogenesis marker of vascular endothelial growth factor (VEGF) were used. Positively stained cells with relevant antigens were analyzed semi-quantitatively in terms of staining intensity (modified H-SCORE analysis)[17]. Thereby rectal localization of these proteins and variance of the staining intensity and regional differences were determined.

Transmission electron microscopic analysis

Rectal segments in size of 1mm^3 were fixed by 2.5% glutaraldehyde and prepared for ultrastructural analysis as described before[18]. Sections were evaluated under a transmission electron microscope (Jeol Jem 1011). The images were transferred to Soft Imaging System Analysis programme by Megaview III digital camera and pictures were taken.

Statistical analysis

Semi-quantitative analysis of cytokines stained in tissue of all rats was performed by modified H-SCORE, given values between 0-300 for five different regions of each slide. Statistical analysis was performed by GraphPad Instat ver. 3.06 (GraphPad Inc, CA, USA) program. Mean values of continuous variables are compared by one way ANOVA and variations between groups were compared by Tukey-Kramer Multiple Comparison Test. Categorical variables were evaluated by Chi-square test. $p < 0.05$, $p < 0.01$ and $p < 0.001$ were accepted as statistically significant for different values of the groups.

■ Results

Histopathological examination

Light micrographs of rectal tissues stained with H&E were given in Figure 2. As a result of light microscopic evaluations, histological structure of the mucosa and submucosa, cryptic glands and other epithelial components were observed to be preserved in the sham group, as expected. However, the micrographs of the control group presented a damaged mucosal structure, high levels of epithelial degenerations with decreased amount of cryptic glands and exacerbated surface. Histopathological evaluations disclosed that the thickness of mucosa increased locally due to cryptitis and cryptic apsis; leucocyte infiltration and fibrosis were observed in the submucosa. The prophylaxis and treatment groups established the normal structures of the mucosal and submucosal components, similar appearing with the sham group. The surface epithelium, cryptic glands and lamina propria were approximately well-preserved. However, these components could not show improvements enough in the high-dose group, compared to the prophylaxis and treatment groups (Figure 2).

Figure 2 Light micrographs of rectum samples from experimental rat model of

radiation proctitis, H&E, x100. a: Sham group, b: Control (radiation) group, c: Prophylaxis group, d: Treatment group, e: High-dose. E: Surface epithelium, C: Cryptic glands, S: Stroma with lamina propria, Su: Submucosa, L: Lumen of rectum.
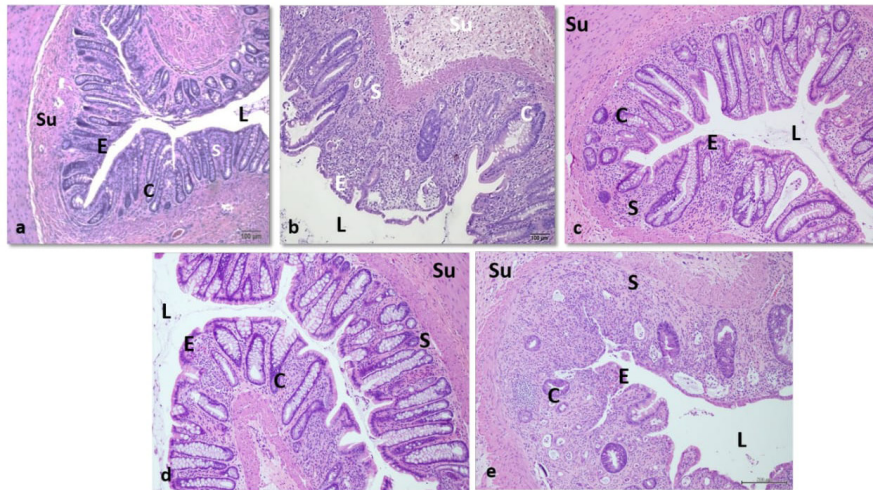

Figure 2 - Light micrographs of rectum samples from experimental rat model of radiation proctitis, H&E, x100. **a**: Sham group. **b**: Control (radiation) group. **c**: Prophylaxis group. **d**: Treatment group. **e**: High-dose. (**E**: Surface epithelium, C: Cryptic glands, S: Stroma with lamina propria, Su: Submucosa, L: Lumen of rectum).

Inflammation, lesion severity, cryptic apsis, cryptitis, cryptic distortion in lamina propria, reactive atypia of epithelial cells and inflammatory infiltration depth were evaluated in H&E stained tissues and lesion severities were graded in 0-3 points (Table 1). Lesion severity of sham group was found as 0 (no lesion) while its mean was estimated as 2.38 (moderate-severe lesion) in the control and treatment groups; 2 (moderate) for prophylaxis group and 2.86 (moderate-severe lesion) for high-dose group. So it was observed to significantly decrease only in the prophylaxis group compared to the control group (p<0.05).

There were statistically significant increases in cryptic apsis, cryptitis and cryptic distortion in the control group, compared to the sham group (p<0.001) while significant decreases were revealed in cryptic apsis and distortion of the treatment group (p<0.001) and in cryptitis and cryptic distortion of prophylaxis and high-dose groups (p<0.01). Reactive atypia could not be ameliorated in any of sildenafil applied groups (Table 1).

A significant increase was detected for infiltration depth in mucosa of the control group measured by light microscopy, compared to the other groups (p<0.01). There was not any difference between sham, treatment and prophylaxis groups (Table 1) but there was a significant difference between sham and high-dose group (p<0.001). Thus, treatment with sildenafil significantly decreased the inflammatory infiltration depth, compared to the radiation group.

Table 1 - Histopathological findings of five experimental groups in the model of acute radiation proctitis.

GROUPS	Lesion Severity (Mean±SD)	Cryptic Apsis n (%)	Cryptitis n (%)	Cryptic distortion n (%)	Reactive atypia n (%)	Infiltration Depth (μm) (Mean±SD)
Sham	0[a]	0 (0)[a]	0 (0)[a]	0 (0)[a]	0 (0)[a]	102.78±17.1
Control	2.38±0.5	8 (100)[c]	8 (100)	7 (87,5)[c]	3 (37,5)	509.63±158.9[c]
Treatment	2.38±0.5	5 (62.5)[d]	8 (100)	4 (50)[d]	3 (37,5)	224.71±25.9
Prophylaxis	2.0±0.8[b]	7 (87.5)	7 (87.5)[e]	5 (62.5)[b]	4 (50)[e]	212.27±55.7
High-dose	2.86±0.4	7 (87.5)	7 (87.5)[e]	6 (75)[e]	6 (75)[e]	278.15±46.4[a]

[a]$p<0.001$ Sham group *vs.* other groups
[b]$p<0.05$ *vs.* high-dose group
[c]$p<0.01$ Control group *vs.* other groups
[d]$p<0.001$ *vs.* prophylaxis and high-dose groups
[e]$p<0.01$ *vs.* control and treatment groups
SD: Standard deviation

Immunohistochemical analysis

H-SCORE analysis of immunoreactivities for inflammation markers of IL-1β, FGF-2, TNF-α, HIF-1α, and angiogenesis marker of VEGF were given in Table 2. Immunoreactivities on rectal tissues were evaluated for three regions: Epithelium, crypts and stroma.

Immunoreactivity of the first cytokine, IL-1β, showed a significant increase in epithelium, crypts and stroma of the control group compared with other groups while the treatment, prophylaxis and high-dose groups indicated significant decreases (p<0.05) (Table 2). These decreases in immunoreactivities were also detected for FGF-2 cytokine in the epithelial and cryptic regions of the treatment, prophylaxis and high-dose groups compared with the control group (<0.01), except the stromal regions of three experimental groups showed no difference for FGF-2 immunoreactivity (Table 2).

Immunoreactivity of the third cytokine,

TNF- α increased significantly in the epithelium and crypts of the control group, compared to the other groups (p<0.01) and decreased significantly in the treatment, prophylaxis and high dose groups (Table 2). Stroma had not such significant differences (Table 2), even for the control group.

There was significant increases in immunoreactivity of HIF-1α in epithelium, crypts and stromal regions of the control group, in regard to the other groups (p<0.001) and significant decreases in the treatment, prophylaxis and high dose groups (p<0.001) (Table 2).

Comparing the number of vessels stained for angiogenesis marker, VEGF in the stroma and submucosa, the control group showed significant increase (p<0.01) and other groups had significant decreases (Table 2). In other words, the sham, treatment, prophylaxis and high-dose groups had no significant differences for VEGF immunoreactivity (Table 2).

Table 2 - Immunohistochemical findings for reactivity of inflammatory marker on epithelial, cryptic and stromal regions of the rectum sections of five experimental groups.

Mean±SD	IL-1β	FGF-2	TNF-α	HIF-1α	VEGF
EPITHELIUM					
Sham	0	152.5±43.7	81.25±15.3	137.5±44.3	181.25±39.8
Control	62.5±19.45[a]	268.75±59.4[b]	160.0±39.3[b]	275.0±46.3[e]	203.75±45.5
Treatment	0	275.0±46.3[b]	168.75±53.0[b]	237.5±58.2[f]	175.0±50.1
Prophylaxis	0	108.75±27.2[c,d]	62.5±14.4[c,d]	108.75±28.6[c,d]	77.5±19.1[c]
High-dose	3.75±1.23	164.29±55.6[c,d]	97.14±19.8[c,d]	185.71±47.6[c]	192.3±28.5
CRYPTS					
Sham	0	32.50±3.2	18.75±5.9	0	25.0±6.7
Control	27.5±5.3[a]	200.0±80.2[a]	76.25±10.9[a]	168.75±16.3[g]	131.25±20.4[f]
Treatment	0	100.0±35.5	43.75±7.2	12.50±5.4	81.25±13.6[f]
Prophylaxis	0	56.25±16.3	25.0±7.2	2.50±1.0	37.5±5.5
High-dose	0	65.71±23.4	17.14±3.6	2.86±1.0	45.6±13.4
STROMA					
Sham	0	46.25±3.4	275.0±37.8	0	8.38±3.1
Control	96.25±7.3[a]	212.5±79.1[f]	256.25±62.3	125.0±45.5[g]	43.13±6.5[a]
Treatment	12.5± 3.1	181.25±75.3[f]	243.75±82.1	36.25±3.8	15.5±4.7
Prophylaxis	25.0±7.8	162.5±15.7[f]	225.0±70.7	12.5±3.1	13.38±5.4
High-dose	7.14±1.8	164.29±78.8[f]	207.14±81.8	28.57±6.7	16.4±2.8

[a]*p*<0.01 *vs.* other groups
[b]*p*<0.01 *vs.* sham group
[c]*p*<0.01 *vs.* control group
[d]*p*<0.001 *vs.* treatment group
[e]*p*<0.001 *vs.* sham group
[f]*p*<0.05 *vs.* sham group
[g]*p*<0.001 *vs.* other groups

Electron microscopic analysis

Electron microscopical results were parallel to the light microscopy as there were histopathogical changes in the control group related to acute radiation (Figure 3b1 and b2). Decreased number of microvilli on the epithelium and diminished length and also apical vacuolization were remarkable on the epithelium (Figure 3b1). With atrophy and distortion on the glands, the glandular epithelium was diminished and corroded, compared to the sham group (Figure 3b1);

migrated nuclei of the epithelium to the lumen side and dilatation of intercellular spaces were observed (Figure 3b2). The number of Goblet cells decreased, mucus secretion diminished and epithelial cells shrank and there were a few damaged cryptic stem cells. On some regions, microvilli were totally deleted, nuclei of the epithelium were enlarged, chromatin modelling differed, and even some gained apoptotic appearance.

On the rectum surface epithelium of the prophylaxis group, microvilli projecting through lumen at typical ultrastructure acquired their

normal number but not the height (Figure 3c1). A large number of tight junctions also protected integrity of the epithelium. The basement membrane protected its continuity and homogeneity more or less and the intercellular area had normal digitations. Normal number but irregular distribution of the fibers was remarkable at subjacent lamina propria, compared to the sham group (Figure 3c2).

On the rectum surface epithelium of the treatment group, microvilli (white arrow) projecting through lumen at typical ultrastructure acquired their normal number and height, better than the prophylaxis group (Figure 3d1). Intercellular borders protected their distance and a large number of tight junctions protected the integrity of the epithelium. The basement membrane of the cryptic epithelium did not lose its continuity and homogeneity; but abnormal digitations were observed in the intercellular spaces.

Ultrastructure of mitochondrial (M) crista at the suprabasal locations was observed to be impaired (Figure 3d2).

In the high-dose group, the epithelium of rectum was in normal thickness, number and height, as in other treated groups; regular Goblet cells were also observed in normal ultrastructure (Figure 3e1). Intercellular spaces were observed in normal distance and number and height of microvilli projecting to the lumen were preserved with their normal structure, compared with the sham group (Figure 3e1). Many tight junctions protected the integrity of the epithelium. Unlike the control group, high-dose group preserved the continuity and homogeneity of the basement membrane and had normal digitations in the intercellular spaces; however, the treatment group had abnormal digitations. The lamina propria was remarkable to have a little increase in the extracellular matrix (Figure 3e2).
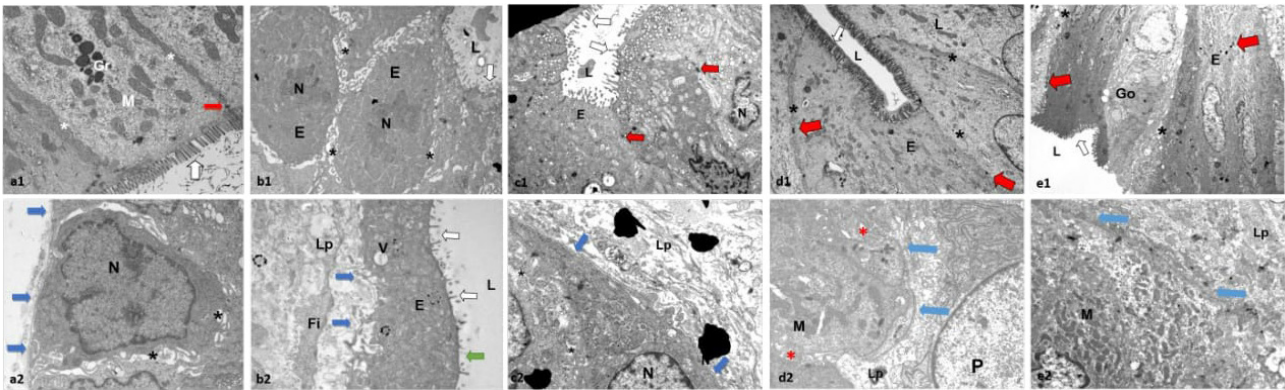

Figure 3 - Electron micrographs of rectum sections from experimental rat model of radiation proctitis. **a**: Sham group; **a1**: x12000, **a2**: x10000. **b**: Control group; **b1**: x7500, **b2**: x7500. **c**: Prophylaxis group; **c1**: x7500, **c2**: x12000. **d**: Treatment group; **d1**: x6000, **d2**: x15000. **e**: High-dose group; **e1**: x4000, x12000. (L: Lumen, White arrow: Microvilli of rectum surface epithelium, asterisks: epithelial digitations, red arrows: tight junctions, M: mitochondria, Gr: intracytoplasmic granules, N: Cryptic epithelium, blue arrows: basement membrane, E: surface epithelium, V: vacuoles, green arrows: locally nude membrane, Fi: fibrin augmentation, Lp: lamina propria, P: plasma cells, Go: Goblet cell).

■ Discussion

Preventing ARP, an inducible inflammation, has paramount importance since ARP gives rise to many difficulties in the radiation therapy. Decreasing the radiation dose or excluding the rectum from radiation area are judicious and alleviating procedures which are advised in radiation therapy, but these are not enough to ameliorate manifestations of the patients totally. Some agents such as rectal or oral misoprostol, rectal sucralfate and intravenous amifostine can be used[19]. However, there has not been any proven method in the literature yet, showing protective or preventive effects in ARP.

A part of the ARP can be ameliorated by supportive anti-inflammatory therapies[19]. Using sildenafil would be an effective approach to prevent this pathological situation whose treatment has such drawbacks[19-23]. Sildenafil is a selective and potent inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE)-5, which catalyzes the hydrolysis of cGMP and has a relaxant effect on the smooth muscle cells of the arterioles supplying the human corpus cavernosum[24]. The data of a recent study have shown that sildenafil, acting via a nitric oxide (NO)-dependent mechanism, prevented indomethacin-induced gastropathy, possibly through a reduction of leukocyte adhesion and maintenance of gastric blood flow[25]. Two studies by Iseri and Yildirim *et al.*[26,27] demonstrated the beneficial anti-inflammatory actions of sildenafil on acetic acid-induced acute colitis and bleomycin-induced lung fibrosis models in rats via prevention of lipid peroxidation, oxidant generation, cytokine production and neutrophil accumulation. As observed by Wang *et al.*[28] and Jeong *et al.*[29] sildenafil attenuated airway inflammation and mucus production in the rat model, through the NO/cyclic guanosine 3',5'-monophosphate pathway and significantly prevented renal damage by ameliorating oxidative and inflammatory injuries in diabetic rats.

Given the importance of inflammation involved in the ARP, there is a need for a treatment and protection modality against ARP that can block the inflammatory processes, and advances in the understanding of the pathogenesis of ARP have created a trend of novel biological therapies that specifically inhibit the molecules involved in the inflammatory cascade[30]. Considering the mode of action of sildenafil in inflammation, we investigated the possible protective and therapeutic effects of sildenafil on the histology and ultrastructure of rectal tissue and inflammatory and angiogenic status induced by radiation in a rat model of ARP. In this study, markers of IL-1β, TNF-α and HIF1α cytokines were investigated to show the inflammation; FGF2 was used as a marker to determine the fibrosis in the rectal tissue, and VEGF marker was used to present the angiogenesis.

The cytokine IL-1β, initiator of prostaglandin synthesis in polymorphonuclear leucocytes and connective tissue cells, has co-effects with TNF. In addition, it activates the vascular endothelial cells[23.] TNF has an effect of oxidative damage on the endothelial cells and increases the number of adhesion molecules; also induces angiogenesis and arranges hematopoiesis. Moreover, angiogenesis and endothelial cell proliferation can also be induced by FGF or VEGF[23].

HIF-1 detects the changes in oxygen concentrations at the cell level and as a response, induces formation of new vessels and organizes many metabolisms such as anaerobic glycolysis[23].

VEGF overexpression has been shown to be associated with neovascularization in neoplastic tumors, and both chemotherapy and

radiotherapy have been found to increase VEGF expression within tumors[24]. It is commonly known that radiation involves the formation of reactive oxygen species and reactive nitric oxide species, which induce stress responses and inflammation, as well as the release of cytokines, growth factors and chemokines. The production of free radicals following exposure to ionizing irradiation causes DNA damage[25].

In our study, immunoreactivities of IL-1β, FGF-2, TNF- α and HIF-1α increased in the control group of ARP especially in the epithelial and cryptic regions, compared to the treatment groups while VEGF reactivity of the control group also increased in stromal vessels, compared to other groups. On the contrary, sildenafil application caused significant decreases of inflammatory markers in all treatment groups, specifically better results in the prophylaxis group. Thus, it shows that sildenafil has anti-inflammatory effects on ARP, as well as protective effects against ARP.

Our histopathological evaluations indicated significant increases in lesion severity, cryptic apsis, cryptitis, cryptic distortion, reactive atypia and infiltration depth of the control (proctitis) group. On the other hand, treated groups showed different results in histopathological findings. While the prophylaxis group had significantly lower scores for lesion severity, cryptitis, cryptic distortion and infiltration depth, the treatment group had lower scores for cryptic apsis, distortion and infiltration depth. High-dose group showed similar results as prophylaxis group, except the infiltration depth was not significantly different from the control group. Generally considering these light microscopic evaluations, histopathological findings of the prophylaxis group was more significant than the treatment group. In other words, the protective effect of sildenafil surpasses its therapeutic effect histopathologically.

Many agents like sildenafil that can be effective on the damage of acute radiation has been investigated in several studies. In a study by Ito *et al.*[33] searching the effect of C vitamin against lethal intestinal damage induced by the radiation, pre-radiation and post-radiation application of C vitamin caused decrease in cytokine levels (TNF and IL-6) in the intestine of rats. Our immunohistochemical analysis demonstrated that sildenafil application resulted in decreased levels of IL-1β and TNF markers, compared to the proctitis group.

Although there is an unfortunate lack of literature about ultrastructure of the rectum in ARP, in a report by Haboubi *et al.*[34], rectal biopsies of six patients with ARP have been evaluated by electron microscopy. As a result, epithelial changes, fibrosis, variations in the lamina propria and cryptic distortion were detected ultrastructurally. These findings are parallel to our electron microscopic results of the experimental rat model of ARP. Moreover, this is the first study that evaluates and indicates ultrastructural changes in a therapeutical and protective model of sildenafil against ARP.

There are also other agents that have been used as treatment or protection against ARP. In an experimental ARP model by Sezer *et al.*[16], histopathological data have been evaluated and hesperidine and diosmin have been proven to be effective. In addition, Doi *et al.*[21] have investigated the effects of polaprezinc in an experimental report and found as to be histopathological and endoscopical effective on ARP.

In parallel to aforementioned light microscopic findings, our electron microscopic analysis showed histopathological changes in the control group due to the acute radiation. Both treatment and prophylaxis groups demonstrated improvements in ultrastructure of the mucosa and submucosa and most of the damage depending on ARP was ameliorated.

Even the high-dose group presented alleviations except the densified extracellular matrix in the lamina propria. To sum up, electron microscopic analysis of the prophylaxis and treatment groups indicated the therapeutical and protective effects of sildenafil on ARP and even the high-dose of sildenafil was non-toxic for histological and ultrastructural integrity of the tissue.

As a result, this study is the first in terms of histopathological, anti-inflammatory and ultrastructural effects of sildenafil on the rectum of an ARP rat model. Yet, many clinical studies, especially biochemical and molecular studies are needed to support these effects of sildenafil. Our study may be a pilot research in this respect.

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