Application of vascular endothelial growth factor at different phases of intestinal ischemia/reperfusion: What are its effects on oxidative stress, inflammation and telomerase activity?

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Abstract

Background. Intestinal ischemic reperfusion injury (IRI) represents a great challenge in clinical practice, with high morbidity and mortality. Vascular endothelial growth factor (VEGF), as a signal protein, contributes to vasculogenesis and angiogenesis.

Objectives. To evaluate the local effectiveness of VEGF following intestinal IRI and its relation with application time.

Material and methods. Thirty Wistar albino rats were allocated to 5 groups and underwent laparotomy. The superior mesenteric arteries (SMA) were dissected in 4 groups, while the control group (Gr C) underwent a resection of small and large intestines. The VEGF group (Gr V) received VEGF following SMA dissection, with no further intervention, and the remaining 3 groups were subjected to ischemia for 90 min through occlusion of SMA and reperfusion for 4 h. Ischemic reperfusion group (Gr I/R) received no additional medication, while the remaining 2 groups received VEGF just before ischemia (Gr V+I/R) and during reperfusion (Gr I/R+V).

Results. Both applications of VEGF caused decreases in plasma levels of interleukin 6 (IL-6), tumor necrosis factor α (TNF- α), intestinal malondialdehyde (MDA), oxidized glutathione, protein carbonyl levels, and increases in intestinal total glutathione and superoxide dismutase (SOD) levels. Telomerase activity, which disappeared for Gr I/R, was found to be elevated following both treatment groups. Similarly, the histopathological scores were found better for both treatment groups, but Gr V-I/R represented best outcomes.

Conclusions. The findings of our study revealed that VEGF, applied either before ischemia or during reperfusion, is effective on local damage following intestinal IRI. By interpreting the biochemical analysis and histopathological findings, we conclude either treatment option to be considered according to the reason of intestinal IRI.

Key words: inflammation, VEGF, telomerase activity, intestinal ischemic reperfusion injury, oxidative stress

Introduction

Intestinal ischemic reperfusion injury (IRI), due to the occlusion at the level of superior mesentery artery (SMA), is connected to parenchymal damage at the intestine as a result of hypoxia, resultant adenosine triphosphate (ATP) depletion and intracellular acidosis at the early phase. The intestinal mucosa and submucosa show deterioration resulting from ischemic damage and ischemia, which also causes stagnation in the bowel and damage to the intestinal barriers. If the condition is untreated, the initial damage is exacerbated and ischemia of the muscle layers becomes inevitable, possibly leading to intestinal perforation, sepsis and even death.¹ As the clinical problem is resolved in IRI, the main problem at the tissue level is the additional inflammatory process taking place due to reperfusion.^{2,3} Evidence supports local and systemic effects of IRI, as many injurious substances and factors are activated during IRI and are released to systemic circulation through venous and/or lymphatic routes.^{2–5} Moreover, IRI has been shown to induce apoptosis of not only intestinal cells but also remote organs as a result of reactive oxygen species (ROS) and pro-inflammatory cytokine production.⁴

Vascular endothelial growth factor (VEGF) is a participant of vital signaling proteins. As a mitogen agent for endothelium, VEGF induces vascular growth and increases microvascular permeability. It acts via nitric oxide (NO) dependent pathways and stimulates vasodilation.⁵ Vessel growth is induced by means of pre-existing vasculature and this expansion is important, especially in case of inadequate blood supply. Hypoxia contributes to angiogenesis, as it is the major stimulant producing VEGF and its receptors.⁶

The VEGF was demonstrated as a recuperative agent of IRI of various organs like myocardium, liver and spinal cord.^{7–11} Thus, we wanted to establish whether it is as effective on intestinal IRI as other organs – and if so, does the timing of its application matter. We designed a study in order to evaluate the effectiveness of VEGF on intestinal IRI used either before ischemia or during reperfusion.

Material and methods

The permission for the experimental study was granted by Animal Ethics Committee of Gazi University (Ankara, Turkey) and the study was completed due to the dictates of the Research Committee at the Experimental Research Center of Gazi University School of Medicine.

Thirty male adult Wistar albino rats weighing 220–270 g (mean weight 240 ± 10 g) were included in the study. All animals were housed under standard temperature and humidity with light and dark cycles of 10 h and 14 h, respectively. No water, food and light restrictions were applied to animals. During the experiments, humane care was given to all animals according to Principles of Laboratory Animal Care and the Guide for the Care and Use

of Laboratory Animals of National Health Institutes (NHS; Bethesda, USA). The animals were anaesthetized using xylazine hydrochloride (5 mg/kg) (Alfazyne; Ege Vet, Izmir, Turkey) and ketamine hydrochloride (40 mg/kg) (Ketalar; Eczacibasi, Istanbul, Turkey), and every surgical procedure was performed under sterile conditions by the surgeon of the team (AP).

All the rats were randomly allocated to 5 groups and underwent a median laparotomy:

- Control group (Gr C, $n = 6$) underwent excision of small and large intestines with no further intervention.

- VEGF group (Gr V, $n = 6$) underwent only dissection of SMA and received VEGF (0.8 $\mu\text{g/kg}$) through the caudal caval vein.

- IRI group (Gr I/R, $n = 6$) underwent dissection and occlusion of SMA. After the dissection of SMA, the vessel was occluded for 90 min using a microclamp at the point where it branches from abdominal aorta. Pulseless mesenteric artery accompanying pale jejunoileal segments confirmed the ischemia.

- VEGF-IRI group (Gr V-I/R, $n = 6$) received VEGF (0.8 $\mu\text{g/kg}$) through caudal caval vein before the surgical procedure. The animals in this group underwent dissection, and occlusion of SMA for 90 min following drug administration.

- IRI-VEGF group (Gr I/R-V, $n = 6$) underwent dissection, and occlusion of SMA for 90 min. The animals in this group received VEGF (0.8 $\mu\text{g/kg}$) through the caudal caval vein at the beginning of reperfusion.

The infusion dose for VEGF was chosen as the minimum effective dose as defined according to the previous study by Oz Oyar et al.⁷ Animals in all groups but Gr C underwent a relaparotomy at the 4th hour of reperfusion, and small bowel and proximal large bowel segments of the animals were removed. The rats were sacrificed with aspiration of cardiac blood and the blood samples were placed in EDTA tubes. Plasma samples were separated by centrifugation ($3000 \times g$ for 10 min at 4°C) and frozen at -80°C . The excised bowel segment was washed in cold 0.9% NaCl, wiped, and weighed. To perform biochemical analysis and histopathological evaluation, the bowel was cut into 3 equal pieces. The 1st (representing the most proximal portion) and 3rd (representing the most distal portion) pieces were fixed in liquid nitrogen and kept frozen at -80°C , whereas the 2nd (representing the middle portion) piece was laid in 10% neutral buffered formalin.

Biochemical analysis

Western blot analysis

Intestinal tissues were weighed, diluted with lysis buffer and homogenized at a ratio of 1/10 (w/v). Homogenates were centrifuged at 2500 rpm for 10 min at $+4^\circ\text{C}$. To quantify the protein levels, BCA Assay Kit (Pierce, Waltham, USA) was used. The heat shock protein (HSP70) (Rabbit,

SPA810; Stressgen, San Diego, USA) and catalase (CAT) (Rabbit, ab1877; Abcam, Cambridge, UK) primary antibodies, used for western blotting analysis and β -actin, were used as housekeeping proteins in order to quantify the other primary antibodies.

Determination of intestinal malondialdehyde levels

Lipid peroxidation was measured using the test published by Gérard-Monnier et al.¹² The malondialdehyde (MDA) levels were also recalculated from our MDA standards produced by the acid hydrolysis of 1,1,3,3-tetramethoxypropane.

Determination of intestinal superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured using nitroblue tetrazolium (NBT) reduction in order to inhibit SOD activity. A superoxide generator (xanthine and xanthine oxidase) was used to trigger inhibition and the amount of SOD required to produce 50% inhibition was accepted to define 1 IU.¹³

Determination of intestinal protein carbonyl group levels

Protein carbonyl group (PC) levels were measured and described by Buss et al.¹⁴ Carbonyl residues were determined using 2,4-dinitrophenylhydrazine (DNPH) and carbonyl contents were calculated by obtaining the enzyme-linked immunosorbent assay (ELISA) of the DNPH-treated samples at 480 nm.

Determination of intestinal total and oxidized glutathione levels

Total glutathione (TGSH) and oxidized glutathione (GSSG) levels were determined with glutathione reductase (GR) as described by Tietze.¹⁵

Determination of intestinal telomerase activity

Telo TAGGG Telomerase PCR ELISA Plus kit (Roche, Mannheim, Germany) was used to determine the telomerase activity. Reactive telomerase activity was quantified with photometric enzyme immunoassay using TRAP and presented as percentage.

Determination of intestinal hypoxia inducible factor 1 α levels

Hypoxia-inducible factor 1 α (HIF-1 α) levels were defined with a solid phase sandwich ELISA (Rat HIF-1 α ELISA Kit; USCN Life Science Inc Wuhan, China; Cat No. E90798Ra, Lot No. 111011285).

Plasma tumor necrosis factor α and interleukin 6 measurements

Plasma tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) levels were also determined by a solid phase sandwich ELISA (Rat TNF- α ELISA Kit; Cat No. ER3TNFA, Lot No. LF144120, Rat IL-6 ELISA Kit; Cat No. EH2IL-6, Lot No. KL138035; Thermo Fisher Scientific, Waltham, USA).

Histopathological evaluation

The intestinal pieces laid in 10% buffered formalin were fixed for 72 h and set in paraffin blocks. Afterwards, five-micrometer-thick sections were cut axially using a microtome (RM 2245; Leica, Wetzlar, Germany). Hematoxylin and eosin (H&E) staining was performed for the sections and the slides were evaluated under a light microscope (DMI 4000 B; Leica) by the histologist of the team (NLU) who was blind to the study groups. In order to quantify the ischemic changes at the mucosal level, a scoring system described by Chiu et al. was used.¹⁶

Immunohistochemical evaluation

After fixing in formalin, embedding in paraffin and cutting sections 5 μ m in thickness, tissue sections were placed in an incubator at 37°C for a night and at 60°C for 1 h. Xylol was applied twice for 15 min. The slides were then placed in 96% absolute alcohol, 80% ethanol for 10 min and distilled water twice for 5 min. For caspase-3 immunoperoxidase, the slides were placed in a high temperature microwave oven in 10% citrate buffer. After the tissue was left for 20 min at room temperature, it was marked with a hydrophobic pen (Super PAP PEN IM3580; Immunotech, Beckman Coulter Co., Brea, USA). In order to block endogenous peroxidase activity, the tissue was washed with distilled water and 0.02 M PBS (pH 7.2) (Lab Vision Corp., Fremont, USA) and 0.3% hydrogen peroxide (Thermo Fisher Scientific) was applied thereafter for 10 min. After washing with PBS, ultra V block (TA-125-UB; Thermo Fisher Scientific) was utilized. Primary antibody including caspase-3 (rabbit polyclonal antibody; Lab Vision/Neo Markers Corp.) was applied for 1 h, the samples were washed with phosphate-buffered saline (PBS), and a post-PBS level was applied (biotinylated goat anti-polyvalent and streptavidin peroxidase, respectively). After having been rewashed with PBS, the specimens were placed for 10 min in AEC (3-amino, 9-ethylcarbazole) (TA_125-HA; Thermo Fisher Scientific) chromogen. Finally, the counterstain with Mayer hematoxylin was performed for 2 min. All the slides were evaluated using a light microscope (DMI 4000 b; Leica) by the same histologist (NLU). The relative intensity of immunoreactivity staining was quantified as described by McCarty et al., focusing on the intensity and the distribution of a specific stain.¹⁷ A histological score (H-score) was formulated as the sum of the percentages

of positively stained cells multiplied by the weighted intensity of staining. The H-score was calculated with the formulation of $\sum Pi(I + 1)$, as I represented intensity of staining (0 – none; 1 – mild; 2 – moderate; and 3 – intense) and Pi represented the fraction of stained cells for each intensity.

Statistical analysis

Statistical package SPSS for Windows v. 18.0 (SPSS Inc., Chicago, USA) was used for analyses with the descriptive statistics presented as mean $\pm$ standard deviation (SD). Kruskal–Wallis test and Mann–Whitney U test were used for statistical analyses and Spearman's correlation test was used to evaluate the correlation between variables. A p-value less than 0.05 was considered to be statistically significant.

Results

All the animals survived the experiment. The results obtained from each group are presented in Tables 1, 2.

Intestinal MDA levels

The MDA levels for proximal and distal intestinal segments of Gr I/R were elevated when compared to Gr C ($p < 0.05$). All treatment groups, not being superior to one another, represented a significant decrease of MDA level at the proximal segment whereas only Gr V-I/R represented a significant decrease at the distal segment (Table 1).

Table 1. Biochemical analysis of intestinal segments and plasma

Parameter	Group				
	Control	VEGF	I/R	V-I/R	I/R-V
MDA-proximal [μ M]	4.08 $\pm$ 0.40	4 $\pm$ 0.18	8.16 $\pm$ 1.56*	4.87 $\pm$ 0.58 [#]	4.99 $\pm$ 0.68 [#]
MDA-distal [μ M]	3.29 $\pm$ 0.46	3.3 $\pm$ 0.71	8.44 $\pm$ 2.97*	4.84 $\pm$ 1.19 [#]	6.84 $\pm$ 1.51
TGSH-proximal [μ M]	326 $\pm$ 32.78	317.2 $\pm$ 13.6	183.16 $\pm$ 31.5*	299.66 $\pm$ 86.48 [#]	250.33 $\pm$ 48.74
TGSH-distal [μ M]	488.45 $\pm$ 48.76	467.26 $\pm$ 60.55	160.63 $\pm$ 35.77*	341 $\pm$ 42.78 [#]	290.32 $\pm$ 60.9 [#]
GSSG-proximal [μ M]	3.93 $\pm$ 1.39	3.91 $\pm$ 0.76	8.29 $\pm$ 1.46*	3.62 $\pm$ 1.19 [#]	4.66 $\pm$ 0.83 [#]
GSSG-distal [μ M]	5.28 $\pm$ 1.36	5.5 $\pm$ 1.37	14.03 $\pm$ 2.19*	6.07 $\pm$ 0.97 [#]	6.58 $\pm$ 1.01 [#]
SOD-proximal [AU]	6.15 $\pm$ 1.32	6.9 $\pm$ 0.31	3.32 $\pm$ 0.75*	5.92 $\pm$ 0.89	5.33 $\pm$ 1.71
SOD-distal [AU]	8.54 $\pm$ 2.07	8.65 $\pm$ 1.52	3.54 $\pm$ 0.94*	7.02 $\pm$ 2.28 [#]	5.35 $\pm$ 1.31 [#]
PC-proximal [AU]	0.103 $\pm$ 0.013	0.101 $\pm$ 0.005	0.114 $\pm$ 0.011	0.097 $\pm$ 0.010	0.105 $\pm$ 0.006
PC-distal [AU]	0.191 $\pm$ 0.004	0.194 $\pm$ 0.002	0.199 $\pm$ 0.003*	0.172 $\pm$ 0.027 [#]	0.184 $\pm$ 0.008 [#]
CAT-proximal [AU]	0.83 $\pm$ 0.06	0.84 $\pm$ 0.16	0.47 $\pm$ 0.09*	0.55 $\pm$ 0.1 [#]	0.67 $\pm$ 0.15
CAT-distal [AU]	1.13 $\pm$ 0.14	1.18 $\pm$ 0.13	0.69 $\pm$ 0.12	0.79 $\pm$ 0.04	0.85 $\pm$ 0.12
HSP70-proximal [AU]	0.51 $\pm$ 0.24	0.49 $\pm$ 0.2	0.82 $\pm$ 0.18*	0.67 $\pm$ 0.2	0.64 $\pm$ 0.39
HSP70-distal [AU]	0.74 $\pm$ 0.13	0.75 $\pm$ 0.16	1.06 $\pm$ 0.55	0.81 $\pm$ 0.21	0.79 $\pm$ 0.17
Telomerase activity [%]	100	83.33	0*	50 [#]	66.67 [#]
Intestinal HIF-1 α [ng/mL]	9.62 $\pm$ 4.02	13.89 $\pm$ 2.45	15.01 $\pm$ 0.51*	13.84 $\pm$ 2.02	13.56 $\pm$ 3.51
Plasma TNF- α [pg/mL]	124.22 $\pm$ 20.70	110.39 $\pm$ 19.90	326.27 $\pm$ 84.93*	139.06 $\pm$ 5.05 [#]	114.76 $\pm$ 14.93 [#]
Plasma IL-6 [pg/mL]	16.30 $\pm$ 1.81	29.78 $\pm$ 5.41	280.24 $\pm$ 89.96*	131.69 $\pm$ 24.87 [#]	73.65 $\pm$ 6.67 [#] &

Biochemical analysis of intestinal segments and plasma, results presented as mean $\pm$ standard deviation (SD); * $p < 0.05$ compared to control group;

[#] $p < 0.05$ compared to I/R; [&] $p < 0.05$ when compared to V-I/R group; VEGF – vascular endothelial growth factor; MDA – malondialdehyde; TGSH – total glutathione; GSSG – oxidized glutathione; SOD – superoxide dismutase; PC – protein carbonyl group; CAT – catalase; HSP – housekeeping protein; HIF-1 α – hypoxia-inducible factor 1 α ; TNF- α – tumor necrosis factor α ; IL-6 – interleukin 6.

Table 2. Mean histopathological scoring, intestinal caspase-3 immunoreactivity and MMP-9 levels for all groups

Parameter	Control	VEGF	I/R	V-I/R	I/R-V
Mean histopathologic scores (grade)	0.16	0.33	2.66*	0.5 [#] &	1.16 [#]
Caspase-3	0.85 $\pm$ 0.37	1.07 $\pm$ 0.41	2.95 $\pm$ 0.45*	1.02 $\pm$ 0.45 [#]	1.2 $\pm$ 0.44 [#]
MMP-9	1.78 $\pm$ 0.35	1.83 $\pm$ 0.42	3.07 $\pm$ 0.20*	2.15 $\pm$ 0.46 [#]	2.32 $\pm$ 0.28 [#]

Intestinal caspase-3 immunoreactivity and MMP-9 levels, results presented as mean $\pm$ standard deviation (SD); * $p < 0.05$ compared to control group;

[#] $p < 0.05$ when compared to I/R group; [&] $p < 0.05$ compared to I/R-V group; VEGF – vascular endothelial growth factor; MMP-9 – matrix metalloproteinase 9.

Intestinal SOD activity

The SOD activity of both proximal and distal intestinal segments of Gr I/R were found decreased when compared to Gr C ($p < 0.05$). Either treatment represented a significant elevation only for the distal segment (Table 1).

Intestinal PC levels

The PC levels of distal intestinal segments of Gr I/R were found increased when compared to Gr C ($p < 0.05$) and either treatment represented significant fall for the same segment (Table 1).

Intestinal CAT activity

Catalase activity was found to decrease significantly at the proximal segment in Gr I/R when compared to Gr C ($p < 0.05$) and the rise of CAT was statistically significant only at the proximal segment for Gr V-I/R (Table 1).

Intestinal TGS and GSSG levels

A statistically significant increase in TGS level and a statistically significant decrease in GSSG level were seen at both proximal and distal intestinal segments of Gr I/R when compared to Gr C. Gr V-I/R represented a significant increase in TGS for both segments, whereas Gr I/R-V represented a significant rise at the distal segment. Both treatment groups represented a significant decrease in GSSG for both segments, but neither treatment was found superior (Table 1).

Intestinal telomerase activity

Telomerase activity was found to decrease to 0 in Gr I/R when compared to Gr C ($p < 0.05$) and all treatment groups recuperated the telomerase activity significantly (Table 1).

Intestinal HIF-1 α levels

The HIF-1 α levels were found increased in Gr I/R when compared to Gr C ($p < 0.05$), but the decrease in treatment groups were insignificant (Table 1).

Plasma TNF- α , and IL-6 levels

Both of the parameters were found significantly increased in Gr I/R when compared to Gr C. Either treatment group represented a significant decrease for TNF- α , and IL-6 when compared to Gr I/R. Despite no changes in TNF- α levels, Gr I/R-V had a more significant decrease to Gr V-I/R in terms of decrease in IL-6 levels ($p < 0.05$) (Table 1).

Histopathological evaluation

Gr I/R represented significant ischemic changes, such as generalized desquamation and moderate to severe mucosal congestion, when compared to Gr C. Both treatment groups represented enhanced histopathological findings, such as focal desquamation and mild mucosal congestion (Fig. 1). The comparison of histopathological scoring of treatment groups revealed a better outcome for Gr V-I/R when compared to Gr I/R-V with a statistically significant difference (Table 2). In terms of caspase activity and matrix metalloproteinase 9 (MMP-9) scores, Gr I/R represented the highest score and both treatment groups represented better scores when compared to Gr I/R ($p < 0.05$) (Table 2).

Discussion

The VEGF is a key signal protein that stimulates expansion of new vasculature from surviving vessels in case of hypoxia.⁷ This angiogenic activity is also supported by the vasodilator effect of protein through nitric oxide (NO) production. The VEGF is also shown to have antioxidant effects and to modulate endothelial dysfunction during IRI. In this study, we aimed to evaluate the effectiveness of VEGF on intestinal IRI during the different phases of the injury. For this purpose, experimental intestinal IRI model was established as described by Dwivedi et al. and an intravenous VEGF dose of 0.8 $\mu\text{g/kg}$ was used for treatment, as this dose was proven to be the minimal effective concentration in an earlier study.^{11,18} The VEGF was applied either before ischemia or during the reperfusion of the experimental intestinal IRI model and it was found that the drug was effective in both situations. This is also the first study that evaluated the changes of the bowel at separate levels (proximal and distal bowel segments) after ischemia and reperfusion. Both of the treatment groups represented enhancement in terms of tissue MDA, TGS/GSSG, SOD, PC, and CAT levels for both intestinal segments. However, some of the parameters showed different distributions – for example, better results with distal intestinal segments and better results with VEGF treatment before intestinal IRI. The differences between proximal and distal intestinal segments can be attributed to the anatomic settlement. Marginal artery (of Drummond) is the major anastomosis between superior and inferior mesenteric arteries. The marginal artery forms a continuous arterial circle along the inner border of the colon and it is probable that during SMA occlusion and reperfusion period the distal segments of the intestine was supported by inferior mesenteric artery (IMA) and presented better results. We observed that some parameters were recuperated only for Gr V-I/R (like MDA for distal segments, TGS and MDA for proximal segments). Application time of VEGF resulted in a difference that is of interest and difficult to justify.

Fig. 1. Histopathological view of intestinal segments stained with H&E in all groups. Panel (a) represents intestinal structures of Gr C within normal limits; (b) represents generalized desquamation (D) within intestinal epithelium and mucosal congestion (*) in Gr I/R; (c) represents intestinal structures of Gr V within normal limits; (d) represents localized desquamation (D) and mucosal congestion (*) with intact muscular layer in Gr I/R-V; (e) represents intestinal structures within normal limits of Gr V-I/R; and (f) represents localized desquamation (D) and mucosal congestion (*) of Gr V-I/R. Arrow – intestinal epithelium; L – intestinal lumen; G – intestinal secretory glands; LP – lamina propria; M – muscular layer; Bar = 1000 μ

Tissue hypoxia is inevitable as a result of capillary perfusion failure. Activation of hypoxia-inducible factor (HIF-1), which is the main regulator of pathophysiological response to hypoxia, has been shown to play an important role in inducing intestinal damage. This is why HIF-1 α , the subunit of heterodimeric transcription factor HIF-1, is accepted as the main transcriptional regulator for cellular hypoxia. However, HIF-1 α , constantly produced and kept low under normoxic conditions, increases rapidly during hypoxia and dimerizes with constitutively present HIF-1 β to stimulate VEGF production.^{19,20} Studies evaluating the effects of IRI on HIF-1 α at separate organs revealed that the ischemic injury is related to elevation of HIF-1 α .^{21,22} Also, Poynter et al. represented the relation between HIF-1 α and VEGF in an experimental ischemic myocardial injury.²¹ The findings of our study supported HIF-1 α elevation in Gr I/R, but apart from the other studies, even the treatment groups represented a decrease in HIF-1 α levels; such decrease was statistically insignificant. As mentioned previously, HIF-1 α is increased rapidly during hypoxia and dimerized with HIF-1 β in order to stimulate VEGF production and induce angiogenesis. Although a decrease in HIF-1 α levels is expected in the case of exogenous administration of VEGF, no such relationship was found in the findings of our study. It is probable that either HIF-1 α production is not affected by VEGF levels or the appearance of free radicals resulted in HIF-1 α being suspended in intestinal tissues despite its short half-life period.

Telomere is a DNA-protein complex consisting of 6 base repetitive sequences and telomere binding proteins at the end of the chromosomes of eukaryotic organisms. Telomeres protect against events such as disruption of the terminal regions of chromosomes. However, telomere segments are shortened by the cell cycle and cell division, which triggers cellular apoptosis.²³ Telomerase is a ribonucleoprotein reverse transcriptase that adds telomeric DNA repeat sequences to the 3'-end of chromosomes. Despite its essential role for telomere homeostasis, its activation leads to unlimited cell proliferation, probably resulting in neoplastic transformation. However, this reverse transcriptase also reactivates during tissue repair.²⁴ Eckers et al. reported that oxidative stress and elevated ROS are closely related to endothelial cell apoptosis and endothelial dysfunction, loss of telomerase activity and telomere shortening.²⁵ Werner et al. presented the protective effect of upregulated telomerase activity against endothelial apoptosis in case of oxidative stress.²⁶ Zaccagnini et al. showed that there was a positive regulation of VEGF and telomerase activity in a hind limb ischemia study in rats.²⁷ The IRI related oxidative stress was also obtained in our study and we found that telomerase activity disappeared in the I/R group, and both treatment groups exhibited elevated telomerase activity, supporting reactivation of telomerase against injury.

The heat shock proteins are a class of molecular chaperons that exist in cell cytoplasm in order to prevent

the formation of nonfunctional structures. It is thought to act as a cytosolic chaperone that plays a role in facilitating protein folding, degradation, complex assembly, and translocation. The best known members are the heat-inducible form, also known as HSP72 or HSP70i, and are also present in the structural form, also called HSC70, HSP73 or HSC73.²⁸ HSP70 interacts with hydrophobic peptide parts of proteins that are not ATP-bound. These cytoprotective proteins are induced by many factors, such as infection, oxidative stress, osmotic stress, hyperthermia, ultraviolet exposure, and heavy metals. In the presence of these inducers, chaperone properties have been reported to reduce protein aggregates and intracellular inclusions. The catalytic unit of telomerase (TERT) is known to interact with chaperone proteins. The 70 kDa heat shock proteins (HSP70), as a chaperone protein was shown not to be vital for telomerase activity but also to be important for telomerase stability.²⁴ The literature review revealed a number of IRI studies on various organs representing elevation of HSP70.²⁹ We also evaluated the HSP70 levels in our study and found the significant elevation of HSP70 at only the proximal intestinal segment, where IMA does not supply in Gr I/R, supporting the vascular compromise in that region. HSP70 was shown to be a cell-protector for apoptosis, but its overexpression was generally proven to be related to poor prognosis.³⁰ Despite insignificant changes in HSP70 in our treatment groups, caspase activity was found to be elevated with IRI and the treatment with VEGF normalized this activity. Even though the last step of either intrinsic or extrinsic pathways of apoptosis is caspase-3 activation, DNA damage and heat shock proteins are mainly involved in intrinsic pathway. However, the increase of HSP70 and caspase-3 in Gr I/R is rational, but the presence of caspase activation in the treatment groups despite low HSP70 is beyond our predictions. These findings supported the claim that the extrinsic pathway stimulated abundantly during I/R is somehow less affected.

Intestinal IRI is a clinical entity related to life-threatening conditions, such as mesenteric artery occlusion, intestinal transplantation, intussusceptions, acute blood loss, and trauma. Each of these conditions, however, causes the same physiological consequence and requires separate approaches in the clinical settings. In this study, we applied VEGF either before ischemia or during the reperfusion of the experimental intestinal IRI model and aimed to evaluate whether any changes occur. We found that the drug was effective in terms of enhancing intestinal IRI in both situations; apart from that, the treatment before ischemic process was found slightly superior to the other therapy. In the light of these findings, we can probably speculate on the benefits of VEGF usage of different timetables for different causes of intestinal IRI. It is obvious that further studies are needed to support this inference.

Conclusions

Intestinal IRI is a clinical entity related to life-threatening conditions like mesenteric artery occlusion, intestinal transplantation, intussusception, acute blood loss, and trauma. Each of these conditions, however, causes the same physiological consequence and has separate approaches in the clinical settings. That is why, the use of VEGF at different timetables of the ischemic reperfusion process could serve different outcomes for different reasons of IRI.

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