MEZENTERİK İSKEMİ-REPERFÜZYONLA İNDÜKLENEN İNTESTİNAL DOKU HASARINA KARŞI EVODİAMİN'İN ETKİSİ: OKSİDATİF STRESİN ROLÜ

The Effect of Evodiamine Against Intestinal Tissue Injury Induced By Mesenteric Ischemia-Reperfusion: Role of Oxidative Stress

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ÖZET

Amaç: Bu çalışma, evodiaminin mezenterik iskemi-reperfüzyonun neden olduğu intestinal dokudaki oksidatif hasara karşı muhtemel yararlı etkisini göstermeyi amaçlamıştır.

Materyal ve Metod: Bu amaçla çalışmamızda deney hayvanlarında mezenterik iskemi-reperfüzyon modeli yapıldı. İskemi-reperfüzyon ve evodiamin düşük ve yüksek doz tedavi gruplarında, superior mezenterik arter 1 saat süreyle klemplendi. Daha sonra klemp açıldı ve 2 saatlik reperfüzyon başlatıldı. Deney sürecinin sonlandırılmasından sonra, tüm hayvanlar sakrifiye edildi ve intestinal doku örnekleri toplandı.

Bulgular: TOS, OSI, MDA düzeyleri ve MPO aktivitesinin sham grubuna göre mezenterik iskemi-reperfüzyon grubunda arttığı görüldü. ''Ayrıca mezenterik iskemi-reperfüzyon grubunda SOD aktivitesi ve TAS seviyesinin sham grubuna göre azaldığı tespit edildi. ''Düşük ve yüksek dozlarda evodiamin (10 ve 20 mg / kg) uygulanan gruplarda mezenterik iskemi-reperfüzyon grubuna göre TAS değeri ve SOD aktivitesi artarken, TOS, OSI değerleri, MPO aktivitesi ve MDA düzeyi azaldığı belirlendi.

Sonuç: İki farklı evodiamin dozu mezenterik iskemi-reperfüzyonun neden olduğu intestinal dokunun oksidatif hasarına karşı yararlı etkiler göstermiştir.

Anahtar Sözcükler: *Mezenterik iskemi-reperfüzyon; Evodiamin; İntestinal doku hasarı; Sıçan*

ABSTRACT

Purpose: This study aimed to show possible benefical effect of evodiamine against oxidative damage of intestinal tissue induced by mesenteric ischemia-reperfusion.

Materials and Methods: For this purpose, in our study mesenteric ischemia-reperfusion model was conducted in experimental animals. In low and high doses treatment of evodiamine and ischemia-reperfusion groups, superior mesenteric artery was clamped for 1 h. Then, the clamp was opened and reperfusion was started for 2 h. After the termination of the experimental prosses, all animals were sacrificed and intestinal tissue samples were collected.

Results: It was seen that TOS, OSI, MDA levels and MPO activity increased in mesenteric ischemia-reperfusion group compared to sham group. Moreover, SOD activity and TAS level reduced in mesenteric ischemia-reperfusion group compared to sham group. When compared to mesenteric ischemia-reperfusion group, TAS value and SOD activity increased while TOS, OSI values, MPO activity and MDA level decreased in groups treated with low and high doses of evodiamine (10 and 20 mg/kg).

Conclusion: Two different doses of evodiamine revealed benefical effects against oxidative damage of intestinal tissue induced by mesenteric ischemia-reperfusion.

Keywords: *Mesenteric ischemia-reperfusion; Evodiamine; Intestinal tissue damage; Rat*

INTRODUCTION

Mesenteric ischemia is a vascular and gastrointestinal surgical emergency in patients that results from either venous thrombotic and arterial thromboembolic occlusions or non-occlusive reasons due to mesenteric hypoperfusion with different pathologies such as sepsis and low cardiac output (1, 2). Intestinal ischemia reperfusion (I/R) has high morbidity and mortality rate because of the hassles in diagnosis and clinical treatment. Intestinal I/R is a medical case results from blood flow restriction or low perfusion and it is common in sepsis, vascular and abdominal procedures, hernias, intra-abdominal adhesions, hemorrhagic shock, major trauma, necrotizing enterocolitis and intestinal transplantation (1-4). In I/R, different pathophysiological results such as decreasing in adenosine-triphosphate production and oxygen supply, increasing in generation of reactive oxygen species (ROS), reactive nitrogen species (RNS) and accumulation of lactic acid may emerge (5, 6). ROS/RNS leads to the damage of biomolecules such as nucleic acids, enzymes/proteins, lipids in the cells. These situations are defined as reperfusion injury. Moreover, it adheres to polyunsaturated fatty acids of membrane and peroxidation prosses starts (7). Intestinal tissue damage occurs as a result of peroxidation.

In many scientific studies to date, it has been tested various pharmacologic drugs or agents to reduce ROS formation and protect/treat bowel tissue against ischemic damage (8, 9). Evodiamine as a natural product, quinazoline alkaloid isolated from plants, is shown to be harmless to human health. Moreover, it was benefited in Traditional Chinese Medicine for treatment of many clinic cases such as abdominal pain, amenorrhea, headache, dysentery and postpartum hemorrhage (10-12). In previous studies, it has been shown that evodiamine has anticancer and antioxidant efficiencies (13, 14).

As a result of our literature research, it was seen no scientific study about the use of evodiamin in the treatment of intestinal ischemic injury. Therefore, in this study, we aimed to investigate the possible beneficial effects of evodiamine in the treatment of experimental intestinal injury induced by mesenteric I/R.

MATERIALS AND METHODS

Ethical approval and drugs

All the experiments of presented study were conducted at Atatürk University Experimental Animals Research and Application Center (ATADEM). Atatürk University Experimental Animals Local Ethics Committee approved this experimental study (28.06.2018/147). All experimental animals were maintained in standard laboratory conditions such as temperature 25 degree, moisture %55 and 12-h light/dark cycle. Evodiamine was obtained from Sigma Aldrich USA. Ketamine (Ketalar 500 Mg Injectable Flakon) and Xylazine (Alfazyne %2 Injectable) were purchased from Pfizer İlaçları Ltd. Şti. İstanbul, Turkey and Ege vet Hayvancılık San. ve Tic. Ltd. Şti. İzmir, Turkey.

Experimental Design

Thirty two female Wistar albino rats weighing (200-250 gr) were used in this study. All rats were weighed and divided into four groups (8 rats in each groups). Groups were designed as sham, mesenteric ischemia-reperfusion, low dose of evodiamin and high dose of evodiamine groups.

1. Sham group (Sham): The abdominal area of the animals were shaved and cleaned, then an abdominal area was opened with an incision and closed under anaesthesia.
2. Mesenteric ischemia-reperfusion (MIR) group: The abdominal regions of the animals were opened, the superior mesenteric artery was detected and clamped for 1 hour. Then, the clamp was opened and allowed to reperfusion for 2 hours.
3. Low dose of evodiamine (Low dose of Evo) group: Evodiamine was administered to animals by oral gavage at the dose of 10 mg/kg for 15 days. Later, I/R model was conducted as described in MIR group. The dose of evodiamine was selected with the reference of a previous study (15).
4. High dose of evodiamine (High dose of Evo) group: Evodiamine was administered by oral gavage at the dose of 20 mg/kg for 15 days. Later, I/R model was conducted as described in MIR group. The dose of evodiamine was selected with the reference of a previous study (10).

After all experiments were completed, the rats were sacrificed under high-dose anesthesia (ketamin/xylazin

50/10 mg/kg). At the end of the experiment, the intestinal parts were taken quickly and kept at -80 °C.

Biochemical Analysis

Tissue samples were weighed for 100 mg and homogenized with 2 mL of phosphate buffer. Homogenized tissues were centrifuged at 5000 rpm for 20 minutes at +4 °C. The supernatant was carefully transferred to tubes and maintained at -80 °C. Malondialdehyde (MDA) measurement principle, as a result of lipid peroxidation, is based on measuring the absorbance at 532 nm of the pink color compound formed as a result of the reaction of MDA and thiobarbituric acid (TBARS) (16). Total antioxidant status (TAS) value was determined with the commercially available kit (Rel Assay Diagnostics, Turkey). Total oxidant status (TOS) measurement was performed with commercially available kit (Rel Assay Diagnostics, Turkey). The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). OSI value was calculated as follows: OSI = [(TOS, µmol H2O2 equivalent L)/(TAS, mmol Trolox equivalent/L) × 10]. The measurement of myeloperoxidase (MPO) activity is based on the kinetic measurement of the absorbance at 460 nm wavelength of the yellowish-orange colored complex as a result of the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide (17). Xanthine oxidase enzyme catalyzes the uric acid from xanthine. The resulting superoxide radical forms the molecular oxygen and hydrogen peroxide with the superoxide dismutase (SOD) enzyme. The resulting superoxide reacts with the tetrazolium salt to form a formazan dye in situations where the effect of the SOD is insufficient, and the SOD activity is measured with the inhibition degree of this reaction (18).

Statistical Analysis

IBM SPSS Statistics 22 package program was used for statistical analysis of the results. One-Way ANOVA and Tukey tests were applied for all the results to determine the statistical significance among the groups. The results were presented as mean ± standard deviation. $p < 0.05$ was considered statistically significant.

RESULTS

The mean±standard deviation results of TAS (mmol/L), TOS (µmol/L) and OSI (TOS/TAS) values of all experimental groups were summarized in table 1. It is noteworthy that the TAS value significantly decreased in the MIR group compared to sham group, and it is decreased in low and high dose treatment of Evo groups compared to MIR group ($p<0.05$). TOS and OSI values increased in MIR group compared to sham group ($p<0.05$). But these values decreased in low and high dose of Evo groups compared to MIR group.

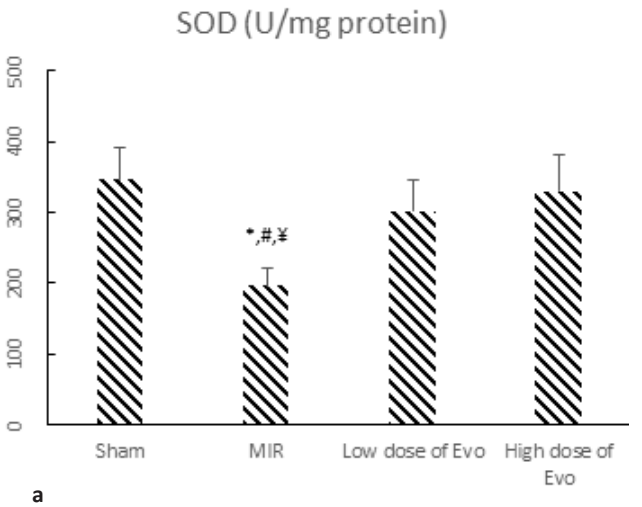
The mean±standard deviation results of SOD (U/mg protein), MPO (U/g protein) activities and MDA (µmol/g protein) level of all experimental groups were presented in figure 1. MDA level and MPO activity increased in MIR group compared to sham group ($p<0.05$). But these values decreased in low and high dose of Evo groups compared to MIR group. In contrast to them, SOD activity decreased in MIR group compared to sham group ($p<0.05$). But it increased in low and high dose of Evo groups compared to MIR group.

Table 1: The mean±standard deviation results of TAS (mmol/L), TOS (µmol/L) and OSI (TOS/TAS) values of all experimental groups are summarized in the table.

	TAS (mmol/L)	TOS (µmol/L)	OSI
Sham	1,64±0,09	4,06±0,31	0,24±0,01
MIR	0,76±0,13 *,#,¥	6,24±0,88 *,,#,¥	0,84±0,22 *,,#,¥
Low dose of Evo	1,29±0,14	5,48±0,45	0,43±0,07
High dose of Evo	1,47±0,20	5,91±0,67	0,4±0,06

$p<0.05$ was considered statistically significant. *: Statistically significant relationship between sham and MIR groups. #: Statistically significant relationship between MIR and Low dose of Evo groups. ¥: Statistically significant relationship between MIR and High dose of Evo groups.

Figure 1.a: The results of SOD (U/mg protein) is presented as mean±standard deviation, b: The results of MDA (µmol/g protein) is presented as mean±standard deviation and c: The results of MPO (U/g protein) is presented as mean±standard deviation. p<0.05 was considered statistically significant. *: Statistically significant relationship between sham and MIR groups. #: Statistically significant relationship between MIR and Low dose of Evo groups. ¥: Statistically significant relationship between MIR and High dose of Evo groups.



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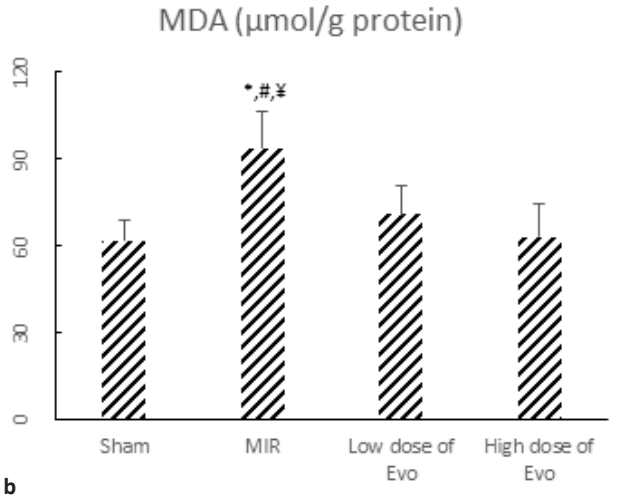

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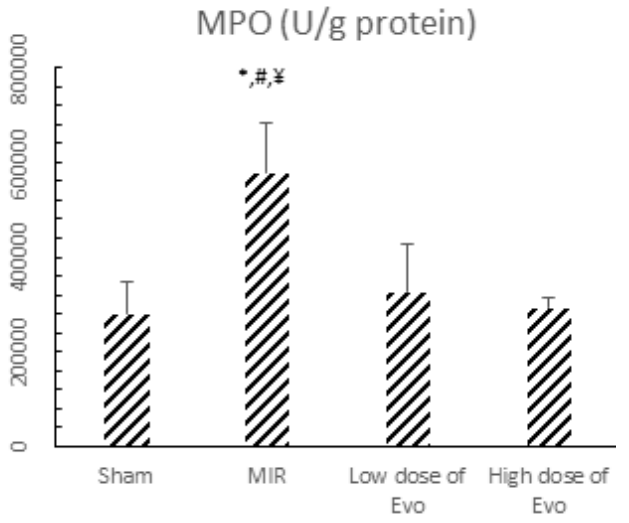

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DISCUSSION

Intestinal ischemia may occur by some clinical situations including invagination, volvulus, mesenteric embolism and small intestine transplantation (19). The intestines are the most sensitive organs for I/R (20). The early diagnosis of MIR induced by intestinal tissue injury is diffucult and it can turn into a life-threatening abdominal emergency due to the high mortality rate (21). In many studies, ROS derived from xanthine oxidase in endothelial cells have been reported to be effective in the pathophysiology of intestinal I/R injury (22, 23). In intestinal I/R damage, activated neutrophils cause generation of the proteases and MPO in addition to ROS. Oxidative stress plays a serious role in intestinal I/R injury. Intestinal mucosa has been shown to be vulnerable against oxidative stress response particularly caused by I/R-induced increasing in ROS generation.

Due to I/R, cells produce a variety of reactive products such as hydrogen peroxide, superoxide anion, hydroxyl radical and peroxynitrite, which severely damage different cellular molecules such as DNA, lipids and proteins (6, 24). The measurement of MDA level, an important end product of lipid peroxidation, and MPO activity reflect the amount of ROS formed in cells and thus it is considered as a major indicator for oxidative stress. The measurement of oxidant and antioxidant levels may not be sufficient individually to reveal most of the oxidative stress status clearly. Therefore, in order to be able to express the state of oxidative stress

absolutely, measurement of TAS and TOS, which have more good precision values, is often preferred in order to give more accurate and inclusive results (25, 26). OSI is the ratio of TOS to TAS and considered significant because it is an important result that simply and easily reveals the balance between antioxidants and oxidants (27). Several endogenous antioxidant enzymes, such as SOD and catalase (CAT), are vital components of cellular defense against oxidative and/or nitrosative stress (28).

For this reason, antioxidants are considered as a significant component of therapeutic agents against mesenteric I/R-induced intestinal injury. In different studies, various antioxidants such as ukrain, resveretrol and N-acetylcysteine were used in alleviating of intestinal I/R injury by scavenging ROS (9, 29, 30). It has been demonstrated that the severity of oxidative damage decreased with the antioxidant treatments used in these studies. Previous scientific studies have also reported that evodiamine, that we used in this study, has anti-nociceptive, anti-obesity, antioxidant, vasodilator, anti-tumor and anti-inflammatory effects (10, 14, 31, 32). Besides these, it was detected that evodiamine prevents obesity and improves glucose tolerance (33). Zhao and colleagues evaluated therapeutic effect of evodiamine and showed that it significantly reduces the amount of MDA and promotes antioxidant activity (34). In another study, it was suggested that evodiamine treatment showed a preservation against myocardial I/R injury in experimental animals (35).

In our study, we aimed to evaluate the beneficial effects of evodiamine in order to alleviate intestinal oxidative damage due to mesenteric I/R. When we evaluate some oxidative stress indicators; MDA, TOS, OSI levels and MPO activity were significantly elevated in the mesenteric I/R group. However, we observed that these findings were significantly reduced in evodiamine-treatment groups and therefore the severity of oxidative damage was reduced. Different doses of evodiamine treatment were effective via supporting the antioxidant system against mesenteric I/R damage. Our findings are consistent with data of previous studies (29, 30, 34, 35).

In the light of all these results, we can say that treatments with low and high doses of evodiamine have been effective by reducing free radical formation and supporting antioxidant defense against mesenteric I/R-induced intestinal oxidative damage.

Conflicts of interest

The authors declare no conflict of interest.

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