EXPERIMENTAL STUDY

Effects of piperine in experimental intestinal ischemia reperfusion model in rats

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ABSTRACT

BACKGROUND: Piperine is a spice principle, and its protective role against oxidative damage and lipid peroxidation has been reported. In this study, we aimed to investigate the effects of piperine in the prevention of ischemia-reperfusion injury to the small intestine.

METHODS: Rats were allocated to three groups of 8 rats each. Rats in the sham group underwent laparotomy and observation only. Animals in the control and study groups underwent 45 minutes ischemia followed by 60 minutes reperfusion. In the study group, 10 mg/kg piperine was administered intraperitoneally just before the reperfusion procedure. Blood samples were obtained for measurement of lactate levels, and resection of the terminal ileum was performed to evaluate the histopathologic specimens and tissue malondialdehyde, superoxide dismutase, and glutathione activities. All results were expressed as mean±SD. Comparisons between groups were made by using the one way analysis of variance (ANOVA).

RESULTS: Lactate and malondialdehyde levels were significantly higher in the control group than the study and sham groups ($p<0.001$). In the study group, superoxide dismutase, and glutathione activities were significantly higher than in the control group ($p<0.001$). The sham group had the highest activities. Histopathologic examination showed disruption of villous pattern and lamina propria in the control group.

CONCLUSION: Intraperitoneal administration of 10 mg/kg piperine just before the reperfusion may reduce ischemia-reperfusion injury to the small intestine.

Key words: Ischemia; piperine; reperfusion; small intestine.

INTRODUCTION

Intestinal ischemia-reperfusion is a common clinical event associated with both clinical and experimental distant organ injury. Arterial ischemia initiates alterations in tissues by blocking the oxygen supply, thus impeding aerobic energetic metabolism. During the ischemia process, there is an accumulation of metabolites, which, directly or through mediators, can cause cellular injury.[1,2] Depending on the time and intensity of the ischemia, when oxygen is reintroduced to the tissues, tissue injury can be further exacerbated (oxygen paradox).[3] Reperfusion leads to an aggravation of ischemic cell damage, especially reactive oxygen species (ROS) derived from activated Kupffer cells and neutrophils.[4] ROS and reactive metabolic intermediates generated from various oxidative factors are known to have an important role in cell damage and in the progression of ischemia-reperfusion injury (IRI).[5] Despite intensive researches that have aimed to investigate the pathogenesis and find a way to prevent or decrease the additional deleterious effect of IRI, the underlying mechanisms remain to be elucidated.

There are many reports indicating that various spice principles form an important group as antioxidants. Piperine is a pungent alkaloid present in black and white pepper (Piper nigrum), long used as a spice and preservative.[6] Piperine has been shown to alter both the bioavailability[7] and biotransformation[8,9] of xenobiotics, and to alter lipid peroxidation and availability of glutathione in the liver[10] and intestine.[11] Piperine has also been demonstrated in in vitro experiments

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to protect against oxidative damage by inhibiting or quenching free radicals and ROS and inhibiting lipid peroxidation.[12] We report here the results of an experimental study to evaluate whether intraperitoneal administration of piperine could be an effective strategy to reduce intestinal IRI.

MATERIALS AND METHODS

This study was performed at Erciyes University Experimental Research Center. Twenty-four male Wistar-Albino rats weighing 250 to 300 g were used for this study. Pre- and postoperatively, the animals were maintained under controlled conditions of temperature (21-24°C), humidity (40%-60%), and light (12-hour light/dark cycle) and fed ad libitum on rat cubes and tap water. Our institutional ethical committee approved the experimental procedures of this study. The animals were divided randomly into three groups containing 8 rats each as follows: group 1 (sham), animals were sacrificed at the end of 105 minutes (min) observation after laparotomy; group 2 (control), operative procedure without further treatment; and group 3 (study), operative procedure with intraperitoneal piperine administration (10 mg/kg).[6] Intestinal ischemia was induced by microvascular clip occlusion of the superior mesenteric artery (SMA) for 45 min. Reperfusion was performed for 60 min after SMA clip removal.[13] In the study group, intraperitoneal piperine (Sigma, P49007, 10 mg/kg) was administered at the end of 45 min ischemia just before the removal of the microvascular clip.

Operative Procedures

The rats were anesthetized using intraperitoneal ketamine hydrochloride (Ketalar; Parke Davis-EWL, İstanbul, Turkey; 20 mg/kg body weight) and xylazine (Rompun, Bayer, İstanbul, 10 mg/kg). After the abdomen was shaved and cleansed with povidone iodine solution, a 5-6-cm midline laparotomy was performed. All procedures were performed under sterile conditions by one surgeon who was blinded to the animal allocations. In groups 2 and 3, 1 ml heparinized blood for serum lactate analysis was obtained from the inferior vena cava after reperfusion, while in the sham group, it was obtained after the end of the observation period. For tissue analysis, a 3-cm ileal segment 10 cm proximal to the ileocecal valve was removed, and 2 cm was fixed in 10% neutral buffered formalin and embedded in paraffin for histopathological evaluation. Paraffin sections 5 µm in thickness were cut and stained with hematoxylin and eosin. Assessment of ileal injury was performed by light microscopy using a scoring system devised by Chiu et al. without knowledge of the study groups. The remaining 1 cm was conserved in an aluminum foil at -80°C for tissue malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH) activities.

Statistical Analysis

All results were expressed as mean±SD. Comparisons of MDA, SOD and lactate between groups were made by using the one way analysis of variance (ANOVA). Post-hoc comparisons of parameters were performed using the Tukey procedure. Statistical significance was set at $p<0.05$. All analyses were performed with the Statistical Package for Scientists (SIGMASTAT) Windows version 3.50.

RESULTS

Lactate levels were 1.29±0.07 mmol/L in the sham, 3.29±0.06 mmol/L in the control and 1.86±0.07 mmol/L in the piperine groups. The sham group had the lowest level while the control group had the highest, and the differences were statistically significant ($p<0.001$ for all) (Fig. 1a). Tissue MDA contents were 0.16±0.02 µM/mg in the sham, 0.38 ± 0.05 µM/mg in the control and 0.22±0.04 µM/mg in the piperine groups, and the content was significantly lower in the sham group. The control group had significantly higher content than the others ($p<0.001$) (Fig. 1b).

Tissue SOD levels were 5.13±0.57 U/ml/g in the sham, 2.70±0.57 U/ml/g in the control and 3.77±0.48 U/ml/g in the piperine groups. The sham group had the significantly highest level while the control group had the significantly lowest level ($p<0.001$) (Fig. 1c). Tissue GSH levels were 89.06±11.78 nmol/ml/g in the sham, 32.89±6.55 nmol/ml/g in the control and 64.16±10.45 nmol/ml/g in the piperine groups. The level in the sham group was significantly higher than in the others. The control group had the significantly lowest level ($p<0.001$) (Fig. 1d).

Histopathologic examination of the specimens showed well-preserved villi and intact intestinal mucosa in the sham group. Disruption of villous pattern, increased cellularity in the lamina propria, extension of the subepithelial space with the epithelial layer lifting up, and dilated capillaries were found mostly in the control group. Chiu scores were 0, 1 and 3, respectively, in the sham, study and control groups ($p<0.001$).

DISCUSSION

Ischemia and reperfusion of the small intestine provoke the rupture of the mucosal barrier, bacterial translocation and the activation of inflammatory responses,[1] as well as hydroelectrolytic and acid-alkaline equilibrium disturbances, which are manifested in distant organs.[14] With the return of blood perfusion, the influx of calcium into the intracellular medium increases, which leads to an expressive increase in phospholipase A2 activity. Arachidonic acid released by phospholipase A2 is metabolized during reperfusion by the enzyme cyclooxygenase, generating prostaglandins, thromboxane and prostacyclins, and by the enzyme lipoxygenase, which generates leukotrienes.[15] Another factor that induces intestinal injury after reperfusion is the generation of free radicals from oxygen molecules, derived from the electron transport chains of the mitochondria, xanthine-oxidase metabolism, endothelial cells, prostaglandins, and activated neutrophils.[4]

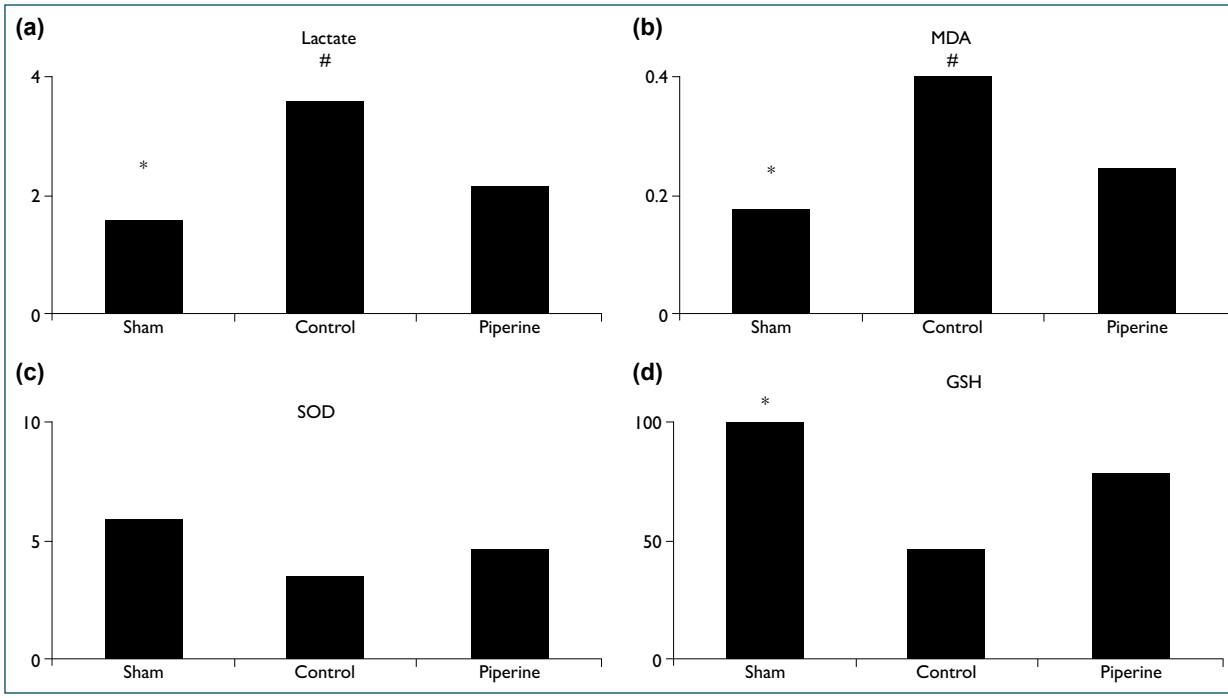

Figure 1. (a) The comparison of serum lactate levels. **(b)** The comparison of MDA levels. **(c)** The comparison of serum SOD levels. **(d)** The comparison of GSH levels. *: p<0.001 Sham group versus Control and Piperine groups; #: p<0.001 Control versus Piperine group.

Many studies in the literature have evaluated a lot of materials to prevent or decrease the effects of IRI. Many radical scavengers, interestingly naturally occurring antioxidants, have been found to be effective in inhibiting the induction of lipid peroxidation and ROS.[16-18] Studies have also indicated that various spice principles form an important group as antioxidants.[19-21] In this study, we investigated whether piperine might have a protective role in an experimental intestinal ischemia-reperfusion model in rats. Piperine has been demonstrated in in vitro experiments to protect against oxidative damage by inhibiting or quenching free radicals and ROS and inhibiting lipid peroxidation.[12,22] The aqueous extract of black pepper as well as piperine have been examined for their effect on human PMNL 5-lipoxygenase (5-LO), the key enzyme involved in biosynthesis of leukotrienes. [23] The formation of the 5-LO product 5-HETE was significantly inhibited with 60 μM piperine. Thus, piperine of black pepper might exert an antioxidant physiological role by modulating the 5-LO pathway. Using diabetes mellitus as a model of oxidative damage, Rauscher et al.[6] investigated whether intraperitoneal piperine treatment would protect against diabetes-induced oxidative stress. They demonstrated that treatment with piperine reversed the diabetic effects on glutathione concentration in the brain, on renal GSH and SOD activities, and on cardiac glutathione reductase activity and lipid peroxidation.

Selvendiran recently investigated the impact of piperine on alterations of the mitochondrial antioxidant system and lipid peroxidation. Oral supplementation of piperine revealed a decrease in the extent of mitochondrial lipid peroxidation and concomitant increase in the activities of enzymatic antioxidants (SOD, catalase, GSH) and nonenzymatic antioxidants (reduced glutathione, vitamin E, vitamin C). They reported that piperine modulates lipid peroxidation and increases the antioxidant defense system.[24]

Vijayakumar et al.[25] recently examined the effect of supplementation of black pepper or piperine on tissue lipid peroxidation and enzymic and non-enzymic antioxidants in rats fed a high-fat diet, and they observed that these spices can reduce high-fat diet-induced oxidative stress. They observed that simultaneous supplementation with black pepper or piperine lowered thiobarbituric acid reactive substances and conjugated dienes levels and maintained SOD, catalase, GSH, glutathione-S-transferase, and reduced glutathione levels near to those of control rats.

In this study, the results were consistent with the previous reports. The statistically significant highest MDA and lactate levels and the statistically significant lowest GSH and SOD levels were in the control group. MDA and lactate levels of the piperine group were significantly lower than in the control group, and GSH and SOD levels were significantly higher. These results clearly show that intraperitoneal administration of piperine significantly decreases the effects of lipid peroxidation and protects against oxidative damage. The histopathologic examination revealed similar findings with intact intestinal wall in the sham group and well-preserved, mildly affected structures in the study group, but with disruption of villous pattern and lamina propria with dilated capillaries and increased cellularity in the control group. Although there are numerous reports

about the effectivity of spice species on peroxidation and oxidative stress, since IRI is a very complex process including many yet to be resolved steps in its pathogenesis, new studies are warranted for a further understanding of the biological effects of piperine regarding its inhibitory activities in IRI.

In conclusion, in this experimental IRI model in rats, piperine treatment decreased IRI of the small intestine according to both morphological and biochemical criteria, even though its detailed mechanism of action remains unclear.

Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Sıçanlarda oluşturulan deneysel iskemi-reperfüzyon modelinde piperinin etkileri

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AMAÇ: Piperin oksidatif hasara ve lipit peroksidasyonuna karşı koruyucu etkisi bildirilmiş bir baharat türevidir. Çalışmamızda ince bağırsak iskemi-reperfüzyon hasarının önlenmesinde piperinin etkisini incelemeyi amaçladık.

GEREÇ VE YÖNTEM: Sıçanlar her biri 8 sıçan içeren 3 gruba ayrıldı. Sham grubundaki sıçanlara sadece laparotomi ve gözlem uygulandı. Kontrol ve çalışma grubundaki hayvanlara 45 dakikalık iskemiyi takiben 60 dakikalık reperfüzyon uygulandı. Çalışma grubuna piperin, reperfüzyon işleminden hemen önce 10 mg/kg dozunda periton içi yolla verildi. Laktat seviyelerinin ölçümü için kan numuneleri alındı. Histopatolojik inceleme, doku malondialdehid, süperoksit dismutaz ve glütatyon aktivitesi ölçümleri için de terminal ileum rezeksiyonu gerçekleştirildi. Gruplar arası istatistiki karşılaştırma ANOVA testi ile yapıldı.

BULGULAR: Kontrol grubunda laktat ve malondialdehid seviyeleri çalışma ve sham gruplarına göre anlamlı olarak yüksekti ($p<0.001$). Çalışma grubunda, süperoksit dismütaz ve glutatyon aktiviteleri kontrol grubundan anlamlı olarak yüksekti ($p<0.001$). Sham grubu her iki parametrede de en fazla aktiviteye sahipti. Histopatolojik incelemede kontrol grubunda villöz yapının ve lamina propria bütünlüğünün bozulduğu görüldü.
TARTIŞMA: Reperfüzyondan hemen önce periton içi verilen 10 mg/kg piperin ince bağırsaklarda iskemi-reperfüzyon hasarını azaltabilir.
Anahtar sözcükler: İskemi, piperin, reperfüzyon, ince bağırsak.

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