

THE EFFECT OF BOSENTAN ON PLASMA ISCHEMIA-MODIFIED ALBUMIN LEVELS IN ACUTE MESENTERIC ISCHEMIA

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ABSTRACT

Objective: Our aim in this study was to examine the effects of bosentan, an orally active antagonist of endothelin A and B receptors, on plasma ischemia-modified albumin (IMA) levels in mesenteric ischemia induced rats.

Material and Method: In this randomized, controlled trial 36 mature female wistar rats were divided into three groups. Group 1 (n=12) was the control group which only laparotomy was performed. Group 2 (n=12) was the ischemia group and group 3 (n=12) was the bosentan pretreated (100 mg/kg-1 day, 1 gavage/day for two days before surgery) ischemia group. In the ischemia groups (GR 2 and GR 3) following laparotomy the superior mesenteric artery was clamped using a bulldog clamp

during laparotomy. Blood samples were taken at 30 minutes from all groups and IMA levels were studied.

Results: Plasma IMA levels in the ischemia group (GR 2) was significantly higher compared to those of the control ($p<0.001$) and bosentan pretreated group (GR 3) ($p=0.002$). Serum IMA levels were higher in GR 3 than control group but there were no statistical significance ($p=0.659$).

Conclusion: These preliminary results suggest that bosentan might have a protective effect in mesenteric ischemic conditions.

Key Words: Ischemia-modified albumin, bosentan, acute mesenteric ischemia *Nobel Med 2013; 9(2): 96-99*

AKUT MEZENTERİK İSKEMİDE, BOSENTAN'IN MODİFİYE ALBUMİN SEVİYELERİ ÜZERİNE OLAN ETKİSİ

ÖZET

Amaç: Bu deneysel hayvan çalışmasındaki amacımız; endotelin A ve B reseptör antagonisti olan Bosentan adlı preparatın, mezenterik iskemi oluşturulan ratlarda plazma iskemi modifiye albumin (İMA) üzerine olan etkilerini araştırmaktır.

Materyal ve Metod: Randomize kontrollü olarak planlanan bu çalışmada 36 matür dişi wistar rat kullanılmış ve 3 ayrı gruba ayrılmıştır. 1. grup (n=12) sadece laparotomi uygulanan kontrol grubu, 2. grup (n=12) mezenter iskemi oluşturulan grup ve 3. grup (n=12) mezenter iskemi öncesi bosentan tedavisi (100 mg/kg-1 gün, 1 gavaj/gün, cerrahi öncesi 2 gün

boyunca) alan gruptur. İskemi oluşturulan 2. ve 3. grupta laparotomi sırasında superior mezenterik arter bulldog klemp ile klemlenmiştir. Tüm gruplardan kan örnekleri iskemi sonrası 30. dakikada alınmıştır ve İMA seviyeleri çalışılmıştır.

Bulgular: Plazma İMA seviyeleri 2. grupta 1. grup ve 3. grup ile karşılaştırıldığında belirgin yüksek bulunmuştur. (sırasıyla $p<0,001$ ve $p=0,002$). Serum İMA seviyeleri 3. grup ratlarda kontrol grubundan daha yüksek bulunmasına rağmen istatistiksel olarak belirgin fark saptanmamıştır ($p=0,659$).

Sonuç: Yaptığımız bu ön çalışma sonuçları, bosentanın mezenterik iskemide koruyucu etkisinin olabileceğini göstermektedir.

Anahtar Kelimeler: İskemi modifiye albumin, bosentan, akut mezenterik iskemi *Nobel Med 2013; 9(2): 96-99*

INTRODUCTION

Mesenteric ischemia is an important clinical entity due to its high mortality rate, commonly caused by occlusion, vasospasm, or hypoperfusion of mesenteric vessels that leads to reduction in intestinal blood flow. The diagnosis is challenging and acute interventions are critically important. Ischemia-modified albumin (IMA) has shown to be a sensitive marker of myocardial ischemia, skeletal muscle ischemia, pulmonary embolism and in addition it has recently been shown in animal studies that; serum IMA levels represents a significant parameter in the early diagnosis of mesenteric ischemia. Drugs that inhibit vasoconstriction and increase blood flow are the first line medical therapy in mesenteric ischemia. Bosentan is a potent competitive antagonist of both endothelin receptors A and B and has also shown to inhibit the increase in vascular resistance in strangulated bowel. As endothelins are known to be the most potent vasoconstrictor agents, their inhibition could prevent intestinal damage and reduce morbidity and mortality rate.

Our aim in this study was to examine the effects of bosentan on plasma Ischemia-modified albumin levels which is suggested to be an ischemia serum marker in the diagnosis of mesenteric ischemia.

MATERIAL and METHOD

This is a randomized, controlled, non-blinded interventional animal study. An approval was obtained from the animal use committee. 36 mature female wistar albino rats weighing 200 to 250 gr were used in the study. The animals were kept in cages at room temperature and were fed with standard rat cow until the start of the experiments.

Rats were allocated into three experimental groups. General anesthesia was administered with 50 mg/kg ketamine and 5 mg/kg xylazine applied intramuscularly. 3 ml/kg/hour saline solution were given by femoral vein catheterization during the surgeries. 4 to 5 cm midline incisions were made for laparotomy and were used for all groups. A heating pad was applied during anesthesia to maintain body temperature.

Group 1 (n=12) underwent a simple laparotomy and blood samples were taken in 30 minutes thereafter.

Group 2 (n=12) was the mesenteric ischemia group without any pretreatment. After the laparotomy, superior mesenteric artery (SMA) was clamped with a bulldog clamp at the aortic bifurcation. Three-milliliters of aortic blood specimens were taken from

each rat after 30 min. and placed in citrated tubes and send to the laboratory for IMA levels.

Group 3 (n=12) was the bosentan pretreated (100 mg/kg⁻¹ day, 1 gavage/day for two days before surgery) mesenteric ischemia group. Bosentan was given daily by gavage at the dose of 100 mg/kg. Treatment of the animals started 2 days before the experiments. The dose was given according to recommendation in literature.^{1,2} After the laparotomy, superior mesenteric artery (SMA) was clamped with a bulldog clamp at the aortic bifurcation (Figure 1,2). Three-milliliters of aortic blood specimens were taken from each rat after 30 min. and placed in citrated tubes and send to the laboratory for IMA levels.

IMA concentrations were analyzed by measuring the complex composed of dithiothreitol and cobalt unbound from albumin by the colorimetric method as described by Gunduz et al.³ Specimen absorbencies were analyzed at 470 nm using a spectrophotometer and the results were given as absorbance units (ABSU). All animals were sacrificed by cervical dislocation after blood samples for IMA measurements were taken. 4 rats were died due to general anesthesia before surgery and 2 rats died during surgeries due to the blood loss. New rats were used instead to equal the number of subjects.

RESULTS

Statistical Analysis

Data analysis was performed with SPSS for windows, version 11.5 (SPSS, Chicago, IL). Data were expressed as mean±SD. Comparison of results between experimental groups was performed one-way ANOVA followed by a Tukey's test. A probability value of p<0.05 was considered to be statistically significant. The mean values are summarized in Table 1. Serum IMA levels in the clamped group were significantly higher compared to the levels of control group and bosentan pretreated clamped group. IMA levels in bosentan pretreated and clamped group were slightly higher than the control group but showed no statistical significance (p=0.659). IMA levels in bosentan pretreated clamped group were significantly lower than the non-treated clamped group (p=0.002). Table 2 showing the summary of comparison values between groups.

DISCUSSION

Mesenteric ischemia is due to a reduction in intestinal blood flow, which is most commonly caused by occlusion, vasospasm or hypoperfusion of the →

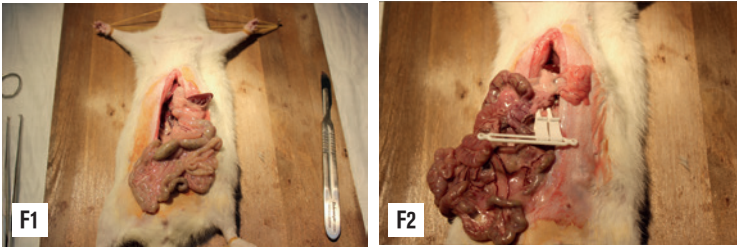


Figure 1: Laparotomy was done and superior mesenteric artery was found
Figure 2: Superior mesenteric artery was clamped with a bulldog clamp at the aortic bifurcation and mesenteric ischemia was generated.

Table 1: The mean values of IMA in groups

	Mean (absorbance units)	Std Dev	SEM
Group 1	0.509	0.0804	0.0195
Group 2	0.726	0.146	0.0355
Group 3	0.551	0.182	0.0442

SEM: Standard error of the mean, IMA: Ischemia modified albumin

Table 2: All pairwise multiple comparison procedures

Comparison	Diff of Means	p	p<0.050
Group 2 vs Group 1	0.217	<0.001	yes
Group 2 vs Group 3	0.174	0.002	yes
Group 3 vs Group 1	0.0428	0.659	no

mesenteric vessels. The superior mesenteric artery (SMA) is most commonly affected.⁴

The underlying causes vary as follows; most commonly occlusive arterial obstruction by emboli or thrombosis of mesenteric arteries, occlusive venous obstruction by segmental strangulation or thrombosis, non-occlusive mesenteric ischemia due severe systemic illness with reduced cardiac output or intestinal vasospasm by cocaine, ergot poisoning, digoxin use. In addition, hypotension from cardiac failure, myocardial infarction, sepsis, severe liver or renal disease might predispose to mesenteric ischemia.⁵⁻⁸

The morbidity and mortality rates are very high unless diagnosis and treatment is done rapidly.⁹ The overall prevalence of acute mesenteric ischemia is 0.1% of all hospital administrations but the mortality rate might be as high as 90% if the diagnosis and treatment is missed until infarction.¹⁰ Additionally, even with appropriate treatment up to 50-80% of the patients die.^{10,11}

As early diagnosis of SMA occlusion before the inception of irreversible intestinal ischemia is extremely important, a specific serum marker is still sought.¹² An increase white blood cell count or serum lactate concentration as well as the high C-reactive protein levels are insensitive and non-specific in the early stages of disease.¹³

In recent years many studies of IMA levels in acute ischemic conditions has been reported. IMA in serum has been shown to be a rapid and low-cost technique in various ischemic conditions such as cerebral ischemia, myocardial ischemia, mesenteric ischemia, and pulmonary ischemia.^{3,14-16}

Human serum albumin consists of 585 amino acids. The first 3 amino acids in the N-terminus, Asp-Ala-His, constitute a specific binding site for transition metals such as cobalt, copper and nickel. Rat serum albumin resembles human serum albumin in its amino acid sequence therefore it has been used in studies based on the decrease in the cobalt binding capacity of the N-terminus of human serum albumin during ischemia to rat serum albumin.^{17,18} During acute ischemic conditions, albumin's metal binding capacity for transition metals are reduced thus giving rise to a metabolic variant of the protein known as IMA.¹⁶ Recently, studies focusing in ischemia modified albumin (IMA) have been published.^{3,18} In these experimental animal studies, IMA levels were shown to represent a significant parameter in the diagnosis of acute mesenteric ischemia. In our study we used IMA as a marker of mesenteric ischemia and its levels to compare bosentan drug effect in mesenteric ischemia.

It has been shown that, structural changes of mucosal cells of intestine occur only after 10 minutes of the onset of mesenteric ischemia caused by superior mesenteric occlusion.¹⁹ Later on the mucosal and serosae layers are effected and the risk of perforation and stricture takes place.²⁰ So as soon as the diagnosis is made, preventive cautions should be commenced. In our study, we first sought bosentan drug effect after 30 minutes of mesenteric ischemia which is enough for acute necrosis and our following studies would be made on time dependent experiments for chronic ischemia results.

In mesenteric ischemia, inhibition of vasoconstriction and increase blood flow may contribute to gain time for surgery and prevent infarction. Many vasodilators and anti-inflammatory drugs were studied for this effect. In our study we used bosentan which is a competitive antagonist of both endothelin receptors ETa and ETb. We considered bosentan might be a suitable drug for these conditions as it inhibits the increase in vascular resistance in strangulated small bowel.²¹ In addition Johnson RJ et al. have demonstrated that mesenteric vessels have endothelin a and b receptors and it is highly possible that their inhibition by bosentan could prevent intestinal damage and reduce morbidity and mortality rate.²² Results of our study supported the idea, as we have shown low IMA levels in bosentan pretreated group. These preliminary results suggest that bosentan has →

a protective effect of mesenteric ischemia by endothelin receptor blockage that results in low IMA levels which suggests less tissue damage in many ischemic conditions.

The underlying mechanism in how the blockage of endothelin receptors might prevent the albumin

N-terminus reduction of transition metal binding needs extreme molecular studies.

This study now needs to be supported by further experimental studies for confirmation and also a time dependent variations with reperfusion studies should be sought.



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