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Role of Ischemia-Modified Albumin in Early Diagnosis of Acute Mesenteric Ischemia in Rats

Sıçanlarda Akut Mezenterik İskeminin Erken Teşhisinde İskemi-Modifiye Albüminin Rolü

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Öz

Amaç: Arteryel ve venöz mesenterik iskeminin erken tanısında iskemi-modifiye albumin (IMA) rolü araştırıldı. Arteriyel ve venöz iskemi gelişen gruplarda ilk 30 dakika, birinci ve üçüncü saatlerde serum IMA düzeylerini değerlendirerek Akut Mesenterik İskemi (AMİ) erken tanı için yeni yöntemleri belirlemeyi amaçladık.
Gereçler ve Yöntem: Çalışmanın deneysel bölümünde, her biri sekiz sıçandan oluşan üç grup; 1. grup: kontrol; 2. grup: süperior mezenterik arter (SMA) ligasyonu; ve 3. grup: süperior mezenterik ven (SMV) ligasyonu. Tüm gruplarda venöz kan örnekleri ilk 30 dakika, birinci ve üçüncü saatlerde alınarak IMA düzeyleri değerlendirildi.
Bulgular: IMA düzeyleri ilk 30 dakika, birinci ve üçüncü saatlerde SMA'nın bağlandığı 2. grupta ve SMV'nin bağlandığı 3. grupta kontrol grubuna göre anlamlı derecede yüksekti. SMA ve SMV'nin ligasyonundan sonraki ilk yarım saatte yüksek IMA seviyeleri ile ilgili olarak istatistiksel anlamlı sonuçların elde edilmesi, iskemi öncesi süreyi işaret ediyor olabilir.
Sonuç: IMA, AMI'nin erken teşhisinde yeni bir belirteç olarak düşünülebilir.

Anahtar Kelimeler: Akut mezenterik iskemi, iskemi-modifiye albumin, erken tanı

Abstract

Aim: The role of ischemia-modified albumin (IMA) was investigated in the early diagnosis of arterial and venous mesenteric ischemia. We aimed to determine novel procedures for the early diagnosis of Acute Mesenteric Ischemia (AMI), by evaluating serum IMA levels during the first 30 minutes, first, and third hours, in groups that have developed arterial and venous ischemia.
Materials and Methods: In the experimental part of the study, three groups, each consisting of eight rats; 1st group: control; the 2nd group: ligation of superior mesenteric artery (SMA); and the 3rd group: ligation of superior mesenteric vein (SMV). Samples of venous blood were withdrawn in the first 30 minutes, first, and third hours in all three groups, and serum IMA levels were evaluated.
Results: IMA levels in the first 30 minutes, first, and third hours were significantly higher in the 2nd group where SMA was ligated, and in the 3rd group where SMV was ligated, compared with the control group. Statistically significant results were obtained regarding the high IMA levels in the first half hour following ligation of SMA and SMV, which might be indicative of the period prior to ischemia.
Conclusion: IMA may be considered as a new marker in the early diagnosis of AMI.

Keywords: Acute mesenteric ischemia, ischemia-modified albumin, early diagnosis

INTRODUCTION

Acute mesenteric ischemia (AMI) is a less frequently occurring disease compared with other causes of acute abdominal pain; however it is an emergent abdomino-vascular pathology with a high rate of mortality (60%-80%), due to the fact that it is often diagnosed too late (1-3). Current studies have demonstrated that AMI exists in one in every 1000 hospitalized patients, and its prevalence has been increasing progressively (4).

High rates of mortality associated with AMI have not changed significantly despite current rapid developments in the diagnostic visualization and laboratory methods, surgical techniques, and perioperative intensive care support (5,6). The main reason for this is that difficulties still exist in diagnosing the disease before intestinal ischemia and necrosis have developed. The role of radiological analysis methods is still limited in the early diagnosis of AMI.

An efficient laboratory method, which can be efficiently used in the early diagnosis of AMI, is not yet available. Various studies have been conducted in the early diagnosis of AMI, investigating the serum levels of biological markers, such as D-dimer, alpha glutathione, S-transferase, D-lactate, L-lactate, LDH, intestinal fatty acid-binding globulin, and alkaline phosphatase. However it is still a matter of debate as to whether these markers have any significance in the

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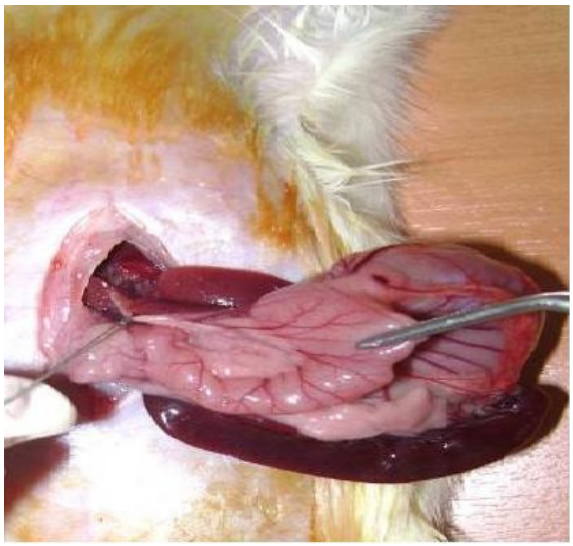

Figure 1. Dissected and ligated superior mesenteric artery was observed

Figure 2. Dissected and ligated superior mesenteric vein was observed

early diagnosis of AMI (7-11).

Our aim was to determine whether ischemia-modified albumin (IMA) can be used as a marker in the early diagnosis of AMI, and regarding its etiology, to establish its origin as arterial or venous.

MATERIAL AND METHODS

The study was conducted in the laboratory at Selçuk University Experimental Animals Research Center (SUERC), with the approval of Selçuk University School of Medicine, Experimental Animals Ethic Committee (2011/54).

Animals, experimental design and study groups

A total of twenty-four adult Sprague Dawley rats weighing 250-300 g were used in this study. Rats were randomly selected, and each cage contained four rats, regardless of their gender. The rats were housed at constant temperature (22±2°C), humidity (51%), and 12 hours of light/dark cycle, and were fed ad-libitum.

A total of 24 rats were separated to three experimental groups. The rats in each control group were marked for blood withdrawal at three different times. The order of the rats in the groups were indicated; (C) the control group, (A) the group of arterial mesenteric ischemia, and (V) the group of venous mesenteric ischemia.

Sample collection

Rats underwent mid-line laparotomy under ketamine hydrochloride anesthesia (2 mg/kg, 10 mg/ml). The rats in the control group were applied laparotomy only. The superior mesenteric artery (SMA)s of rats in the second group were dissected, and ligated (Figure 1). superior mesenteric vein (SMV) s of rats in the third group were dissected, and ligated (Figure 2). Blood samples were withdrawn from vena cava in all the three groups for the measurement of IMA, at the first 30 minutes, 1st, and the 3rd hours. All rats were sacrificed at the end of the fourth hour.

Laboratory analysis

2 mL of blood samples were withdrawn in all groups in the first 30 minutes, 1st, and the 3rd hours, and were centrifuged, and stored at -80°C. Samples for the measurement of IMA blood concentrations were pipetted into the Eppendorf tubes, and stored at -80°C. Vacutainer tubes, free of anticoagulant, were

Table 1. Biochemical values (ABSU)* of rats at group 1

Values/rats	1	2	3	4	5	6	7	8
½ hour	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2
1st hour	0.3	0.1	0.1	0.1	0.1	0.2	0.2	0.2
3rd hour	0.2	0.3	0.2	0.1	0.2	0.2	0.3	0.2

***ABSU:** Absorbance unit.

Table 2. Biochemical values (ABSU)* of rats at group 2

Values/rats	1	2	3	4	5	6	7	8
½ hour	0.4	0.5	0.3	0.3	0.4	0.4	0.4	0.4
1st hour	0.6	0.6	0.5	0.6	0.5	0.6	0.6	0.7
3rd hour	0.8	0.9	0.8	0.7	0.9	0.8	0.9	0.9

***ABSU:** Absorbance unit.

used for obtaining sera. Sera were separated by centrifugation at 3000 rpm, for 15 minutes. IMA levels were determined using the albumin cobalt-binding test. The decreased binding capacity of cobalt to albumin was evaluated by the rapid and colorimetric method developed by Bar et al. (6), 200 µL of sera were added to glass tubes; 50 µL of 0.1% $CoCl_2.6H_2O$ (Sigma) was pipetted on the sera, mixed slowly, and then waited for ten minutes for maintaining efficient binding of cobalt to albumin. 50 µL of 1.5 mg/mL Dithiothreitol (DTT) was added as the coloring agent. After waiting for two minutes, reaction was stopped by adding 1 mL of 0.9% NaCl, for ending the cobalt-albumin binding. Sample-blind was prepared for each sample. During the phase of DTT addition, serum cobalt-blind free of DTT was prepared by adding 50 µL of distilled water instead of 50 µL of 1.5 mg/mL Dithiothreitol (DTT). Sample absorbances were measured by the spectrophotometer (Shimadzu, UV1601), at 470 nm. Coloring of the samples with DTT, were compared with coloring of the blind solutions, and the results were expressed in absorbance units (ABSU).

Statistical analysis

Statistical analyses of the study findings were performed using the SPSS (Statistical Package for Social Sciences) for Windows 18.0 software. Results were expressed as the mean±standard deviation. The relationship between the IMA levels of the groups in the first 30 minutes, 1st, and 3rd hours, were analyzed using the Friedman test, and a Mann-Whitney test was used for the variance analysis between the groups.

RESULTS

No rats were died during the experimental period. No significant differences were determined between the mean IMA levels of the control group in the first 30 minutes, and their levels in the 1st, and the 3rd hours. The mean IMA levels of the control group in the 1st, and the 3rd hours also did not differ significantly. IMA levels of each control subject in the 1st, and the 3rd hours, did not show any significant differences with the same levels of other subjects in the group (Table 1).

The IMA levels of subjects in group 2, in the first 30 minutes were determined to be higher than the IMA levels of the subjects in control group in the first 30 minutes, 1st and the 3rd hours, and IMA levels of subjects in group 3, in the first 30 minutes, and the 1st hours ($P < 0.05$). The IMA levels of subjects in group 2 in the 1st hour was higher than the IMA levels of subjects in the control group and group 3, at the first 30 minutes, 1st, and the 3rd hours ($P < 0.05$). IMA level of subjects in group 2 in the 3rd hour was higher than the IMA levels of subjects in the control group and group 3, at the first 30 minutes, 1st, and 3rd hours ($P < 0.05$). The IMA levels of subjects in group 2 in the 3rd hour was significantly higher than the IMA levels of subjects in group 2, at the first 30 minutes, and 1st hour ($P < 0.05$). The IMA levels of subjects in group 2 in the 3rd hour was significantly higher than the IMA levels of subjects in group 2, in the first 30 minutes and the 1st hour ($P < 0.05$). The IMA level of each subject in group 2 at the first 30 minutes did not show any significant differences with the same levels of other subjects in the group (Table 2).

The IMA levels of the subjects in group 3 at the first 30 minutes, 1st and 3rd hours, were higher than the IMA levels of the subjects in the control group, at the first 30 minutes, 1st, and 3rd hours. The IMA levels of the subjects in group 3 in the 3rd hour was significantly higher than the IMA levels of this group at the first 30 minutes and the 1st hour ($P < 0.05$) (Table

Table 3. Biochemical values (ABSU)* of rats at group 3

Values/rats	1	2	3	4	5	6	7	8
½ hour	0.3	0.2	0.2	0.3	0.3	0.2	0.4	0.3
1st hour	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3
3rd hour	0.4	0.3	0.4	0.4	0.4	0.5	0.4	0.4

***ABSU:** Absorbance unit.

3).

DISCUSSION

The main reason for the increases in morbidity and mortality in patients with acute mesenteric ischemia is the delay in diagnosis, and hence markers are needed to indicate mesenteric ischemia, particularly in the period prior to ischemia. Human serum albumin is the protein that exists in the highest concentration in blood; it is synthesized in the liver, and consists of 60% plasma proteins.

David Bar and Bhagavan first identified Ischemia-modified albumin (IMA) in 1990 (6). They indicated the changes in circulatory albumin in their studies evaluating the hypoxic heart tissue. During myocardial ischemia, IMA is indicated by the determination of a decrease in the binding of cobalt to the N-terminal region of the human serum, albumin (12, 13).

Human serum albumin consists of 585 amino acids. Heavy metals like cobalt, copper and nickel, bind to the N-terminal region of albumin. Cellular changes, like the dysfunction of sodium-calcium pump during ischemia, or the reperfusion resulting in lactic acidosis and increased free radicals, leading to damage in the N-terminal site of albumin, and thus decrease the metal-binding capacity of albumin; a variant metabolic protein exists as a consequence of this process. This change can be measured, and it's known as IMA (12-18).

Ischemia-modified albumin is not only specific to acute coronary syndrome. Studies have shown that it increases in the late stages of cancer, advanced liver failure, acute strokes, terminal renal failure, pregnancy, and in the ischemic muscle (19-21).

In the present study, we investigated whether ischemia-modified albumin could be used in the early diagnosis of mesenteric ischemia that originated from arterial and venous mesenteries. Two experimental groups were developed besides the control group; superior mesenteric artery was ligated in one group, and the superior mesenteric vein was ligated in the other. In the groups where the artery or vein was ligated, we determined that IMA level increased with time. Our results indicated that IMA levels at the first 30 minutes, 1st, and 3rd hours increase in the group in which SMA was ligated, and the IMA levels in the 1st and the 3rd hours increased in the group that SMV was ligated. In light of these results, we concluded that IMA could be used as a marker for the early diagnosis of mesenteric ischemia of arterial or venous origin. We suggest that the time duration of high IMA levels may be a clue when considering arterial/venous etiologies.

Ligation of the SMA led to an earlier and a higher rate of IMA elevation, compared with the group of SMV ligation. This finding undoubtedly supports the diagnosis, and gives the surgeon the advantages of early diagnosis and intervention, when correlated with the patient's anamnesis. IMA analysis in the cases with AMI may be precious, as a marker regarding the early diagnosis. Similar suggestions exist in the very limited number of experimental studies related to the use of IMA in the diagnosis of AMI.

The rate of change in IMA levels with the degree of intestinal ischemia, its significant or nonsignificant increase in the segmentary ischemias, and the level of IMA in the non-occlusive mesenteric ischemias, are the primary issues that it is essential to reveal in future experimental and clinical studies.

CONCLUSION

In conclusion, we obtained significant results in the present experimental study, indicating that IMA may be used as a marker in the early diagnosis of mesenteric ischemia of arterial or venous origin. Comparative experimental and clinical studies over a wider spectrum are undoubtedly required on this subject. We suggest that IMA may be used as a marker in the early diagnosis of AMI, and in indicating mesovascular pathology prior to ischemia.

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