The effects of diltiazem on superior mesenteric artery ligation

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The effects of diltiazem on superior mesenteric artery (SMA) ligation were studied experimentally on three rats. The study groups consists of 5 control, 8 SMA ligation, 8 SMA ligation and diltiazem (0.25 mg/kg). The levels of creatine Phosphokinase, lactic dehydrogenase, aspartate transaminase and alanin transaminase were significantly decreased after diltiazem injection compared with the SMA ligation group Histopathologic examinations revealed that diltiazem has protected small intestine from ischemic changes. [Turk J Med Res 1995; 13(5):165-168]

Key Words: Superior Mesenteric Artery, Ischemia, Diltiazem

Intestinal necrosis due to acute mesenteric ischemia is still an important problem in spite of developed surgical techniques and intensive care units (ICU). With aging and increasing the incidence of cardiovascular diseases, the incidence of acut mesenteric ischemia is increasing. The incidence of operations for mesenteric ischemia performed in surgical clinics is as low as 0.4%, but the mortality rate is high as 70-90% and is very important. In order to investigate the subject we planned an experimental study to observe, biochemical and histopathological changes after superior mesenteric artery ligation and protective effect of diltiazem.

MATERIALS AND METHODS

We used 21 wistar albino type female rats weighing 200-220 g in the study. Animals were left hungery for a 12 hour period before giving anesthesia with 25% urethane (1.25 g/kg subcutaneously) and catheterized by external jugulary vein.

Abdomen was opened by a midline incision in five rats from control group. SMA was skeletionized and not ligated and then after blood samples were taken in the 1th, 30th, 45th and 90th minutes and at the same time feces cultures were taken and small intestine was completely resected. At the end, all rats were sacrificed.

Received: July 22,1995 Accepted: Aug 5,1995

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Abdomen was opened by a midline incision in eight rats from second group. SMA was prepared for ligation by circulating a 3/0 silk at the aortic origin: tips of silk were passed through a three cm plastic catheter and then taken out of the abdomen and SMA ligation was completed before abdomen was closed. Blood samples were taken in 30th and 45th minutes after ligation. SMA ligation was opened at the 45th minute and blood sample was taken at 90th minute. Feces culture was taken and whole small intestine was resected and then all animals were sacrificed.

Blood samples were taken in the first minute and SMA ligation were performed in eight rats from the third group. Blood samples were taken in 30th and 45th minutes again and SMA ligation was opened at the 45th minutes in every rat, and then 0.25 mg/kg diltiazem (Diltizem ®) was infused by external jugulary vein line. At the 90th minute, blood samples and feces culture were taken and whole small intestine was resected. All rats were sacrificed.

Tissue samples were fixed by 10% formaldehid and parafine blocks were prapered for investigation. Serum creatine phosphokinase (CPK), lactic dehydrogenase (LDH), aspartate transaminase (ALT) levels were measured in the blood samples.

Kruskal-Wallis test was used for the statistical analysis.

RESULTS

There were no pathological changes in the first minute blood samples in all groups and single layer columnar

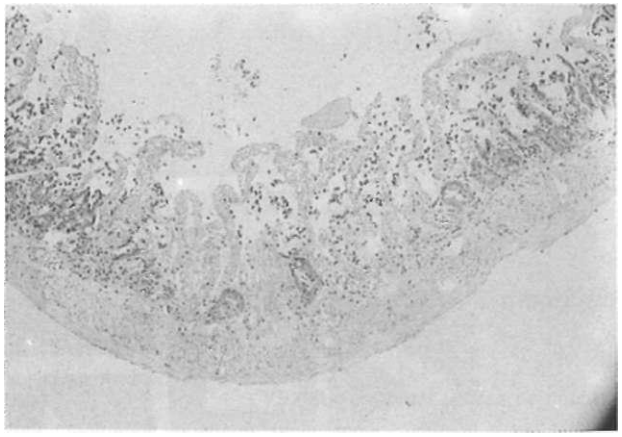

Figure **1.** Necrosis of criptae and heavy damage (HEx40). Moderate-heavy necrosis was seen in the all layers of wall in the right side, and 2/3 of upper part of villus in the left side

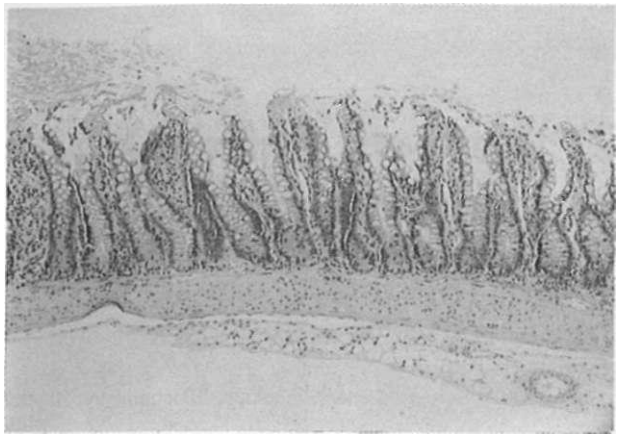

Figure 2. Mild and moderate degree of mucosal columnar epithelial necrosis was found in the tip of villi.

epithelial cells and villi lining small intestinal mucosa from control group.

We observed gangrenous and necrotic changes in the small intastine and hemorrhagic fluid collection in the lumen after relaparatomy in the 45th minute of SMA ligation. In the histological examination, necrosis was found in the single layer columnary epithelium and villi. In the some areas, necrotic changes and erythrocytic accumulation were including cripta and muscularis propria (Figure 1).

In the histopathological evaluation of intestinal tissue samples from diltiazem group; there was necrosis in the single layer columnary epithelium lining tip of villi, and other areas were completely normal (Figure 2).

We can not isolated any microorganism in the feces cultures.

Mean serum phosphokinase levels were measured every groups. CPK levels were 319±64.7 IU/L in the first minute. At the 45th minute of SMA ligation it increased to 4010±1350 IU/L Forty-five minutes after diltiazem (after 90 minute from the beginning) CPK levels were decreased to 2545±791 IU/L (Table 1) ($p<0.001$).

LDH levels were 812±116 IU/L in the control group and after SMA ligation it increased to 5363±1414 IU/L in the 45th minute. LDH levels were decreased to 2462±1615 IU/L after giving diltiazem ($p<0.001$).

There were similar changes in the AST and ALT levels as CPK and LDH (Figure 1,2). The decreases in the two transaminase levels were statistically significant ($p<0.05$).

DISCUSSION

In early 1970's, the diagnostic value of creatinin kinase (CK), alkaline phosphatase (AP), lactic dehydrogenase (LDH), aspartate transferase (AST), and their isoenzymes have been investigated in different diseases (1). Creatinin kinase has three different isoenzymes in various tissues. CPK-MM isoenzyme, originated from skeletal muscle, is the biggest part of CK. CPK-BB isoenzyme is being originated from brain and smooth muscle. CPK-MB is being originated from myocardium and it has a spesific diagnostic value in myocard infarction (2). Its diagnostic role is not obvious in gastrointestinal infarcts. Graber et al performed an experimental mesenteric ischemia in dogs and measured high CPK levels, but CPK-MM, CPK-BB and CPK-MB levels showed some differences (3,4). CPK-BB was the first elevating isoenzyme. It reached a peak level in 6 hours. CPK-MM isoenzyme reached the peak level in 9 hours and CPK-MB isoenzyme reached the peak level in 24 hours.

We could not measured CPK-isoenzymes, but our CPK levels were as high as Graber's results (3,4). This result supports that; serum CPK elevations in acute mesenteric arterial occlusion has a diagnostic value.

DeToma et al (5) found that LDH elevations occured before CPK elevations after SMA ligations in dogs. We also found a similar elevations in LDH levels as DeToma's study. However DeToma et al (5) did not find any elevation in AST levels, but we found a moderate elevation in AST levels after SMA ligation ($p<0.05$).

Histopathological findings were similar to biochemical parameters. There was necrosis in all villi, and in some criptae, and a heavy necrosis in a small part of muscularis propria in SMA ligation group. On the other hand, there was only a small necrosis and epithelial desquamation on the tips of villi in diltiazem group.

Table 1. Biochemical changes after SMA ligation

	t*	CPK IU/L	LDH IU/L	AST IU/L	ALT IU/L
Control	1'	319±64.7	812+116	86±51.6	63±27.6
SMALIg.	30'	3601.5±428	5371.5+1709	495.5H38	152±89
SMALig.	45'	4010+1350	5363±1414	546±160	175±44.7
Diltiazem	90'	2545±791	2462.5+1615	279.5±140	103±48

(p<0.05, p<0.001)

This study shows that, diltiazem prevents the destructive effect of ischemia partly and stops the destruction going into an irreversible pathway.

Successfull results have been reported after the use of calcium channel blocking agents in the regulation of post ischemic myocardial functions (6), protecting the kidneys from ischemia (7), and in the treatment of ischemic skin flaps (8). The protective effects of calcium canal blocking agents had been defined in some experimental liver ischemia-reperfusion injury models (9,10). In our study, diltiazem as a calcium channel blocking agent reduced CPK an LDH levels and protected mucous membrane from injury after SMA ligation. The mechanism of this effect is not clear, but calcium may has a protease activating role during ischemic period and by the way of this effect it converts xanthine dehydrogenase to xanthine oxidase in intracellulary compartment (11,12). Thus, free oxygen radicals are reduced and mucosal injury is avoided. Oxygen radicals are constantly produced as a result of cellular metabolism of oxygen in a living cell. The organism save himself by means of superoxide dismutase, catalase and glutation peroxidase enzymatic defence systems from these radicals.

It has been documented that, free oxygen radicals produced in a living organism play an important role in many diseases as cardiac diseases, cancer, cerebrovasculary diseases, rheumatoid arthritis etc (13).

Diltiazem has also another potential protective mechanism; vasodilatation. Calcium channel blocking agents make vasodilatation in mesenteric vasculary area; as smooth muscle relactants (14). It also reduces stagnation phenomenon in post-ischemic organ hypoperfusion (15).

Intestinal mucosa has a barrier role against bowel flora and their toxins besides absorption of nutrients. Recent studies have shown that experimental ischemia break mucosal barrier in animal models. Bacterial translocation and toxin diffusion due to increased mucosal permeability after ischemia and reperfusion injury were investigated in many study.

Reperfusion after a complete ischemia in the bowel increases mucosal permeability. Mucosal barrier is lost and bacterial translocation develops (16,17). Chui et al showed that mucosal permeability is related to the duration and severity of ishemia (18). All feces cultures were negative in our study. This can be explained by short ischemic period. To break mucosal barrier and to get bacterial translocation from bowel flora need a longer ischemic period than our study.

In conclusion, we showed that diltiazem protected bowel from an irreversible ischemic process after SMA ligation.

Superior mezenterik arter ligasyonunda diltizemin etkisi

Bu deneysel çalışmada raflarda superior mezenterik arter (SMA) ligasyonu yapılarak oluşturulan iskemi üzerinde diltizemin etkileri araştırıldı. Çalışmada 5 tanesi kontrol, 8 tanesi SMA ligasyonu, 8 tanesi de SMA ligasyonu ve diltiazem (0.25 mg/kg) verilen 21 rat kullanıldı. Biyokimyasal incelemeler yapılarak kreatin fosfokinaz, laktik dehidrogenaz, aspartat transaminaz ve alanın transaminaz değerlerinde Diltiazem verildikten sonra kontrol değerlerine göre anlamlı düşme saptandı. Histopatolojik incelemeler sonunda Diltiazemin barsağı iskemik harabiyetten kısmen koruduğu gözlendi. [TurkJMedRes 1995; 13(5):165-168]

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