Effects Of Sodium Nitroprusside On Ischemia-Reperfusion Injury

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ABSTRACT

***Aim**: The aim of this study was to investigate the effects of sodium nitroprussid (SNP) on ischemia-reperfusion injury in rats.*

***Method**: Twenty-four Sprague-Dawney rats were divided into four equal groups: group I without ischemia-reperfusion (I-R); group II with ischemia; group III with I-R; group IV with I-R and SNP. Complete bilateral hindlimb ischemia was produced by means of tourniquet occlusion of the upper thigh.*

***Result**: Blood creatine phosphokinase, lactate dehydrogenase, aspartate transaminase, urea, creatinine levels were not significantly different between groups 3 and 4. Malondialdehyde (MDA) levels were not significantly different between groups 3 and 4 in liver, soleus muscles and lung tissue. However, MDA levels were significantly lower in group 4 when compared with group 3 in renal tissue. Histological examination of the soleus muscles revealed that neutrophil leukocyte infiltration in group 3 was significantly less prominent than in group 4.*

***Conclusion**: These results have shown that SNP was not able to improve muscle ischemia-reperfusion injury in our study.*

***Key words**: Sodium nitroprusside, ischemia, reperfusion*

INTRODUCTION

It is known that long lasting ischemia for a definite period can cause cell death and tissue necrosis due to energy deficiency. Recent studies have shown that this tissue injury developes not only during ischemia period, but also continues during reperfusion period. To date, many studies have been performed in order to prevent reperfusion injury; but a certain treatment modality couldn't be determined because of the complex mechanism of reperfusion injury (1). In clinical settings, we can see skeletal muscle ischemia-reperfusion (I-R) injury during vascular surgery after releasing across clamp, during orthopedic surgery after releasing tourniquet, after crush syndrome or extremity retransplantation (1).

Nitric oxide (NO) is one of the main moderators in the pathophysiology of I-R injury. Different experimental studies have shown that NO can act dually and has both cytotoxic and cytoprotective effects. Treatment time and duration of NO and derivatives are not still clearly defined (2). In the present study, we aimed to search regional and systemic protective effects of sodium nitroprusside (SNP) as a NO donor on muscle I-R injury.

MATERIAL AND METHODS

This study was performed in Dicle University Health Sciences Research Center, Diyarbakir, Turkey. The study was approved by Dicle University Ethical Committee for Animal studies. We used 24 Sprague-Dawney male rats weighing 200-250 gr. A total of 24 rats were divided into four equal subgroups:

Group 1: Control group. Only anesthesia was applied to rats. Ischemia was not achieved.

Group 2: Ischemic group. Ischemia was applied to bilateral lower extremities for four hours following anesthesia.

Group 3: Ischemia-reperfusion group. After anesthesia, ischemia was applied to bilateral lower extremities for four hours. Afterwards, tourniquet was released for four hours to induce reperfusion. Bolus isotonic serum physiologic (SP) solution (0.5 mg/kg) was applied before 30 minutes before reperfusion and following reperfusion SP infusion (0.5 mg/kg/hr) was applied for 240 minutes.

Group 4: In this group, I-R and SNP was applied. After anesthesia, ischemia was applied to bilateral lower extremities for four hours. Afterwards, tourniquet was released for four hours for reperfusion. SNP was given by intravenous infusion initiated before 30 minutes and continued for 240 minutes (0.5 mcg/kg/min)

We achieved anesthesia in all subjects with ketamine (Ketalar, Pfizer) at 130 mg/kg dose and xylasine (Rompun, Bayer) at 20 mg/kg dose via intraperitoneal line. Ketamine HCL (50 mg/kg) was used for maintenance of anesthesia. At right neck area, right jugular vein was cannuled with cutaneous and subcutaneous incision and serum physiologic (SP) solution (0,5 mg/kg) was applied during the procedure. Arterial pressure was monitored with catheterization of right carotid artery. The model of ischemia-reperfusion was performed with tourniquet method. Ischemia was achieved by applying tourniquet to left or right back legs of subjects for four hours at the level of hip joint. Before tourniquet application, 100 IU/kg heparin was infused intravenously (liquemine, Roche). During tourniquet, extremity was controlled with doppler ultrasonography and perfusion was observed. At reperfusion group, after four hours of ischemia the tourniquet was cut and the extremity was reperfused for four hours. At the end of the experiment, all of the subjects were sacrified with lethal dose of sodium thiopental (200 mg/kg pentobarbital, intraperitoneal way).

When all procedures were accomplished, we obtained tissue samples from soleus muscles, liver, kidney and lungs of all subjects for measurement of malondialdehid (MDA) concentration that is the end product of lipid peroxidation. Tissues were homogenized with 1.15 % potassium chloride. Afterwards, homogenized material was treated with 0.8 tiyobarbiturate at 100oC for 60 minutes. Later, chromogen n-butanone was extracted. Molar absorptive coefficient factor (1,56 x 10 5 m-1 cm- 1) was used for the calculation of malondialdehide concentration and expressed as nmol malondialdehyde/mg tissue.

Before ischemia and after reperfusion, blood (2 ml) was obtained from juguler vein. From these samples, we measured serum levels of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate transaminase (AST), urea and creatinine. Blood samples were centrifuged and stored at –70 oC. Obtained blood amounts were replaced with SP. So, the amounts of obtained blood were balanced with infused SP.

For histological study, tissue samples obtained from soleus muscle and lung were fixed in 10 percent buffered formalin prior to the routine processing of the paraffin-embedded block. Four-micrometer-thick sections were cut and stained with hematoxylin and eosin. All glass slides were examined under light microscope. For the muscle, neutrophil leukocyte infiltration was pointed from zero to 3: zero: absent (less than 5% of maximum pathology), 1: mild (10 %), 2: average (15-20 %), 3: intense (20-25%)

During histopathological examination of the lung tissue, edema and congestion were evaluated separately and graded from zero to 3 according to intensity of edema or congestion. For the edema: zero: no edema; 1 positive: focal mild edema; 2 positive: focal intense edema; 3 positive: intense edema. For the congestion: zero: no congestion; 1 positive: focal mild congestion; 2 positive: focal intense congestion; 3 positive: intense congestion. The results were expressed as mean ± standard deviation. We used Mann-Whitney U-test for

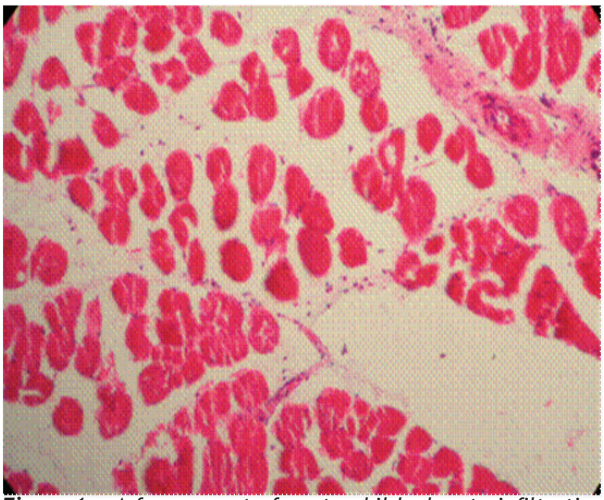

Figure 1a. *A few amount of neutrophil leukocyte infiltration between muscle fibrilles (HEX200) (Group 3)*

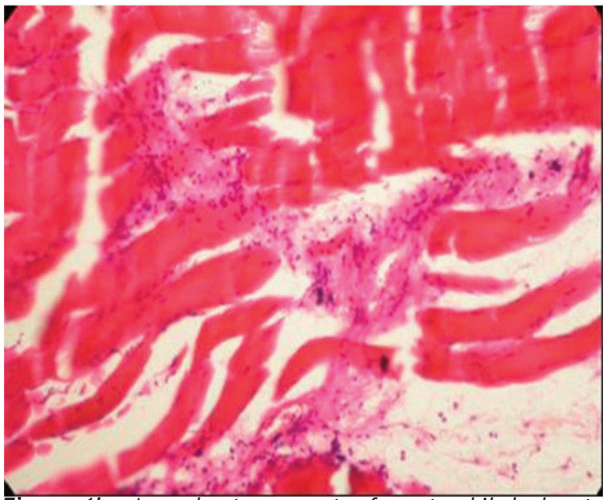

Figure 1b. *A moderate amount of neutrophil leukocyte infiltration between muscle fibrilles (HEX200) (Group 4)*

comparisons between two groups and Kruskal-Wallis test for comparisons of all groups. A p value less than 0.05 was considered statistically significant.

RESULTS

Data belonging to serum CPK, LDH, AST, urea, creatinine measurements from blood samples obtained from jugular vein after reperfusion are shown in Table 1. No statistically significant differences were found in pre-ischemic period measurements between groups ($p>0.05$).There were no statistically significant differences of serum CPK levels when groups 1 and 2 and groups 3 and 4 were compared with each other ($p>0.05$); however, significant differences were found in CPK levels between groups 1 and 3 ($p<0.05$), between groups 1 and 4 ($p<0.05$), between groups 2 and 3 ($p<0.05$) and between groups 2 and 4 ($p<0.05$).

In the view point of serum LDH levels; significant differences were found between groups 1-3, 1-4, 2-3 and 2-4 ($p<0.05$); but not betwen groups 1-2 and groups 3-4 ($p>0.05$). When serum AST levels were compared; there were not statistically significant differences between groups 1-2 and groups 3-4 ($p>0.05$); but between groups 1-3, 1-4, 2-3 and 2-4 ($p<0.05$). For serum urea levels, there were statistically significant differences between groups 1-2, 1-3, and 1-4, ($p<0.05$); but not between groups 2-3, 2-4 and 3-4 ($p>0.05$). When creatine levels were compared, no significant differences were found between all groups ($p>0.05$).

There was statistically significant difference between MDA levels of soleus muscle tissue and lung tissue ($p<0.05$). When MDA levels of liver tissue were evaluated, statistically significant differences were found between groups 1-2 and 1-3 ($p<0.05$); but no differences were found between groups 1-4, 2-3 and 3-4 ($p >0.05$). When MDA levels of kidney tissue were compared, there were statistically significant differences between groups 1-2, 1-3, 2-4 and 3-4 ($p<0.05$); but not between groups 1-4 and 2-3 ($p>0.05$) (Table 2). With histopathological examination, a few neutrophil leukocyte infiltration was observed among muscle fibers. In the I-R SNP group, there were average amount of neutrophil leukocyte infiltration in the muscle fibers in the I-R group (Figure 1a and 1b). For the lung tissue, there was evident perivascular edema and focal infiltration in the I-R group. Mild focal pneumatic infiltration was observed in the I-R SNP group.

DISCUSSION

The role of nitric oxide in the ischemia and reperfusion is still contraversial. In recent studies, L-arginine as an endogenous preproduct of NO donor products were used with positive effects in the ischemic muscles (3). These effects were suggested to occur because of decrease in leukocyte and thrombocyte aggregation. The involvement of NO in ischemia-reperfusion injury remains controversial and has been reported to be both beneficial and deleterious, depending on the tissue and model used [4-6]. On the other hand, hyperemic reperfusion, which may cause more oxygen free radical generation, could possibly impair muscle function. Ikebe et al (7) have shown that postischemic blood flow

Table 1. *Results of biochemical analysis in all groups*

	Group 1	Group 2	Group 3	Group 4
Urea	*50.8±7.0*	*77.4±7.6*	*87.3±16.1*	*79.5±11.3*
Creatine	*0.61±0.18*	*0.75±0.11*	*0.65±0.19*	*0.55±0.17*
CPK	*599±199*	*626±254*	*6752±3684*	*9614±1460*
LDH	*613±93*	*629±65*	*1627±472*	*1380±493*
AST	*75.3±29.9*	*85.6±30.5*	*420.7±94.4*	*481.5±148.1*

CPK: creatinine phosphokinase, LDH: lactate dehidrogenase, AST: aspartate aminotransferase

is significantly decreased and the contractile function of the reperfused skeletal muscle is well preserved by nitric oxide synthase (NOS) inhibitor infusion. Other studies proposed that these conflicting results could result from dosage differences (8). The beneficial effects of inhaled NO on myocardial I-R injury and left ventricular functions were shown in adult cardiac surgery patients (9).

Ischemic period as short as possible could help to decrease injury that could occur after reperfusion period. Similarly, preferred materials should have pharmacokinetic structure that they could reach the free oxygen radicals production area. Two characteristic factors in the reperfusion chain are period of ischemia and infusion of the materials that are thought to be antioxidant before oxygenation and after reperfusion period (1). In present study, SNP was used as a NO donor and its effects were investigated.

End products of lipid peroxidation are MDA, aldehydes and hydrocarbons. With the effects of oxygen radicals, MDA showing increased lipid peroxidation is one of the best indicator of composed oxygen radicals. In our study, regarding MDA levels in the lung tissue, there was no statistically significant difference between study and control groups (P>0.05). At histopathological study, there was evident perivascular edema and focal infiltration in the I-R group and mild pneumatic infiltration in focal regions in the I-R SNP group.

There are few studies about the effects of NO and its donors on lung injury. Study by Della and coworkers (10) indicated beneficial effects of inhaled NO on patients with pulmonary hypertension and hypoxia. Waisman et al. (11) studied the effects of NO to lung injury in rats, due to intestinal ischemia reperfusion. At the end of this study, they observed that NO decreased lung inflammation (11). On the other hand, Kao et al. observed that, exogenously given NO can increase I-R injury of the lung (12).

In our study, MDA levels of liver tissue were compared between groups 3 (IR) and 4 (IR and SNP), and no significant difference was found related to SNP infusion. Levels of ALT and LDH also not changed after SNP infusion and were found to be similar in groups 3 and 4. Kuroki and coworkers studied the effects of SNP on liver I-R injury. They indicated improvement of liver microcirculation and hepatocyte injury in the early period of reperfusion (13). In another study supporting this finding, SNP infuse after short period of liver ischemia also decreased liver I-R injury (14). However, we didn't show similar beneficial effects of SNP on liver tissue in the present study.

Further researches have been performed about the effects of I-R injury on kidneys. Kuru et al. (15) suggested that increase of renal injury occured with decrease of NO synthesis after chronic NOS inhibition. In another study (16), endogenous NO donor (molsidomine) was shown to improve kidney functions after abdominal aorta surgery. In our study, creatinine and urea levels were compared, and no significant difference was found between IR and IR+SNP groups. Comparison of MDA levels in kidney tissue between groups 3 and 4, showed significant

Table 2. *Malondialdehyde values of the groups*

	Group 1	Group 2	Group 3	Group 4
Soleus muscle	*60.9±8.8*	*82.4±18.6*	*65. 9±17.0*	*68.2±25.9*
Lung	*81.2±12.7*	*75.8±32.1*	*77.5±15.1*	*76.5±14.2*
Liver	*90.3±7.2*	*108.4±12.6*	*108.7±12.0*	*100.5±31.2*
Kidney	*115.5±16.0*	*151.5±33.6*	*157.9±37.8*	*108.2±22.9*

difference ($p<0.05$). According to our results, we observed positive effects of SNP on kidneys only from the view point of MDA levels.

In present study, MDA levels of soleus muscle tissue and serum CPK levels of IR and IR+SNP groups were found to be similar. At histopathological study of I-R group, there was a few neutrophil leukocyte infiltration in the muscle fibers, while in the I-R SNP group, average amount of neutrophil leukocyte infiltration in the muscle fibers were present as a worse situation observed. Gowda et al. (17) investigated the effects of N (omega)-propyl-L-arginine as a NO donor on muscle I-R injury and they have not observed positive effects (17). In another study, iNOS inhibition was found to be efficactive in denervated muscle I-R injury (18).

In conclusion, in this study, we used SNP as a NO donor to decrease local and systemic I-R injury in the muscle and other organs. However, beneficial effects of SNP on local and systemic I-R injury was not clear. We obtained worse results with SNP infusion at histopathological examination. Only, we observed positive effects of SNP infusion on kidneys in the MDA levels. Further studies are needed to clarify the effects of SNP infusion, its beneficial dosage and most effective infusion time and duration in IR injury of different tissues.

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