ORIGINAL ARTICLE

How effective are alprostadil and hydrocortisone on reperfusion injury in kidney after distant organ ischemia?

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Background: After reestablishment of blood flow to ischemic limb recirculation of free radicals may cause ischemia-reperfusion injury in many organs. This study designed to investigate effects of hydrocortisone and alprostadil distant injury to kidneys by both measuring biochemical markers of oxidative stress and histopathologic examination in an experimental rat model of hind limb ischemia-reperfusion. **Materials and Methods:** This study conducted in Isfahan University of Medical Sciences during 2011–2012. Ischemia was established by infra renal aortic clamping for 60 min in 32 male Wistar rats. Animals were divided into those receiving alprostadil (group ischemia-reperfusion plus alprostadil (IR/A), n = 8), those receiving hydrocortisone (group ischemia-reperfusion plus hydrocortisone (IR/H), n = 8), control group (group ischemia-reperfusion (IR), n = 8), and sham group (n = 8). After 120 min of reperfusion both kidneys were removed. Levels of superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) as indirect markers of oxidative injury was measured. Finally all data in different groups were compared using the analysis of variance (ANOVA) test by Statistical Package for Social Sciences (SPSS) version 16. **Results:** Administration of alprostadil or hydrocortisone does not improve the biochemical parameters of oxidative injury including MDA and SOD. However, statistically significant difference was seen in GSH level among sham and IR groups. Mean (± standard deviation (SD)) concentration of GSH in IR, IR/A, IR/H, and sham groups were 1028.77 (72.65), 924.82 (70.66), 1000.28 (108.77), and 846.69 (163.52), respectively (P = 0.015). Histopathological study of specimens did not show any significant changes between groups. **Conclusion:** Alprostadil and hydrocortisone do not improve the kidney GSH, SOD, and MDA level and kidney releases its GSH reserve during ischemia-reperfusion event, and another point is that, 3 h of ischemia-reperfusion does not develop injury in kidney.

Key words: Alprostadil, hydrocortisone, ischemia-reperfusion injury, oxidative stress

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INTRODUCTION

During and after revascularization of skeletal muscles, acute ischemia followed by reperfusion may be encountered. After reestablishment of blood perfusion edema, necrosis, reactive oxygen species (ROS), and inflammatory mediators will produce.[1,2] Ischemia forces tissues to anaerobic metabolism, by returning blood flow to ischemic tissue, vasculature macrophages are activated and ROS are produced.[3] These components play major role in oxidative stress. It is well-known that ROS are the triggers of reperfusion injury,[3,4] and endothelial injury, over production of proinflammatory cytokines, and leukocyte infiltration are the following steps in both local and systemic ischemia-reperfusion injury.[4] Myocardial tissue, lungs, and kidneys are particular target organs for such injury.[5] In recent years, many studies are available to investigate the protective effect of various drugs for ischemia-reperfusion injury.

Hydrocortisone is a corticosteroid derivative, and has many systemic effects like other corticosteroids such as anti-inflammatory effects, immunosuppressant, anti-insulin effects in glucose and fat metabolism, and catabolic effect in protein metabolism.[6] The anti-inflammatory effects of corticosteroids are attributable to influencing prostaglandin (PG) generation through intracellular receptors and by activating nuclear factor kB (NF-kB), indirect reduction release of hydrolytic enzymes, lipid peroxidation, and production of oxygen radicals.[6,7]

Alprostadil or PGE1 is an active biologic agent. Its mechanism of actions includes vascular dilation, platelet aggregation inhibition, inhibition of the multiple activities of neutrophils; including chemotaxis, adhesion,

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aggregation, activation, and granular enzyme release.[8] It also has the action of reducing the production of oxygen free radicals,[8] and it may reduce ischemia-reperfusion injury.

Due to reactive nature and short lives of free oxygen radicals, indirect markers of oxidative injury can be used instead to evaluate the severity of oxidative damage. Malondialdehyde (MDA) is end product of lipid peroxidation which is increased by ROS.[9]

Glutathione (GSH) and superoxide dismutase (SOD) considered first lines of defense when cells injured by free radicals. These antioxidants are most important intrinsic antioxidant system and the essential agents for maintaining cell integrity because of their reducing capabilities.[10]

The potential protective effect of alprostadil and hydrocortisone as antioxidants by decreasing oxidative stress has not previously been investigated in remote lower extremity ischemia-reperfusion injury model. Therefore, this study was designed to investigate the possible anti-inflammatory action of alprostadil and hydrocortisone on remote kidney injury by measuring biochemical markers of oxidative injury and histopathologic examination in an experimental rat model of hind limb ischemia-reperfusion.

MATERIALS AND METHODS

This study was conducted in Isfahan University of Medical Sciences during 2011-2012. Thirty-two adult male Wistar rats weighting between 200 and 250 g were included in this study. The rats were housed at a temperature of 23-25°C. All rats were fed a standard laboratory chow and had free access to water. Rats were acclimatized to this diet for at least 1 week before beginning of the experimental procedure. The protocol of experiment was approved in advance by the Baqiyatallah and Isfahan Universities of Medical Sciences Ethics Committees.

Experimental procedure

For anesthesia, 75 mg/kg ketamin and 5 mg/kg xylazine hydrochloride intraperitoneal injection were applied. After anesthesia and to facilitate respiration, via cervical incision tracheostomy was performed and a 2 mm plastic tube was inserted as a tracheostomy tube. The right internal jugular vein was cannulated with a fine catheter for intravenous injection through the same neck incision.

Rats were randomly divided into the four groups of experiment, each consisting of eight subjects. The sham group underwent midline laparotomy without infrarenal abdominal aortic clamping, however anesthesia was maintained up to 3 h. The control group (IR) underwent laparotomy and clamping of infrarenal abdominal aorta for 60 min followed by 120 min of reperfusion by removing the aortic clamp with continuous intravenous (IV) infusion of 10 cm^3/kg/h isotonic saline at the end of 30 min of ischemia. The alprostadil group (IR/A) also underwent 60 min of ischemia and 120 min of reperfusion and 30 min after clamping received 0.1 mg/kg/min IV infusion of alprostadil continued for 150 min (after completion of 120 min of reperfusion) in addition to 10 cm^3/kg/h isotonic saline. The hydrocortisone group (IR/H) was the same as IR/A group, but they received continuous IV infusion of 50 mg/kg hydrocortisone, beginning at the end of 30 min of ischemia and continued till the end of 120 min of reperfusion. The dose and timing of infusion of alprostadil and hydrocortisone were decided by taking the studies of Iwata *et al*.,[11] Milcan *et al*.,[12] and Pararajasingam *et al*.,[13] into consideration.

At the end of reperfusion in IR, IR/A, and IR/H groups (completion of 3 h anesthesia), both kidneys were removed rapidly. Right kidney was used for microscopic evaluation and left kidney was applied for biochemical assay. The tissue specimens were stored in a deep freeze -20°C for subsequent analysis, including MDA, SOD, and GSH until the date of analyses.

Biochemical assay

Tissue samples were weighted carefully and then minced with surgical blade and homogenized in four volumes of cold normal saline using a glass teflon homogenizer for 5 min at 5,000 rpm. Frozen tissue and blood samples were melted in room temperature and centrifuged at 20,000 rpm for 10 min. The supernatant was used for biochemical assays. MDA, SOD, and GSH concentrations were evaluated with a commercially available double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits (Glory Science Co., Ltd, China) according to the manufacturer's instructions in ng/ml for SOD and GSH, and mmol/ml for MDA.

Histopathological assay

After standard tissue processing, 5 mm tissue sections were obtained. The slides were stained with hematoxylin and eosin and examined with light microscope (´400). Sections were observed by an expert pathologist with no previous knowledge of the experimental design for tubular dilation, tubular atrophy, loss of brush borders, proteinaceous casts, and interstitial hemorrhage. At least two different slides were evaluated in each sample. The grade of the kidney injury was described according to the following injury score: 0, no damage; 1, mild damage; 2, moderate damage; and 3, severe damage.[14]

Statistical analysis

Due to normal distribution of our data, repeated measure analysis of variance with Tukey post hoc tests was used to study the change in MDA, SOD, and GSH levels during the

study in the four groups. Computer software (Statistical Package for Social Sciences (SPSS) version 16.0; SPSS, Inc., Chicago, IL) was used for statistical analysis. $P < 0.05$ was considered significant. Descriptive measures also calculated by SPSS version 16.

RESULTS

There was no statistically significant difference between the groups according to the body weight (data not shown). Mean (± standard deviation) concentration of GSH in IR, IR/A, IR/H, and sham groups were 1028.77 (72.65), 924.82 (70.66), 1000.28 (108.77), and 846.69 (163.52), respectively. Biochemical analysis shows that administration of alprostadil or hydrocortisone does not improve the study biochemical parameters including MDA and SOD (*P*-values were 0.8 and 0.51, respectively). However, in I/R group GSH level was statistically higher than sham group ($P = 0.015$). GSH levels among other groups showed no significant difference. Detailed data are shown in Table 1 and Figure 1. Histopathological study of specimens did not show ischemic changes and all 32 specimens were grade 0 [Figure 2].

DISCUSSION

The aim of the present study was to determine the possible anti-inflammatory role of alprostadil and hydrocortisone on remote kidney ischemic reperfusion injury by measuring biochemical markers of oxidative injury and histopathological examination.

Our study results show that administration of alprostadil or hydrocortisone does not improve the biochemical kidney markers among study groups. Previous studies did not note the effect of hydrocortisone and alprostadil on kidney antioxidant markers directly. However, some of them mentioned that glucocorticoids indirectly reduce ischemic reperfusion injury in many organs.[7,15] In contrast with these studies and in line with our results, Parra *et al*., performed a rat model study and compared some immunosuppressive drugs such as rapamycin and methylprednisolone; and they found that none of the drugs conferred protection against ischemia-reperfusion injury.[16]

About the PGs, there are previous studies that have shown PGE1 protected and reduced ischemia-reperfusion injury in various organs.[17-19] For instance Mahmoud, *et al*.,[20] study on 56 rats showed that administration of PGE 1 protects kidney against ischemia-reperfusion injury. However, we did not find any protective role.

In our study we found a matter which was not taken into consideration previously; our results show that GSH level was higher in IR group than other groups; it means that by administration of alprostadil or hydrocortisone, kidney GSH

Table 1: Biochemical parameters of study groups

			P-value
GSH (ng/ml)	IR	1028.77±72.65	0.01*
	IR/A	924.82±70.66	
	IR/H	1000.28±108.77	
	Sham	846.69±163.52	
MDA (μmol/ml)	IR	1.35±0.48	0.80
	IR/A	1.51±0.19	
	IR/H	1.46±0.29	
	Sham	1.52±0.47	
SOD (ng/ml)	IR	610.10±85.18	0.51
	IR/A	655.84±45.64	
	IR/H	617.68±68.52	
	Sham	627.76±48.93	

Data are presented as mean ± standard deviation (SD). Sham=The sham group; IR=the lower limb ischemia-reperfusion group; IR/A=IR plus alprostadil group; IR/H=IR plus hydrocortisone group, GSH=glutathione, SOD=superoxide dismutase; MDA=malondialdehyde; *Statistically significant difference ($P < 0.05$)

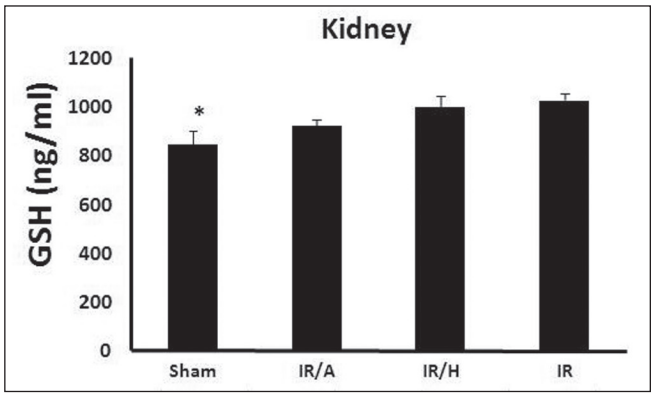

Figure 1: Alprostadil and hydrocortisone effect on glutathione level. Data are presented as mean ± standard deviation. Sham = The sham group, IR = the lower limb ischemia-reperfusion group, IR/A = IR plus alprostadil group, IR/H = IR plus hydrocortisone group, GSH = glutathione. *$P < 0.05$, treatment groups versus control group

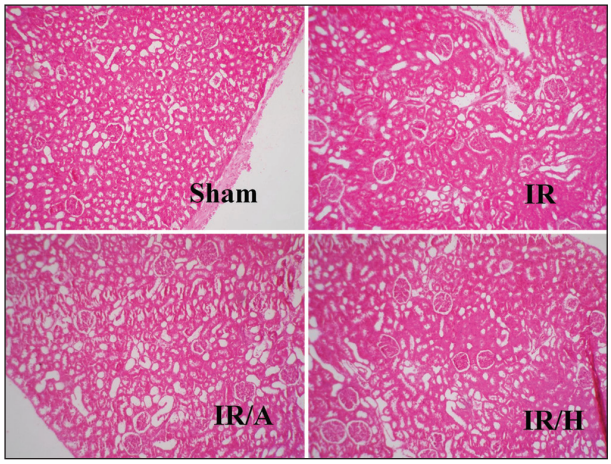

Figure 2: Hematoxylin and eosin (H and E) stained sections of rat kidneys show normal renal morphology in all four groups. Sham = The sham group, IR = the lower limb ischemia-reperfusion group, IR/A = IR plus alprostadil group, IR/H = IR plus hydrocortisone group

level as an antioxidant agent did not increase, even if it rises in IR group more than others. This suggests that in first hours of ischemic-reperfusion event, kidneys may release all GSH reserve due to excess oxidant agent in order to reduce the injury by their valuable potential against free radicals. As depicted in Table 1 the rats of sham group showed lower GSH levels than IR group. A possible explanation is rapid release of all GSH level of kidney affected by I/R injury in first hours prohibited us to show amount of oxidative injury in different groups. Further studies should continue I/R injury for more than 3 h to allow kidneys to produce and release antioxidant potential and thereafter evaluation of severity of injury and both biochemical and histological assays should be considered.

In addition to biochemical assay, histopathological study shows no kidney injury due to hind limb ischemia-reperfusion. By considering these two points that there are no significant changes in biochemical markers and also no change in histological finding. Thus, it is suggested that ischemia-reperfusion event in less than 3 h (60 min ischemia and 120 min reperfusion) does not cause the injury in kidney and I/R injury needs more time to establish.

In conclusion we deduce that in an ischemia-reperfusion event due to hind limb ischemia, Alprostadil or hydrocortisone do not improve the kidney GSH, SOD, and MDA level and kidneys release GSH reserve during ischemia-reperfusion event, and another point is that, 3 h of ischemia-reperfusion of infrarenal aortic clamping does not develop I/R injury in kidney.

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