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Amelioration Effects of Irrigation-Aspiration on Renal Ischemia-Reperfusion Injury in Canine Model

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Abstract : Renal ischemia-reperfusion injury is great clinical important because viability of the transplanted organ depends on the tolerance of the graft to ischemia-reperfusion injury, an inevitable processing during surgery. The purpose of this study was to investigate the effects of irrigation-aspiration in ischemia-reperfusion injury model induced by cross-clamping of renal vessels. Blood samples were collected from these dogs for measurement of kidney function and antioxidant enzyme activity, and RI at the intrarenal artery was measured at different time intervals. And the kidneys were taken for histopathologic evaluation at day 14. Kidney function (Cr and BUN) showed a significant increasing in untreated group compared to treated group. Resistive index of intrarenal artery was no significant difference among the groups. Activity of antioxidant enzymes in plasma was significant decrease in untreated group compare to control group while in treated group was no significant difference compared to control group. In histopathologic finding, treated group was showed less damage than that of untreated group. This result suggests that the processing of irrigation-aspiration is useful to reducing ischemia-reperfusion injury.

Key words **:** irrigation-aspiration, ischemia-reperfusion, kidney, canine.

Introduction

Tissue subjected to a period of ischemia undergoes morphological and functional damage, which increase during the reperfusion phase (17,22). Ischemia-reperfusion (I/R) injury in the kidney is often observed in the renal operation. Thus, to decrease of the degree of tissue damage is important to ameliorate cause of renal cell death, renal failure, delayed graft function, and renal graft rejection. Reperfusion of ischemic kidneys increases the hazardous effect of early ischemic injury by release of reactive oxygen species (ROS) and accumulation of activated neutrophils (8). And also, ROS cause lipid peroxidation of cellular membranes and, hence, disruption of the structural integrity and capacity for cell transport and energy production (5).

Ischemia-reperfusion injury is thought to be related to a variety of circumstances such as donor age, cause of death, prolonged warm and cold ischemic time, and surgical techniques at transplant (7,19,26). The more severe the ischemia-reperfusion injury that occurs initially, the higher the incidence of rejection and graft dysfunction caused by significant regulation of major histocompatibility antigens (7,16,21, 32,34). Therefore, reduction in primary ischemia-reperfusion injury would lead to better outcome for short- and long-term graft survival.

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Many studies investigated effects of antioxidants in the ischemia-reperfusion injury of an organ for the diminution of impair by oxidative stress (2,25,31). Although previous studies were performed after renal ischemia by the cross-clamping of renal vessel or renal transplantation, author thought that irrigation-aspiration with cold perfusion may play a role in reducing of free radicals by a physical mechanism of free radical washing. The objective of the present study was designed to investigate the effects of irrigation/aspiration in the condition as same as the renal transplantation.

Materials and Methods

Animals

Both sexes, adult beagle dogs weighing 10-13 kg (Marshal Farms, USA) were used in this study. These animals were acclimated and maintained on a standard diet, routine lighting cycle and room temperature for 6 months, and demonstrated normal renal function before the surgical procedure.

Experimental groups

Control group (n = 3); only the right kidney is removed. Untreated group (n = 3); after the right nephrectomy, the left kidney is freed from the perirenal tissue and fat. A bolus of 150 IU/kg of heparin is given IV 3 min before ischemia and the left renal vessels are clamped with an atraumatic vascular clamp. After ischemia for 60 min, the clamp is removed and the blood reflows. Treatment group (n = 3); The right nephre-

ctomy is performed. And the left kidney is freed from the perirenal tissue and fat. A bolus of 150 IU/kg of heparin is given IV 3 min before left renal artery and vein are clamped with an atraumatic vascular clamp. After ischemia during 60 min, heparinized saline is irrigated through the renal artery (50 mmHg) and aspirated the fluid from the renal vein, and then the renal vessels are unclamped.

Surgical procedure

After overnight fast, the animals are premedicated with atropine sulfate (Atropine Sulfate, Huons Co, LTD, 0.04 mg/kg, SC) and antibiotic prophylaxis with cefazolin sodium (Cefazolin, Chong Kun Dang Co, 20 mg/kg, IV) and analgesic with meloxicam (Metacam, Boehringer Ingelheim Co, 0.2 mg/kg, IV). The animals are induced with thiopental sodium (Thionyl Dai Han Pharm Co, LTD, 12.5 mg/kg, IV) and then maintained during the procedure with isoflurane 2% and 100% oxygen supply. Laparotomy was performed by midline incision. The left kidney was isolated, and then both the renal artery and vein were clamped. After 60 min of warm ischemia, the vessels were unclamped, and the right kidney was removed. And then irrigation-aspiration was performed in treated groups. During the operation, the dogs were given intravenous fluid. Postoperatively, the dogs were allowed free access to water and food.

Renal function

Blood urea nitrogen (BUN) and creatinine levels were determined on serum samples taken day 0, 1, 3, 5, 7, 14 before and after procedure from jugular vein, using a commercially available kit (VetTest 8008, IDEXX Co, Japan). The results are expressed as milligrams per deciliter.

Resistive Index (RI) measurement

Doppler signals were generally obtained from the interlobar arteries along the border of the medullary pyramids. Peak systolic and end-diastolic velocities of the arteries were recorded and the RI values were measured by built-in computerized calculations. At least 3 measurements were obtained in cranial portion, midportion, and caudal portion, and averaged for each kidney at each session. The waveforms were optimized by adjusting the pulse repetition frequency to the lowest possible level that would not produce aliasing. A spectrum was considered optimal if 3 to 5 consecutive similar-appearing waveforms were noted. All studies were performed with a 5- to 12-MHz linear transducer (SONOACE 8800, Medison Co, Korea). RI was performed preoperatively and postoperatively at day 1, day 3, day 5 and 1 week in ischemia-reperfusion injury induced kidneys respectively.

Antioxidant enzyme activity in plasma

Blood samples were collected using an anticoagulant as EDTA, and centrifuged at 700~1,000 x g for 10 min at 4°C. Then, the samples were pipetted off the top yellow plasma layer without disturbing the white buffy layer, and collected plasma samples were stored plasma on ice until assaying or freeze at -80°C. *Superoxide dismutase* (SOD) activity was determined with a commercial Superoxide Dismutase Assay Kit (Cayman, Co, USA) for the measurement of SOD activity from plasma. The activity was recorded spectrophotometrically at 450 nm. The enzyme activity was calculated as U/ml. *Glutathione peroxidase* (GSHPx) activity was measured with a commercial Glutathione Peroxidase Assay Kit (Cayman, Co, USA). The activity was recorded spectrophotometrically at 340 nm. *Catalase* (CAT) activity was measured with a commercial Catalase Assay Kit (Cayman, Co, USA). The activity was recorded spectrophotometrically at 540 nm.

Histopathologic examination

Tissue samples from the left kidney were taken for histologic examination after euthanasia on postoperative day 14. Tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned in 4 μm slices, and mounted on slides. After being deparaffinized, each specimen was stained with hematoxylin and eosin (H/E) for light microscopic evaluation. The morphological characteristics of the kidneys were determined by blinded histopathologic evaluation.

Statistical analysis

All values are expressed as means ± SD of determinations for all dogs in the group. Data were analyzed using analysis of variance followed by two-way repeated measures analysis (ANOVA) followed by Student *t*-test and a p value below 0.05 or 0.01 was considered statistically significant.

Results

Renal function

Serum creatinine levels, measured as an index of kidney function, increased to 15.01 ± 3.59 mg/dl in the untreated group prior to euthanasia after day 14 of reperfusion while in the control group, treatment group, these levels were nearly normalized by day14 after reperfusion (normal range, 0.50~1.80). We also measured blood urea nitrogen as a second index of kidney function in these experimental groups. Similar to serum creatinine, the levels of BUN in the untreated group increased to 342.25 ± 18.73 mg/dl prior to euthanasia after day 14 of reperfusion. The BUN levels in the other groups increased to 28.63 mg/dl (normal range, 7.0~27.0) after reperfusion and then gradually decreased to normal level by day 21 of reperfusion.

Resistive Index (RI) measurement

The RI values were measured in three interlobar arteries randomly selected at pre-operation day 1, 3, 5, 7, and 14. Although, the RI values increased to 0.70 ± 0.10 (normal range, < 0.7) in treated group prior to euthanasia after reperfusion, there was no significant difference among all groups.

Antioxidant enzyme activity in plasma

The specific activities of SOD, GSHPx, and CAT were significant decreased in untreated group exposed to 60 min of ischemia insult followed by 72 h reperfusion. While, these levels in treated group, resulted in a tendency more less decreasing after 72 h of reperfusion. The levels of SOD activity was 3.33 ± 0.05 (control), 2.12 ± 0.07 (untreated), 2.60 ± 0.24 (treated) nmol/min/ml followed by 72 h reperfusion (Fig 1). The SOD level of untreated group was statistically significant compared to control group. The levels of GSHPx activity was 133.00 ± 7.46 (control), 96.06 ± 10.61 (untreated), 97.11 ± 10.12 (treated) nmol/min/ml followed by 72 h reperfusion (Fig 2). The GSHPx levels of untreated group were statistically significant compared to control group. The levels of CAT activity was 2.49 ± 0.21 (control), 1.44 ± 0.32 (untreated), 1.85 ± 0.25 (treated) nmol/min/ml followed by 72 h reperfusion (Fig 3). The CAT levels of untreated and treated group were statistically significant compared to control group.

Histopathologic examination

At day 14, in control group kidneys normal cellular integrity was observed (Fig 4-A). However, most of kidneys in the untreated group had evidence of tubular necrosis, with intermittent areas of active tubular necrosis and regeneration of tubules and moderate mononuclear cell filtration (Fig 4-B). In contrast, kidney of the treated group (Fig 4-C) demonstrated mild regeneration of tubules and mononuclear cell infiltration. Ischemia and reperfusion for 60 min resulted in widespread damage to the tubule system by H/E staining consisting of regeneration of renal tubule, individual cell death, mineralization, modularly necrosis. In contrast, irrigation-aspiration resulted in preserved cellular integrity.

Discussion

This study was conducted to identify the protective agent with the aim of improving outcome of renal transplantation by attenuating I/R injury and making the model which renal transplantation without reimplantation. We chose 60 min of warm ischemia, which results in sufficient I/R injury to the kidney because of a pilot study which dogs did not survive of 3 days after reperfusion for 90 min of warm ischemia (25). Although, many authors reported a peak wave of renal functional damage at 24 or 48 hours after reperfusion (11,24) and examined the subjects for 3 days after reperfusion (4), some author investigated attenuation of renal I/R injury for 21 days (31). Thus the subjects were examined for 21 days after reperfusion. Contralateral nephrectomy immediately after ischemia-reperfusion was performed, so as to attempt in part to reproduce the renal transplant situation without the presence of a healthy kidney (14). Although this model differs from clinical renal transplantation in many points, the aim of this study was to focus only on reperfusion injury after warm and cold ischemia without the influence of other factors such as technical and manipulative differences during the reimplantation.

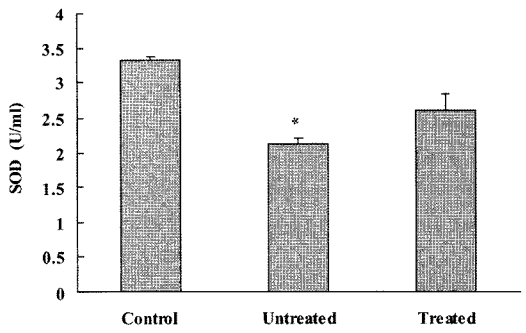

Fig 1. The activity of total SOD in plasma exposed to 60 min of ischemia followed by 72 h reperfusion. The values are expressed as mean ± SD for all groups. *p < 0.01; statistical significances compared to control group.

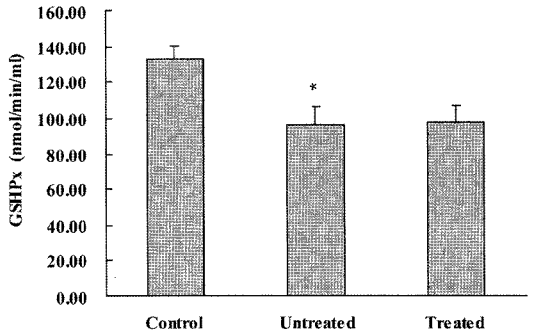

Fig 2. The activity of GSHPx in plasma exposed to 60 min of ischemia followed by 72 h reperfusion. The values are expressed as mean ± SD for all groups. *p < 0.05; statistical significances compared to control group.

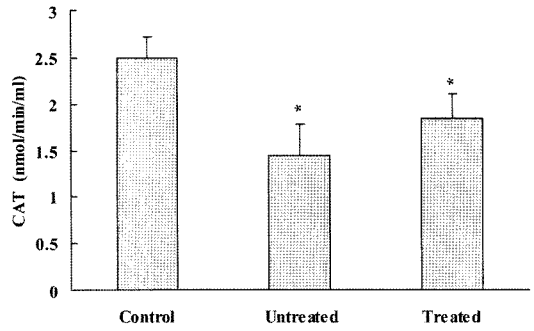

Fig 3. The activity of CAT in plasma exposed to 60 min of ischemia followed by 72 h reperfusion. The values are expressed as mean ± SD for all groups. *p < 0.05; statistical significances compared to control group.

Renal function was evaluated by blood urea nitrogen and serum creatinine levels in this study. The levels of BUN and creatinine were significantly increased in the untreated group at day 1 or 3 of reperfusion. However, there was no significant difference between control group and treated group as well as

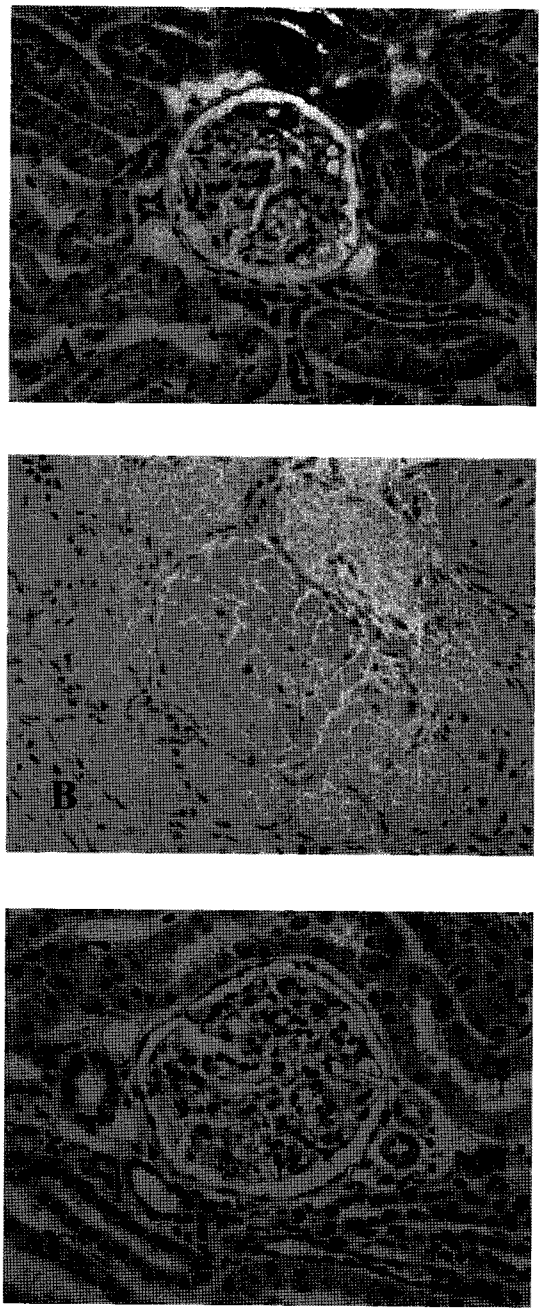

Fig 4. At 14 days, in control group normal cellular integrity revealed (A), while in untreated group it had evidence of tubular necrosis, with intermittent areas of active tubular necrosis and regeneration of tubules and moderate mononuclear cell filtration (B), and in treated group demonstrated mild regeneration of tubules and mononuclear cell infiltration.

maintained normal ranges. These results suggested that renal blood flow was continued by reperfusion or tolerance for the ischemia was increased as condition of ischemic preconditioning. The mechanisms of ischemic preconditioning seem to include a few well-described signal transduction pathways. These mechanisms include adenosine receptor mediated activation of adenosine triphosphate-gated potassium channels (23,28), nitric oxide synthesis (10,20), free radical generation (3,12,35), and the up-regulation of molecular chaperones (30). In fact, in this study, we did not investigate all of these parameters. Although, we measured antioxidant enzymes to scavenger free radical oxygen, these enzymes revealed increasing in the irrigation/aspiration groups than that in the only ischemic-reperfusion group. However, it may be argued that increased antioxidant enzymes may be associated with ischemic preconditioning.

Non-invasive techniques to diagnose episodes of renal dysfunction can help avoid biopsies, which carry some risk of complication and cannot be easily carried out frequently (18).Therefore, duplex Doppler ultrasound has become an important diagnostic tool for the early diagnosis of acute allograft rejection (1,27). However, the information that color Doppler scan provides seems to be limited for detection of vascular complication, so resistive index cannot predict transplanted kidney function(9). The change of renal blood flow after I/R injury plays an important role in the pathophysiology of ischemic renal failure. However, in this study, the resistive index and pulsatility indexmeasured by Doppler were not significantly different among all groups.

In histologic and morphologic examination, changes of untreated group revealed sever necrosis of tubular system, while in treated groups, damage of the tubule revealed less than that of untreated group. Previous studies have demonstrated that ischemia irreversibly damages the distal segments of the proximal tubules (S3) whereas more proximal segments suffer reversible injury after a short period of normothermic ischemia (6,13,15,29,33,36). Although we did not showed differences of segment of tubule in this study, the S3 segment might undergone necrosis and shed into lumen of the tubule which was considered to be the basis for the decrease in the glomerular filtration rate which is indicated by a significant increase in the serum creatinine and serum urea nitrogen levels as results of present study.

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개에서 신장의 허혈-재관류 손상에 대한관류-흡인의 감소효과

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요 약 : 신장의 허혈-재관류 손상은 수술하는 동안 부득이하게 발생하는 허혈-재관류 손상에 대한 이식조직의 내성에 따라 이식된 장기의 생존력이 달려있기 때문에 임상적으로 아주 중요하다. 본 연구의 목적은 신장 혈관을 차단하여 유발한 허혈-재관류 모델에서 관류-흡인의 효과를 알아보고자 실시하였다. 신기능과 항산화 효소를 검사하기 위해 혈액 샘플을 채취하였고 신장내 동맥의 혈류저항을 측정하였다. 14일째 신장을 절제하여 조직검사를 실시하였다. 신기능(Cr, BUN)은 처치군에 비해 비처치군에서 유의성 있는 상승을 보였다. 신장내 혈류 저항은 두 그룹 사이에 유의성이 없었다. 항산화 효소 활성은 대조군에 비해 비처치군에서 유의성 있는 감소를 보였으나, 처치군에서는 대조군과 유의성이 없었다. 조직검사 결과에서도 처치군이 비처치군에 비해 적은 조직손상을 보였다. 이러한 결과는 관류-흡인 과정이 신장의 허혈-재관류 손상을 감소시키는데 유용한 단계임을 시사한다.

주요어 : 관류-흡인, 허혈-재관류, 신장, 개