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Influence of ascorbic acid on BUN, creatinine, resistive index in canine renal ischemia-reperfusion injury

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Renal ischemia as a course of renal transplantation is a common cause of renal dysfunction as renal failure. The purpose of this study was to investigate the influence of ascorbic acid on blood urea nitrogen (BUN), creatinine (Cr) and resistive index (RI) for dog models with renal ischemia-reperfusion (I/R) injury. Renal ischemia was induced on 6 Beagle dogs. The left kidney was exposed to normothermic ischemia for a short period at 30 min followed by reperfusion. On the blood Cr level and RI, there was no significant difference comparing both groups. 14 days after I/R injury a significant reduction on the blood BUN level was observed in the vehicle group (34.06 mg/dl) compared to that of ischemia induced treated group (10.3 mg/dl) ($p < 0.05$). In conclusion, administration of ascorbic acid for renal ischemic-reperfusion injury had influence on blood BUN level, but it was not revealed the influence on blood Cr and RI.

Key words: BUN, creatinine, dogs, renal ischemia-reperfusion, resistive index

Introduction

Ischemic injury results when the blood recycle to a tissue is interrupted, but paradoxically more severe tissue injury occurs when blood flow is restored on reperfusion [3]. Renal warm ischemia-reperfusion (I/R) injury occurs prior to removal of a kidney for transplantation and it is a major factor in resuscitation of the kidney. Unfortunately, oxygen free radical (OFR) injury which occurs in oxygen metabolism can play an important role in the pathophysiology of the kidney ischemic-reperfusion injury [5]. When oxygen free radical is overproduced, administration of antioxidants should be given as a potential scavenger for reactive oxygen species (ROS). Ascorbic acid is a very powerful antioxidant that reacts rapidly with a variety of oxidants, and also represents the first line of antioxidant defense [2]. Therefore, ascorbic acid, a main water-soluble antioxidant, may be effectively recovering the renal dysfunction by ischemia reperfusion injury. The sonography is a commonly used tool for diagnosis of acute renal failure, and also blood urea nitrogen (BUN) and creatinine (Cr) level have been widely used to access renal function. Thus, through the above parameters, we examined the effects of ascorbic acid pretreatment on the kidney damage after induction of renal ischemia followed by reperfusion in a dog model.

Materials and Methods

An experimental study was performed on six 3-year-old, male Beagles with a mean weight of 12.4 kg (range, 11~13 kg). The animals received standard kennel food, routine lighting cycle and room temperature, and demonstrated normal renal function before the study. The animals were divided into two groups, each of the groups contained 3 dogs randomly. Group 1 was the control group in which normal saline solution was given intravenously, and group 2 was the treated group in which ascorbic acid (100 mg/kg) was given intravenously. Following the administration, the animals were subjected to left renal warm ischemia for 30 min, and reperfusion as follows.

The animals were premedicated with atropine (0.04 mg/kg, ID). Anesthesia was induced with thiopental sodium (12.5 mg/kg, IV) and maintained with isoflurane in 100% oxygen. All dogs were administered a balanced electrolyte solution (10 ml/kg/hr, IV), and cefazolin sodium (20 mg/kg, IV) as a prophylactic treatment was administered before surgery. After median laparatomy, the left kidney was freed from the peripheral tissue and fat. For each group, normal saline of the same volume of ascorbic acid (control group) and ascorbic acid (treated group) were given intravenously 10 min before ischemia, and then the left renal artery and vein were clamped with an atraumatic vascular clamp

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during 30 min. After 30 min of normothermic ischemia, the clamp was removed from the left renal vessels and the blood reflow to the kidney was verified visually, and then the right nephrectomy was performed. The abdominal cavity was closed and the wound was sterilized by applying 10% povidone-iodine. The animals were allowed food and water after recovering from the anesthesia.

Blood samples were drawn post-operatively at different time intervals (1, 3, 5, 7, 14 days), and serum creatinine and blood urea nitrogen were measured. Color Doppler sonography (Medison, Korea) with measurement of resistive index (RI) of intrarenal arteries were also conducted at the time of blood sampling. Data was expressed as mean and standard deviation (SD) for each of experiments. Statistical analysis was evaluated by ANOVA and Student t-test. Differences at $p < 0.05$ were taken as statistically significant.

Results

All dogs of both groups had no clinical signs of uremia during the experiments. Serum Cr levels, measured as an index of kidney function, were not significant different in the both groups (Fig. 1). We also measured BUN as a second index of kidney function in these experimental groups. Different to Cr, the level of BUN in the control group increased to 34.06 mg/dl after 14 days of reperfusion.

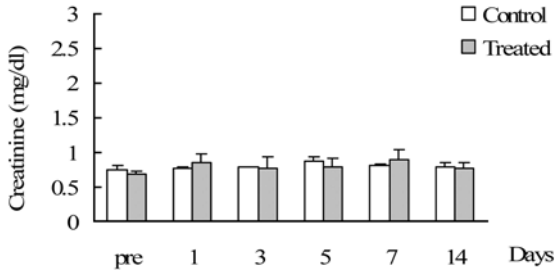

Fig. 1. Changes of Cr concentration after renal ischemia-reperfusion of kidneys subjected to 30 min of warm ischemia. The values are expressed as the mean with SD. Pre; Pretreatment period of atropine.

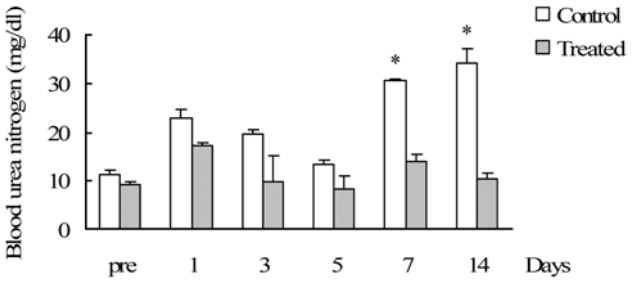

Fig. 2. Changes of BUN concentration after renal ischemia-reperfusion of kidneys subjected to 30 min of warm ischemia. The values are expressed as the mean with SD. *Statistically significant difference ($p < 0.01$) between the both groups. Pre; Pretreatment period of atropine.

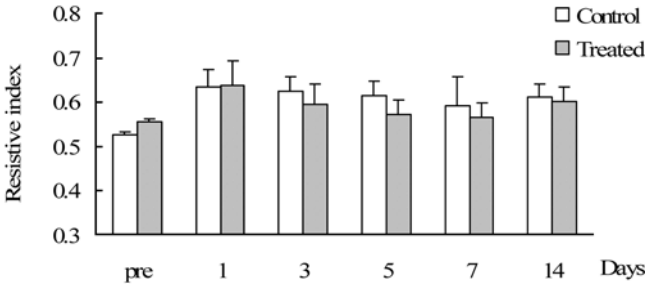

Fig. 3. Changes of resistive index after renal ischemia-reperfusion of kidneys subjected to 30 min of warm ischemia. The values are expressed as the mean with SD. Pre; Pretreatment period of atropine.

The BUN levels in the treated group were 10.3 mg/dl after 14 days of reperfusion (Fig. 2). Although, the level of BUN of control group came within normal range on days 1, 3, 5, and then revealed significant increase on days 7, 14 to compare with pre-treatment. Meanwhile, the level of BUN of treated group revealed within normal range. And also, the RI values of two groups measured at before and 1, 3, 5, 7, 14 days after surgery were normal range. The peak RI values ranged from 0.52 to 0.63 in control group and from 0.55 to 0.63 in treated group and there was no significant difference between control and treated group (Fig. 3).

Discussion

In the present study, the changes of BUN, Cr and RI in the dog model were evaluated after I/R injury. Ascorbic acid is a main water-soluble antioxidant in plasma. This antioxidant which reduces reactive oxidant species intracellularly and extracellularly reverses the endothelial dysfunction by ischemic injury [4]. Significant changes in BUN levels to compare values immediately before and 14 days after reperfusion were demonstrated in control group. In a previous study, renal reperfusion following ischemia for 90 min demonstrated abrupt increase of BUN levels on 4 days [8]. Morphological studies have demonstrated that ischemia irreversibly damages the distal segments of the proximal tubules whereas more proximal segments suffer reversible injury after a short period (30 min) of normothermic ischemia [9]. Therefore, the proximal segment undergoes necrosis/apoptosis and sheds into lumen of the tubule which is considered to be the basis for the decrease in the glomerular filtration which is indicated by a significant increase in the Cr and BUN levels [8]. In our study, although it was not severs than that of previous study due to a short period of ischemia, the levels of BUN in control group increased gradually. BUN levels of treated group was not much changed. This finding suggests that ascorbic acid influenced the renal tissue damaged by ischemia- reperfusion. It seems that ascorbic acid protect renal tissue from the injury by reperfusion and so help recovery of renal function. However,

no significant changes in Cr levels were demonstrated in both groups. It is thought that Cr diffuses more slowly than does BUN. Thus, this finding suggest that ischemic injury time was not long enough for change. Levels of Cr did not show much varied changes than BUN.

Additionally, we have failed to demonstrate correlations between RI and I/R injury. Color Doppler sonography with measurement of RI of intrarenal arteries has been used to evaluate some renal disorders such as obstructive uropathy [6] and to diagnose graft rejection by some authors [7]. However, evaluation of renal function using the parameters measured in Doppler scan such as RI and pulse index is risky [1]. Similarly, the variations of RI in this study are difficult to evaluate renal function.

In conclusion, administration of ascorbic acid before the renal ischemia may play a role of in control of elevation of BUN, although it did not have influence on Cr and RI. These date resulted from short period of ischemic-reperfusion. Thus, further studies are necessary to ascertain the influence of ascorbic acid on long-term of renal ischemic-reperfusion and other parameters.

Acknowledgments

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