NOTE *Laboratory Animal Science*

Erdosteine in Renal Ischemia-Reperfusion Injury: An Experimental Study in Pigs

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ABSTRACT. The aim of the present study was to investigate the effect of erdosteine on renal reperfusion injury. Twelve male Landrace and Yorkshire mixed pigs were randomly divided into two groups: untreated control group (I/R), erdosteine treated group (I/R + erdosteine). Each group is composed of six pigs, and the pigs were unilaterally nephrectomized and their contralateral kidneys were subjected to 30 min of renal pedicle occlusion. The elevations of creatinine and blood urea nitrogen levels were lower in the treated group compared with the control group. The catalase activity and the glutathione peroxidase activity were higher in the erdosteine group. As a result, this study suggests that the erdosteine treatment has a role of attenuation of renal I/R injury recovery of renal function in pig.
KEY WORDS: antioxidant enzyme, erdosteine, ischemia-reperfusion injury, pigs.

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Several agents are proposed to be useful in the clinical setting for renal I/R damage, including free radical scavengers used for the prevention of renal I/R [7]. Erdosteine was introduced as a mucolytic agent for chronic pulmonary diseases more than 10 years ago. The effects of erdosteine might, however, extend much further than modulation of mucus viscosity and increase of tracheobronchial clearance; the blocked suphydryl group, after hepatic metabolization, becomes available for free radical scavenging and antioxidant activity too [9]. However, the effects of erdosteine on antioxidant activities have not been indicated after I/R injury till now. Therefore, the aim of this study is to evaluate the protective effects of erdosteine against oxidative stress during I/R injury of the kidney in pigs, utilizing biochemical parameters.

Twelve male Landrace and Yorkshire mixed pigs (37.6 ± 1.7kg, 3 to 4 month old) were used in the experiments. Pigs were randomly assigned: control untreated pigs (n=6) and erdosteine-treated pigs (n=6). All pigs were obtained from the experimental livestock farm of the College of Agriculture, Chungnam National University (CNU). These experimental and housing protocols were approved by the CNU Animal Care and Use Committee. The animals were acclimated and maintained by being fed with standard swine diet. Additionally, routine lighting cycle and standard room temperatures were maintained. Pigs were housed in an air-conditioned room with a 12 hr light-dark cycle and controlled temperature (24 ± 2°C). Pigs were starved for 24 hr prior to surgery in order to prevent any possible adverse effects associated with anesthesia. The animals were premedicated with atropine sulfate (Atropine Sulfate®, Huons Co., Ltd., 0.04 mg/kg, IM) and xylazine hydrochloride (Rompun®, Bayer Co., Ltd., 4.4 mg/kg, IM) for immobilization. Prophylactic antibiotics, ampicillin sodium (Penbrrok®, Chong Kun Dang Co., Ltd., 20 mg/kg IV) and analgesic agent, meloxicam (Metacam®, Boehringer Ingelheim Co., Ltd., 0.2 mg/kg IV) were administered before surgical operation. Afterwards, thiopental sodium (Thionyl® Dai Han Pharm. Co., Ltd., 6 mg/kg, IV) was administered for tracheal intubation. The anesthesia was maintained with 2% isoflurane under pure oxygen. Laparotomy was performed by midline incision. The right kidney was isolated and then both the renal artery and vein were clamped. After 30 min of ischemia, the vessels were declamped, and the left kidney was removed. Subsequently, irrigation-aspiration of the right kidney of each group was performed. One hundred U/kg of heparin sodium were infused intravenously 10 min prior to renal ischemia injury. During the surgical operation, the pigs were given intravenous fluid (Hartmann sol, 5 m*l*/kg/hr). Pigs were allowed unlimited access to water and food 24 hr after the surgery. Ampicillin sodium (20 mg/kg IM, bid) and analgesic agent, meloxicam (0.2 mg/kg IM, sid) were administered for 13 days. Antibiotics spray (Pink skin®, Sungwon Co., Ltd.) applied to the middle line area once daily for 13 days. The erdosteine treatment (E-drol Cap® Ilsung Pharm. Co., Ltd., 300 mg, SID, PO) was commenced 2 days prior to the induction of I/R injury so as to ensure that adequate level of the drugs would be present in the pig's body at the time of I/R injury. The control pigs were given vehicle alone on the same schedule. Blood samples were collected via venipuncture from the jugular vein in each designated days after surgery. Blood urea nitrogen (BUN) and serum creatinine levels were measured from serum samples obtained from pre-I/R injury, post-I/R injury, 1st POD, 3rd POD, 7th POD, and 14th POD using a blood chemistry analyzer (IDEXX Vetest 8008, Maine, USA). Blood samples for plasma isolation were collected in the tubes containing EDTA and were eventually centrifuged at 1,000 g for 10 min at 4°C and the plasm sample were stored at −80°C. CAT and GPx concentration were measured with Cayman kit (Cayman Chemical Company, Ann Arbor, MI, U.S.A.) using ELISA reader (Synergy™HT and KC4, Bio-

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Tek® Instrument. Winooski, VT, U.S.A.). The pigs were euthanized on day 14 post-operation and the kidneys were obtained for hitopathologic examination. For light microscopic examination, kidney tissue fragments were fixed in 10% formalin solution. Following dehydration in an ascending series of ethanol (70, 90, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 4 μm were stained with hematoxylin and eosin. Each kidney sample was evaluated microscopically with the degree of renal damage include; tubular cell necrosis, cytoplasmic vacuole formation, leukocyte infiltration and glomerular degeneration. To avoid the possibility that the weight of pig affects our experimental results, we controlled the weight of pig in two folds. Each pig is randomly assigned into each treatment, and the variability among weights of pigs are kept as minimized as possible. The sample standard deviation (1.7 kg) is less than 5% of sample mean (37.6 kg) in the weight of pigs used in the whole experiment. Data were expressed as mean ± SD, and Mann-Whitney U-test was used as appropriate. A p-value of <0.05 was considered as significant. All statistics were performed using a computer statistical package (SPSS for Windows, Release 12.0.1 24 Mar 2004, SPSS inc., NY, U.S.A.).

The serum BUN levels are shown in Table 1. It was observed that the serum BUN levels increased significantly in both groups after I/R injury. The peak of the BUN occurred on the first day after renal I/R, which decreased within time of renal reperfusion. At the 14 days after I/R injury, the BUN level of the erdosteine group was significantly different from that of control group. The BUN levels were recovered within normal range 14 days after in the erdosteine group.

Serum creatinine levels increased significantly in both groups (Table 1). The creatinine peak occurred in three days in the control group, while it occurred within a day in the erdosteine group. The elevation of creatinine levels was lower in the erdostein group compared with the control group. At the 14 days after I/R injury, the creatinine level of the erdostein group was significantly lower compared with the control group. Recovery times were as follows: 7 days in the erdosteine group and 14 days in the control group.

CAT activity did not change significantly with time in both groups (Table 1). However, CAT activity increased with time in the erdosteine group, and was significantly higher than in control group at 5 days. GPx activity did not change significantly with time in erdosteine group (Table 1). GPx activity increased with time in erdosteine, whereas it significantly decreased in the control group. Activity of GPx in the erdosteine group was significantly higher than in control group at 1 day and 3 days. Microscopically, the kidney pathology was not significantly different between control and experimental groups (Fig. 1). These results were due to healing process for 14 days post-operation. We decided 30 min of ischemia for propose of test, which results in sufficient I/R injury to the kidney, because pigs showed irreversible cell injuries for 60 min of ischemia in our pilot study.

The results of the present study demonstrate that erdosteine has protective effects on I/R-induced renal damage. Erdosteine preserved renal function and increased glutathione peroxidase and catalase levels. It prevented the rise in BUN and creatinine levels induced by I/R as well. The I/R injury is of paramount importance in organ transplantation and is clearly a major determinant of early graft dysfunction. Loss of blood results in ischemia, which rapidly damages the metabolically active tissues such as the renal tissue [1]. Reactive oxygen species (ROS) have been implicated strongly in the pathogenesis of cellular damage associated with renal I/R [2]. Oxidative damage caused by free radicals and peroxide such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH), play a crucial role in the pathogenesis of many diseases [10]. Inactivation and removal of these highly reactive species depend on the reactions involving the antioxidant defense system [5, 11]. The two main enzymes that control the biological effects of reactive oxygen species are: CAT, which detoxifies H_2O_2, and GPx, which oxidizes reduced glutathione, inactivates H_2O_2, and reduces organic peroxides to their alcohols [12]. BUN and creatinine levels have been widely used to access renal function. In this study, renal I/

Table 1. Blood urea nitrogen (BUN), creatinine (Cr), catalase (CAT) and glutathione peroxidase (GPx) data

	Group	Pre-treatment	Post-OP	Day 1	Day 3	Day 5	Day 7	Day 14
BUN	Vehicle	6.5±1.73	8.0±2.16*	68.3±4.70*	65.5±5.48*	40.3±7.78*	22.8±8.73*	15.3±0.96*
mg/dl	Erdosteine	9.0±2.45	11.0±3.56*	52.5±2.52*	34.3±0.97*	19.3±4.50*	15.0±0.82*	11.0±1.41§
Creatinine	Vehicle	1.2±0.06	1.4±0.04*	4.9±0.54*	5.6±1.02*	3.5±0.92*	2.1±0.85*	1.3±0.28
mg/dl	Erdosteine	1.1±0.05	1.3±0.16*	3.6±0.82*	2.7±0.78*	1.9±0.15*	1.4±0.32*	1.1±0.09§
CAT	Vehicle	2070.3±221.80	1790.1±351.18	1998.5±220.28	1934.9±340.83	2007.5±70.54		
U/ml	Erdosteine	2168.1±315.89	1970.8±411.59	2262.6±457.90	2099.6±524.56	2326.9±157.01§		
GPx	Vehicle	212.35±45.67	140.96±19.53	142.79±22.32	130.50±11.64	126.32±17.86		
nmol/min/ml	Erdosteine	202.21±46.76	234.93±16.14	242.05±15.65§	228.83±10.26§	215.39±36.26		

Data are expressed as mean ± SD (n=6).
* Significantly different from pre-treatment value ($p<0.05$).
§ Significantly different from vehicle group ($p<0.05$).
Pre-treatment; Pre-treatment of erdosteine or vehicle, Post-OP; Post operation.

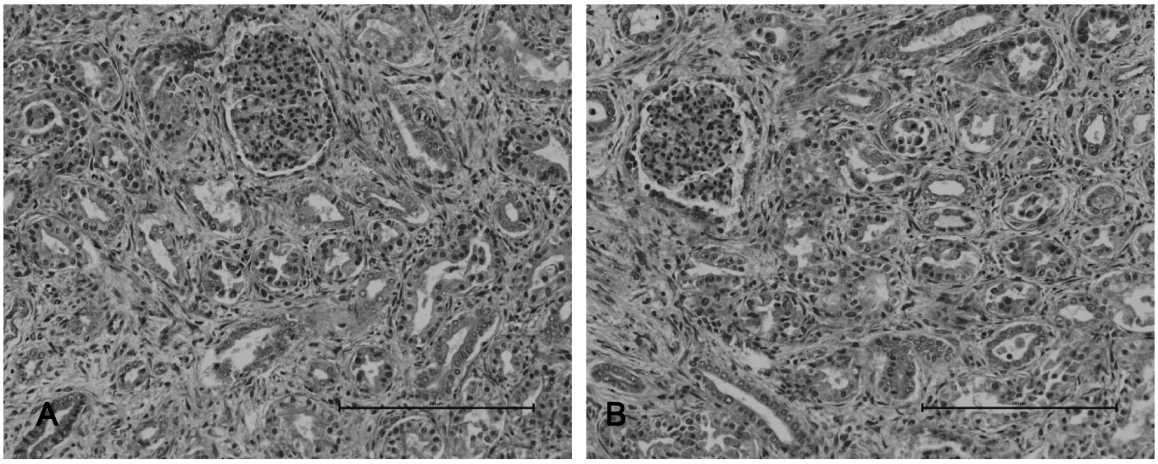

Fig. 1. HE-stained sections of pig kidney. Kidney sections of treated (A) and non-treated (B) pig show mild damage including normal glomeruli, slight interstitial edema and mild tubular damages. Bar=200 μm.

R-induced oxidative stress was associated with impaired renal function leading to a marked increase in serum urea nitrogen and creatinine levels. The levels of BUN and creatinine were significantly increased by I/R. However, the elevation in serum BUN and creatinine levels were depressed in the erdosteine group compared with the control group. Thus, erdosteine tend to show protective effects in renal function after renal I/R, possibly through decreased creatinine and BUN concentration. Hosoe *et al.* [8] demonstrated the selective scavenging activity of erdosteine and its metabolites for H_2O_2 and hypochlorous acid *in vitro*. The CAT and the GPx activities were higher in the erdosteine group. Like other body compartments, kidneys have enzymes (CAT, and GPx) and nonenzymic antioxidant defenses to cope with this potential damage. The antioxidant defense system operates through enzymatic and nonenzymatic components. In normal condition, the antioxidant defense system can detoxify the produced ROS with endogenous antioxidants such as GPx and CAT. However, if there is a pathological condition, like I/R injury, ROS is produced more than usual. High productions of ROS cannot be detoxified by antioxidant enzymes. However, antioxidant therapy prevents the toxic effect of ROS not detoxified by endogenous antioxidant.

In this study, it was observed that GPx and CAT activities decreased in the control, and erdosteine treatment prevented the decrease of enzyme activities. Improvement of antioxidant enzyme activity in the erdosteine group might be a result of the free radical scavenging effect of this drug. The mechanism of effect of erdosteine on GPx activity and CAT activity is unknown, but we suggest that erdostein may act as a stimulating factor in GPx and CAT activity during the reperfusion phase. This effect of erdosteine may be an important factor in decreased oxidative damage in I/R injury. Experimentally studies examined the role of erdosteine in the protection of kidney after I/R in rat [4, 6]. The studies examined the role of erdosteine in the protection of kidney after I/R in a rat models. The study showed a significant decreased antioxidant enzymes activities in the I/R group in comparison with I/R plus erdosteine group. In our study, the CAT and GPx activities were significantly higher in the erdosteine group when compared with the control group. The renal I/R- induced oxidative stress was associated with impaired renal function leading to a marked increased in serum urea nitrogen and creatinine levels. The present study demonstrates that erdosteine, while improving kidney functions, significantly decreased the I/R induced elevations of BUN and creatinine, supporting the notion that the mechanism of action of erdosteine involves its antioxidant activity. Erdosteine may protect the tubular epithelium effectively from reperfusion injury. It is a mucolytic agent which contains two blocked sulfhydryl groups that are released following its metabolic process. It has been shown that its active metabolites have exhibited free radical scavenging and anti-inflammatory activities [3, 9]. In this study, the improved renal function in the pigs treated with erdosteine resulted in the stimulation of antioxidation activities by CAT, and GPx.

In conclusion, the present study demonstrated that renal I/R injury resulted in oxidative damage to cellular injury as seen in biochemical parameter. Following administration of erdosteine prevented renal malfunction and inhibited the generation of free radials, erdosteine appears to play a protective role in the kidney insulted by I/R. Although the exact mechanisms remain to be clarified, erdosteine could be an effective regimen to reduce I/R injury of kidney and enhance the therapeutic efficacy in clinics.

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