Urol Res (2004) 32: 93–96
DOI 10.1007/s00240-003-0382-6

ORIGINAL PAPER

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The effect of dehydroepiandrosterone on renal ischemia-reperfusion-induced oxidative stress in rabbits

Received: 20 June 2003 / Accepted: 30 September 2003 / Published online: 31 October 2003

Abstract Reactive oxygen species (ROS) can play an important role in the pathogenesis of ischemia-reperfusion (I/R) injury. Dehydroepiandrosterone (DHEA) is one of the hormones secreted from adrenal glands, and in some studies it has been shown that DHEA has antioxidant properties. This experimental study was designed to determine the effect of DHEA on I/R-induced oxidative stress in rabbit kidney. Twenty-one rabbits were divided into three groups. Rabbits were subjected to 60 min of left renal pedicle occlusion followed by 24 h of reperfusion. DHEA (50 mg/kg) (I/R + DHEA group) or equal volume of vehicle (I/R group) was administered 3 h prior to ischemia. The control group received only laparotomy without I/R, DHEA or vehicle. At the end of the reperfusion periods, rabbits were decapitated. Renal tissues were taken for determination of malondialdehyde (MDA) levels as an indicator of lipid peroxidation and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities as antioxidant enzymes. In the I/R group, while renal SOD and CAT activities were significantly lower, MDA levels were significantly higher than in the I/R + DHEA group and controls. In the I/R + DHEA group, enzyme activities and MDA levels were similar to the controls. There was no significant difference in terms of renal GPX activity among the groups. DHEA may have a beneficial effect on renal tissue against oxidative damage due to I/R by preventing decreases in some antioxidant enzyme activities.

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Keywords Renal ischemia-reperfusion · Dehydroepiandrosterone · Reactive oxygen species · Antioxidant enzymes

Introduction

Renal ischemia-reperfusion (I/R) injury could arise as a consequence of clinical conditions such as renal transplantation, shock, cardiac arrest, hemorrhage and renal artery surgery [1]. In clinical settings, evidence is emerging that reactive oxygen species (ROS) injury resulting from disorders in oxygen metabolism can play an important role in the pathophysiology of the kidney reperfusion injury following ischemia [2]. In body, formed ROS are eliminated by enzymatic and non-enzymatic antioxidants. But when free radicals are generated in excess or when the cellular antioxidant defense system is defective, they can stimulate chain reactions by interacting with proteins, lipids and nucleic acids, causing cellular dysfunction and even death. In such a situation, administration of antioxidants should therefore give potential benefit by neutralizing ROS [1, 2]. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) are the most abundant steroid hormones in the circulation. Although DHEA is known to have numerous biological and biochemical effects, and beneficial action on atherosclerosis, neuronal injury, diabetes, obesity, cancer, stress, and viral and bacterial infections [3, 4, 5], the mechanism underlying the protective effect of DHEA is not obvious.

Several studies have investigated the effect of DHEA on the cellular antioxidant enzyme activities [6, 7] and DHEA administration is considered to prevent lipid peroxidation of cell membranes and make them more resistant to oxidative stress [5, 6, 7, 8].

In the present study, we aimed to investigate the effects of DHEA pretreatment on lipid peroxidation, superoxide dismutase (SOD), catalase (CAT) and

glutathione peroxidase (GPX) activities as antioxidant enzymes following renal I/R in the rabbit model.

Materials and methods

After obtaining approval from the local ethics committee, we used 21 adult male New Zealand rabbits weighing 2,410–2,900 g (2742.0 ± 159.3 g). Before starting the experimental protocols, the rabbits were weighed and randomly divided into three groups of seven animals: the control group underwent sham operation; the I/R group was subjected to left renal ischemia followed by 24 h of reperfusion; and the I/R + DHEA group was pretreated with 50 mg/kg DHEA and submitted to left renal I/R.

DHEA dissolved in 1 vol 95% ethanol was mixed with 9 vol 16% Tween 80 in 0.9% NaCl to give a 6% (w/v) solution. DHEA was administered subcutaneously (sc) to over-night fasted rabbits at a dose of 50 mg/kg 3 h before operation in group III (I/R + DHEA) animals. Group II (I/R) received the vehicle only.

All of them were kept in the same room under a constant temperature (22 ± 2°C). The animals went through a 12-h fasting period before the experiments. They were anesthetized with 85 mg/kg ketamine HCI and 6 mg/kg xylazine given intramuscularly.

A laparotomy was performed with a vertical midline incision, and the left renal artery was identified by blunt dissection. A hemostatic microvascular atraumatic clamp was applied on the renal artery of the left kidney for 60 min to create complete renal ischemia. The right kidney was left intact to serve as a control. The clamp was removed later to allow restoration of blood flow to the kidney. Animals were sacrificed following 24-h initiation of reperfusion. After that, both right and left kidneys were dissected out and washed by ice-cold 0.9% NaCl solution. All renal tissues were stored at −70°C for biochemical assays.

A portion of each renal tissue for all assays except the MDA assay was homogenized in 0.9% NaCl solution (10% w/v) with an OMNI TH International homogenizer. Tissue homogenates were centrifuged for 15 min at 18,000 g, and then the supernatants were removed for analysis. For the MDA assay, renal tissue samples were homogenized so that each gram of tissue had a 1.15% KCl solution of 9 mL. Protein levels of the homogenates were determined according to the method of Bradford [9]. All of the spectrophotometric measurements were performed using a Beckman DU 530 spectrophotometer (USA). SOD, CAT, GPX activities and MDA levels were measured in the renal homogenates. SOD activity was measured by reduction of nitroblue tetrazolium (NBT) by xanthine-xanthine oxidase system, which is a superoxide generator. Enzyme activity leading to 50% inhibition was accepted as one unit [10]. CAT activity was measured using the method by Sinha [11]. GPX activity was measured by the method of Paglia and Valentine [12]. Results were expressed as units per gram protein. Tissue MDA levels were determined spectrophotometrically according to the method described by Ohkawa [13]. Results were expressed in terms of nanomoles per gram protein.

Data are expressed as means ± standard deviation (SD). The significance of differences between the groups was assessed using the Kruskall-Wallis test and Mann-Whitney U test and $p < 0.05$ was considered as significant.

Results

Mean renal tissue SOD, CAT, GPX activities and MDA concentrations of groups are presented in Table 1. SOD activities of the I/R group were found to be significantly lower than those of the I/R + DHEA and control groups ($p = 0.004$ for both). There was no significant difference in SOD activities between the I/R + DHEA and control groups. CAT activities of the I/R group were found to be considerably lower than those of the I/R + DHEA and control groups ($p = 0.01$ and $p = 0.02$, respectively). The difference in CAT activities between the I/R + DHEA and control groups was insignificant. Renal GPX activity was measured lower in the I/R group compared with the I/R + DHEA and control groups, but the difference did not reach statistical significance ($p = 0.09$ and $p = 0.07$, respectively).

The degree of free radical damage following I/R was assessed by lipid peroxidation, which was measured as MDA levels. MDA levels of the I/R group were significantly high compared with the I/R + DHEA and control groups ($p = 0.01$ for both). There was no significant difference between the I/R + DHEA and control groups in terms of MDA levels.

As expected, in the I/R and IR + DHEA groups, the non-I/R right kidneys had MDA levels of 60.6 ± 14.0 and 42.4 ± 16.3 nmol/g protein, respectively, whereas in the left kidneys subjected to I/R, MDA levels were 79.0 ± 15.3and 52.1 ± 15.4 nmol/g protein, respectively.

Discussion and conclusions

Renal ischemia generally presents in two types of clinical situations: 1) when renal ischemia is anticipated and the physician has the opportunity to prepare the patient for the events such as elective renal artery, tumor-sparing renal, aortic and anatrophic procedures, and 2) when the ischemic event such as acute renal failure, obstructive

Table 1 Activities of antioxidant enzymes (SOD, CAT and GPX) and MDA levels in the study groups

Groups	SOD (U/g protein)	CAT (U/g protein)	GPX (U/g protein)	MDA (nmol/g protein)
Control group	851.5 ± 133.8	1548.1 ± 357.2	489.3 ± 140.8	63.7 ± 14.6
Ischemia/Reperfusion group				
Ischemic kidney	555.0 ± 141.3[a]	1029.8 ± 227.6[b]	334.9 ± 146.1	79.0 ± 15.3[c]
Nonischemic kidney	844.4 ± 157.6[d]	1483.5 ± 429.8[e]	458.1 ± 114.6	58.0 ± 10.3[d]
Ischemia/Reperfusion + DHEA group				
Ischemic kidney	908.2 ± 166.6[f]	1452.8 ± 313.8[g]	465.5 ± 120.2	52.1 ± 15.4[g]
Nonischemic kidney	744.7 ± 161.3	1428.9 ± 339.4	550.4 ± 151.7	42.4 ± 16.3

All values are means ± standard deviation (SD)
[a]$p = 0.004$, [b]$p = 0.01$ and [c]$p = 0.03$ compared with the control group
[d]$p = 0.01$ and [e]$p = 0.02$ compared with the I/R ischemic group
[f]$p = 0.004$ and [g]$p = 0.01$ compared with the I/R group

uropathy or pre-renal failure due to hypotension has already occurred by the time the physician is able to treat the patient [1]. Ischemia also has a significant role in the pathogenesis of acute intrarenal failure in which renal vasocontruction, tubular obstruction, back leakage of filtrate and decreased ultrafiltration coefficient are the proposed mechanisms [1]. Renal cell damage due to ischemia followed by reperfusion is assumed to be partly a result of the lipid peroxidation which in turn leads to membrane damage [1, 2].

ROS (H_2O_2, O_2^-, OH) are normal by-products of cellular metabolic processes. The human body has a complex antioxidant defense system that includes the antioxidant enzymes (SOD, GPX and CAT) and non-enzymatic antioxidant components such as glutathione, α-tocopherol, ascorbic acid and β-carotene. These prevent the initiation or propogantation of free radical chain reactions. During ischemia, adenosine triphosphate is degraded to hypoxanthine and xanthine dehydrogenase is converted to xanthine oxidase (XO). During reperfusion, XO catalyzes the conversion of hypoxanthine to uric acid with release of the superoxide radical anions (O_2^-). Subsequent reactions catalyzed by SOD result in the formation of H_2O_2, which is less hazardous than O_2^-. CAT enhances the degradation of H_2O_2 to oxygen and water. Also GPX functions to remove H_2O_2 [1, 2].

DHEA and/or DHEAS are the most abundant adrenal steroids in the blood, and evidence indicates that at concentrations slightly above those found in human tissues, it possesses a multitargeted antioxidant effect [14, 15]. In one study, it was shown that while in rats treated with streptozotocin the ROS levels were markedly increased, streptozotocin + DHEA treatment did not alter ROS levels [6]. In another study, the same investigators reported that DHEA treatment reduced the oxidative imbalance induced by transient ischemia followed by reperfusion in the synaptosomes obtained from the brain of both normoglycemic and streptozotocin-diabetic rats, suggesting that DHEA might be an excellent scavenger in brain as well as in other tissues [16].

Despite many potentially important roles, it is presently unclear how DHEA exerts these effects. The possibility that inserting DHEA into the lipid membranes makes them more resistant to oxidative stress may be considered. DHEA has been reported to change the fatty acid composition of mitochondrial membrane phospholipids in rats [17, 18].

Aragno et al. [7] reported that DHEA appears to need a "lag phase" before becoming active. They speculated that DHEA might be converted to active metabolites during the "lag phase". This phase was estimated as 3 h. The protective effect of DHEA against pro-oxidant agent is maintained for a long time (17 h) after DHEA administration. In the present study, we administered DHEA 3 h before experimental protocol.

In the present study, administration of DHEA before inducing ischemia significantly attenuated oxidative stress, improved activities of antioxidant enzymes, particularly SOD and CAT, and decreased concentrations of MDA. In rats with hyperlipidemia, DHEA increased platelet SOD activity. Increased SOD activity might protect the organism against atheromatosis. Thus, it is possible that the activation of the antioxidative system is one of the mechanisms of antioxidative DHEA activity in animals [19]. Despite several studies of DHEA, according to our knowledge, there has not been any study related to the antioxidative effects of DHEA on renal ischemia/reperfusion injury.

Aragno et al. [6] investigated both lipid peroxidation and cellular antioxidant defenses during hyperglycemia and they demonstrated that DHEA treatment clearly reduces oxidative stress products in the tissues of diabetic rats. While the total SOD activity decreased in the liver tissue of diabetic rats, DHEA-treatment restored SOD activity to control values. Liver and kidney tissues of diabetic rats showed a marked increase in GPX and CAT activities. DHEA treatment was found to normalize GPX and CAT activities.

We found that the SOD and CAT activities in the renal tissues were higher in both I/R + DHEA and control groups when compared with the I/R group. Although GPX was higher in the I/R + DHEA and control groups than in the I/R group, the difference was not statistically significant. This may be attributed to the small number of the groups.

Our data show that free radical overproduction induced by I/R causes lipid peroxidation of rabbit kidney. Administration of DHEA 3 h before renal I/R decreased MDA levels and prevented decreases in SOD and CAT enzyme activities in the kidney homogenates. Tissue homogenate MDA levels significantly increased in renal I/R compared with the control group. However, pretreatment with DHEA attenuated MDA levels in the renal I/R. Also, it has been demonstrated that treatment with DHEA has a beneficial effect on MDA levels during various substance-induced lipid peroxidation situation [6, 7, 15]. This effect of DHEA may be dependent on the prevented decreases in some antioxidant enzyme activities.

Results of this study show that prophylactic administration of DHEA in ischemia condition prevents reperfusion injuries by eliminating oxygen radicals and inhibiting lipid peroxidation. It is well known that peroxidation of unsaturated fatty acids in membranes may lead to a decrease in membrane fluidity. Consequently, DHEA may be useful in combating diseases of oxidative stress. However, further investigations and clinical studies are needed to explain the effects of DHEA thoroughly.

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