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The effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in comparison with α-tocopherol in rat kidneys

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Abstract Oxygen-derived free radicals have been implicated in the pathogenesis of renal injury after ischemia-reperfusion. Caffeic acid phenethyl ester (CAPE), an active component of propolis extract, exhibits antioxidant and anti-inflammatory properties. To determine whether CAPE offers any advantage over α-tocopherol, we compared their effects on an in vivo model of renal ischemia-reperfusion injury in rats. CAPE at 10 μmol/kg or α-tocopherol at 10 mg/kg was administered intraperitoneally before reperfusion. Acute administration of CAPE suppressed ischemia-reperfusion induced renal lipid peroxidation and tissue injury more than α-tocopherol. CAPE may therefore offer a therapeutic advantage in acute injury settings.

Key words Reperfusion injury · Kidney · Caffeic acid phenethyl ester · α-Tocopherol

Introduction

Ischemia-reperfusion (I/R) injury in the kidney is often observed in the renal operations. Reperfusion of ischemic kidneys increases the hazardous effect of early ischemic injury by release of reactive oxygen species and accumulation of activated neutrophils. This cascade of events is known as reperfusion injury [2]. Reperfusion injury has been studied in the kidneys through changes in malondialdehyde (MDA) content and changes in antioxidant enzyme levels [1].

There have been many studies assessing tissue injury caused by I/R in the kidney and its protection. A number of drugs or chemicals have been used to prevent the I/R injury in kidneys; and of these trimetazidine [12] and dopexamine [6] were found to be effective in prevention of lipid peroxidation. Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis extracts and has been used in traditional medicine for many years. At a concentration of 10 μM, CAPE completely blocks production of reactive oxygen species (ROS) in human neutrophils and xanthine/xanthine oxidase (XO) system [4, 16]. Although CAPE has antioxidant effects [5, 8], its effect in renal I/R injury has not been investigated to date. The objective of the present study was to investigate the effects of CAPE on histopathological changes, lipid peroxidation, and XO levels in comparison with that of α-tocopherol in I/R injury in rat kidneys.

Materials and methods

Thirty adult female albino Wistar rats (210–240 g) were divided into five groups ($n = 6$): group I, sham; group 2, ischemia; group 3, I/R; group 4, CAPE + I/R; and group 5, α-tocopherol (Vit E) + I/R. Rats were anesthetized with ether and right renal vascular pedicles were exposed via a midline laparotomy. Ten minutes after placing an occlusive vascular clamp across the right renal pedicle, either saline, CAPE (10 μmol/kg, from 25 μmol/ml solution) or α-tocopherol (Evigen-AKSU; 10 mg/kg) was injected into the peritoneum. Following unilateral renal ischemia (30 min) and reperfusion (30 min), right and left nephrectomy was performed, and a part of the kidney was preserved in formalin with the remainder stored at –30°C until analysis. CAPE applied in this study was synthesized according to the technique mentioned elsewhere [3]. In the ischemia group, the kidneys were removed after 30 min of ischemia. Animals in the sham group underwent a surgical procedure similar to the other groups but the artery was not occluded.

For biochemical analysis, after weighing the kidneys, tissues were homogenized in four volumes of ice-cold Tris-HCl buffer

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(50 mM, pH 7.4) containing 0.50 ml/l Triton X-100 with a homogenizer (IKA Ultra-Turrax T 25 Basic) for 2 min at 13,000 rpm. All procedures were performed at +4°C. Xanthine oxidase activity and MDA levels were determined in the homogenate. XO activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbancy at 293 nm, according to Prajda and Weber's method [14]. Tissue homogenate was incubated (50 µl) for 30 min at 37°C in 2.85 ml of medium containing phosphate buffer (pH 7.5, 50 mM) and xanthine (0.067 mmol final concentration in each tube). The reaction was stopped by addition of 0.1 ml 100% (w/v) TCA and the mixture was centrifuged at 5000 *g* for 15 min. With resultant clear supernatant, absorption at 293 nm was measured against blank. A calibration curve was constructed by using 10–50 milliunits/ml concentrations of standard XO solutions (Sigma X-1875). All chemicals were obtained from Sigma. One unit of activity was defined as 1 µmol of uric acid formed per min at 37°C, pH 7.5, and expressed in U/g prot. All samples were assayed in duplicate. Tissue malondialdehyde (MDA) levels were determined by the method described by Wasowicz et al. [18]. Briefly, MDA was reacted with thiobarbituric acid by incubating for 1 h at 95–100°C. Following the reaction, fluorescence intensity was measured in the *n*-butanol phase with fluorescence spectrophotometry (Hitachi, model F-4010; excitation at 525 nm, emission at 547 nm), by comparing with a standard solution of 1,1,3,3-tetramethoxypropane. Results were expressed in terms of nmol/g wet tissue.

Protein measurements were made according to the method explained elsewhere [10]. For histological evaluation, formalin-fixed specimens were stained with H&E and the kidneys were examined for the presence of tubular dilatation, interstitial edema and vacuole formation.

All statistical analyses were carried out using SPSS statistical software (SPSS for Windows; Chicago, Ill., USA). The one-way ANOVA analysis of variance and post-hoc multiple comparison test (LSD) were performed on the data of the biochemical variables to examine differences between the groups. *P*-values less than 0.05 were considered to be significant.

Results

MDA levels and XO activities for ipsilateral and contralateral kidneys are shown in Table 1. MDA levels in the I/R group were higher than in sham ($P < 0.026$) and ischemia ($P < 0.011$) groups. However, the MDA level in the CAPE group was significantly lower than in both the I/R ($P < 0.0001$) and α-tocopherol groups ($P < 0.033$). XO activities in both the ischemia and α-tocopherol groups were significantly higher than those of the I/R group in only ipsilateral kidneys. Furthermore, α-tocopherol also caused a significant increase in XO activity compared to levels obtained in the CAPE group. XO activity in contralateral kidneys was not affected by I/R of the ipsilateral kidneys.

Morphological damage ranged from normal (sham group; Fig. 1) to mild (ischemia and CAPE groups; Fig. 2) and severe (I/R; Fig. 3), with α-tocopherol groups (Fig. 4) with cortical rather than medullar tubules, demonstrating the most marked changes. Morphological changes, including tubular dilatation, interstitial edema and vacuole formation, were clearly observed in the kidneys of the I/R group (Fig. 3) and α-tocopherol group (Fig. 4). The most remarkable indices were the overall cortical damage and the degree of cortical tubular dilatation. The glomeruli appeared normal in all groups. Vacuole formation was observed in all groups to some extent except in the sham group. CAPE (Fig. 2) but not

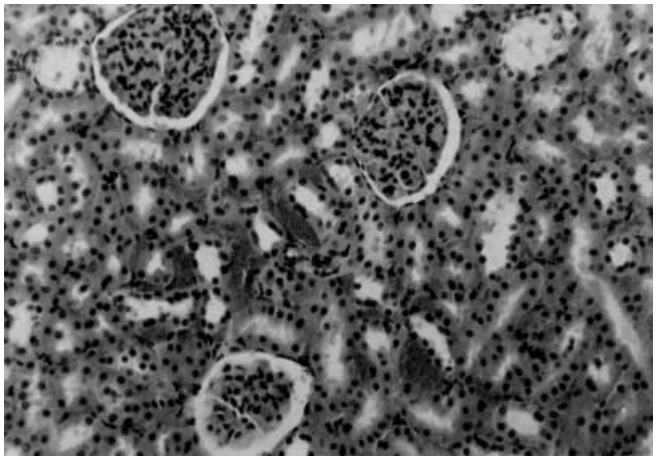

Fig. 1 Photomicrograph of renal cortex in sham group. Tubules and glomeruli appear normal. H&E, ×245

Table 1 The mean renal tissue malondialdehyde (*MDA*) levels and xanthine oxidase (*XO*) activities in sham, ischemia, ischemia/reperfusion (*I/R*), caffeic acid phenethyl ester (*CAPE*) + I/R and vitamin E (*Vit E*) + I/R groups of rats (*ips* ipsilateral kidney, *cont* contralateral kidney, *n.s.* not significant)

Groups	MDA (nmol/g wet tissue)		XO (U/g prot)	
	ips	cont	ips	cont
I. Sham ($n = 6$)	1086 ± 482	1154 ± 333	0.307 ± 0.211	0.210 ± 0.086
II. Ischemia ($n = 6$)	1009 ± 175	982 ± 239	0.299 ± 0.125	0.245 ± 0.107
III. I/R ($n = 6$)	1577 ± 482	1519 ± 616	0.111 ± 0.070	0.230 ± 0.131
IV. CAPE + I/R ($n = 6$)	664 ± 220	825 ± 280	0.126 ± 0.059	0.183 ± 0.095
V. VIT-E + I/R ($n = 6$)	1135 ± 386	1299 ± 246	0.314 ± 0.141	0.271 ± 0.156
I–II	n.s.	n.s.	n.s.	n.s.
I–III	0.026	n.s.	0.010	n.s.
I–IV	n.s.	n.s.	0.016	n.s.
I–V	n.s.	n.s.	n.s.	n.s.
II–III	0.011	0.016	0.013	n.s.
II–IV	n.s.	n.s.	0.022	n.s.
II–V	n.s.	n.s.	n.s.	n.s.
III–IV	0.0001	0.002	n.s.	n.s.
III–V	0.044	n.s.	0.007	n.s.
IV–V	0.033	0.032	0.013	n.s.

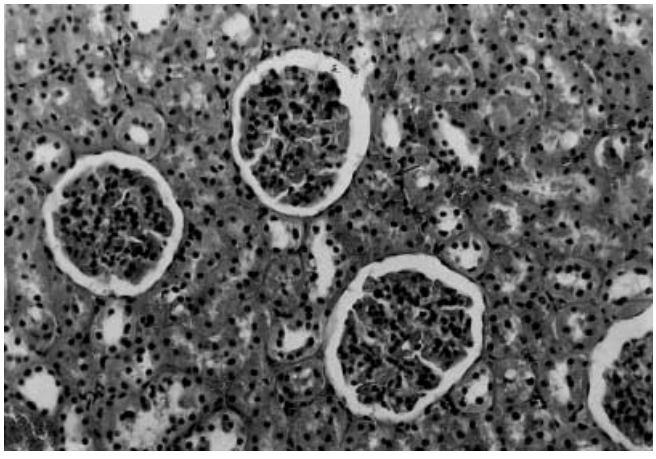

Fig. 2 Photomicrograph of renal cortical tubules after I/R following pretreatment with CAPE. The tubules and the interstitium are almost normal, but the cytoplasm of some of the tubular epithelial cells are filled with fine vacuoles (*arrow*). H&E, ×245

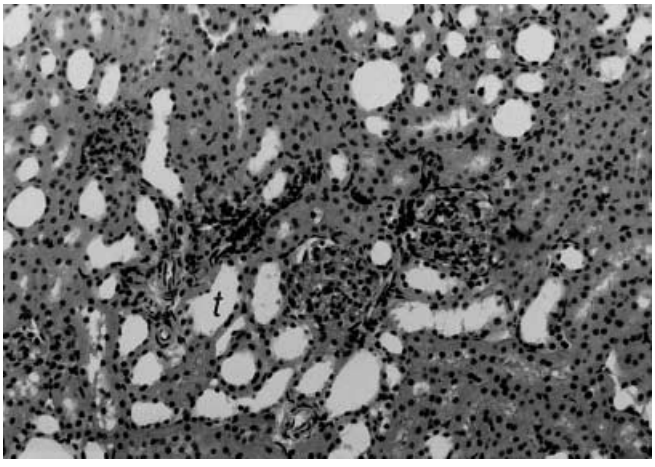

Fig. 4 Photomicrograph of renal cortical tubules after I/R following pretreatment with α-tocopherol. Notice the dilated tubules (*t*) and hardly visible two glomeruli due to edematous interstitium. H&E, ×245

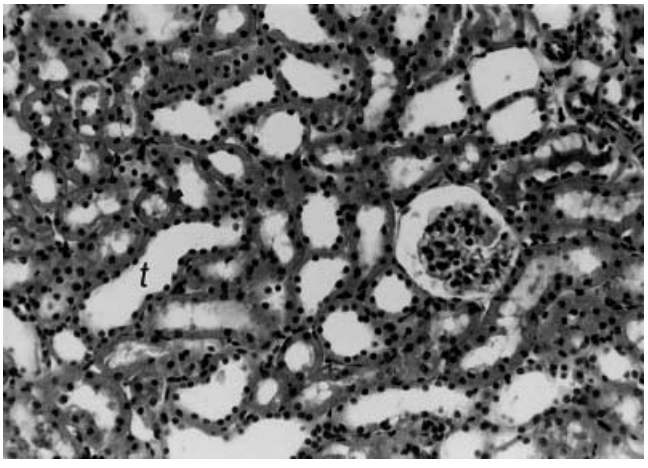

Fig. 3 Photomicrograph of renal cortical tubules after ischemia-reperfusion period. The glomeruli still appear normal while the tubules appear dilated (*t*) and lined by flattened epithelial cells. *Arrow:* vacuoles. H&E, ×245

α-tocopherol apparently reduced the renal tissue damage. Contralateral kidneys presented a normal morphology except in the I/R group. Contralateral kidneys in the I/R group showed mild morphological changes with slight tubular dilatation and vacuole formation.

Discussion

Despite many studies concerning the prevention of reperfusion injury in kidneys, this is the first study in which CAPE was used for preventing I/R injury in kidney. MDA is, in particular, released as a result of toxic effects of active oxygen radicals which destroy unsaturated fatty acids in the cell membrane. Neutrophil infiltration might be regarded as another source of free radicals in the ischemic tissue since activation of neutrophils results in the production and release of potentially toxic oxygen metabolites [2]. Although tissue MDA levels were clearly decreased by CAPE, its mechanism is not clear. CAPE may eliminate free oxygen radicals or decrease myeloperoxidase activity in neutrophils or directly increase the antioxidant enzyme activity and prevent the inhibition of these enzymes. Since antioxidant properties of CAPE are well documented, decrease in MDA levels in the CAPE group is probably due its antioxidant effect.

Since α-tocopherol was a proven agent for prevention of tissue injury in kidney caused by free oxygen radicals [17, 19], MDA and XO levels in the Vit E + I/R group were compared to those of CAPE + I/R. Results of the present study demonstrated that CAPE administration had prevented the lipid peroxidation in the kidneys more than α-tocopherol had.

Tissue XO and neutrophils appear to be a source of superoxide. In general, neutrophils act as a secondary amplifier rather than an initial trigger of reperfusion injury. In our study, XO activity was significantly lower in the I/R group than in the ischemia group. Marked decrease in XO means that superoxide radicals stem from neutrophils rather than the XO system in kidneys. Thus, we suggest that the neutrophil-based generation of superoxide is the major source of reperfusion injury in kidneys and the cytoprotective effect of CAPE may also stem from the interaction with neutrophils attenuating neutrophil-mediated injury. Our finding is consistent with that of others who compared the conversion of xanthine dehydrogenase (XD) to XO with the time course of lipid peroxidation in an I/R model of rat kidney and found no significant conversion of XD to XO in contrast to enhanced lipid peroxidation [7]. Therefore, XO-derived superoxide anion radicals can not be considered causative for lipid peroxidation in the reperfusion interval of the experimental I/R model of rat kidneys. α-Tocopherol causes an elevation in XO activity after reperfusion, while CAPE kept XO activity

at the I/R level. CAPE may act as an inhibiting factor in the process of XD→XO conversion during renal reperfusion while α-tocopherol induces XO conversion.

Histological findings were in parallel with the degree of lipid peroxidation. In I/R and α-tocopherol groups, apparent tubular dilatation may stem from precipitated proteins which obstructed tubules, raising intratubular pressure. This suggestion is consistent with the result of studies in animal models which showed increased intratubular pressure in ischemic injury [13]. Interstitial edema in these two groups may result from filtrate leaking back into the peritubular circulation via injured tubular cells. Studies on the clearance of inulin in man and animals showed that leakage of tubular fluid occurs in ischemic renal injuries [13]. Fine vacuoles observed in the cytoplasm of some of the proximal tubular epithelial cells may result from osmotic changes in tubular fluid. Histological examination also confirmed the protective action of CAPE. In the CAPE group, the damage was less severe than in the I/R and α-tocopherol groups. CAPE may protect tubular epithelium effectively from reperfusion injury.

Contralateral kidneys were examined as to whether ROS or other metabolites may reach them and have deleterious effects on them. Renal I/R and CAPE administration changed the MDA levels in both ipsi- and contralateral kidneys, which suggests that contralateral kidneys are sensitive to the effects of ROS derived from ipsilateral kidneys. Similarly, many laboratories have previously shown that unilateral testicular torsion had an adverse effect on the contralateral testes [9, 15]. Nagler and De Vere-White designed an experimental animal model which seemed to indicate that the causative mechanism was immunologically mediated and that the damage to the contralateral testis was proportional to the duration of torsion [11].

According to the biochemical and histopathological findings, we demonstrated that prophylactic administration of CAPE protects kidneys from reperfusion injuries more than α-tocopherol. In conclusion, it is important to inhibit lipid peroxidation to prevent renal I/R injury and we suggest that parenteral administration (intravenous or intraperitoneal) of CAPE would be helpful in humans undergoing reconstructive renal surgery and transplantation.

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