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A novel antioxidant agent caffeic acid phenethyl ester prevents shock wave-induced renal tubular oxidative stress

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Abstract The aim of this study was to evaluate the effects of the novel free radical scavenger caffeic acid phenethyl ester (CAPE) on extracorporeal shock wave lithotripsy (ESWL) induced renal impairment. The study was performed using 30 rabbits which were divided into two groups, each exposed to 3,000 shock waves at 18 kV: (1) control group, (2) ESWL + CAPE treated group. Malodialdehyde (MDA), urine N-acetyl-β-glucosaminidase (NAG) activity, uric acid and white cell counts were used as markers of oxidative stress. Following shock wave exposure there was a significant rise in MDA, NAG and uric acid and white cell counts. CAPE reduced the rise in MDA, NAG, uric acid and white cell counts. Thus CAPE treatment to a great extent prevented the induction of these renal changes. Our results suggest that the antioxidant capacity of the kidney tissue was reduced after ESWL treatment and that the tissue was exposed to oxidant stress. We conclude that CAPE treatment provided significant protection against ESWL induced free radical damage.

Keywords Shock wave induced renal oxidative damage · Caffeic acid phenethyl ester

Introduction

Extracorporeal shock wave lithotripsy (ESWL) is a non-invasive routine treatment modality for urolithiasis. However, it is not completely free from side effects. The mechanism of ESWL induced cellular damage is still controversial. One of the mechanisms discussed for tissue damage is free radical formation during ESWL [1–4]. In addition to the mechanical fragmentation of the calculus, each shock wave that is generated by thermal effects of 18–24 thousand volts at the second focus may result in some biochemical events, and homolytic cleavage of molecules may take place leading to the formation of free radicals [5, 6]. Because of their very high reactivity, free radicals can cause serious damage to the macromolecules in cells.

Free radical formation has been reported during ESWL treatment. Morgan et al. [1–6] adapted the Fricke ferrous sulphate radiation dosimeter to examine the chemical effects of high energy shock waves, and significant free radical production was documented. Serel et al. [7] presented intra- and extracellular in vitro measurements of free radicals and investigated cell viability after shock wave treatment. They demonstrated an elevated concentration of intracellular free radicals during such treatment in suspended cells in vitro. The source of oxygen radicals in the tissue are neutrophils recruited into the necrotic region, as well as metabolic transformation of hypoxanthine and xanthine to uric acid. Subsequent reactions generate lipid peroxides as well as cytotoxic and oxidation products, of which one is malondialdehyde (MDA). MDA, an indicator of free radical generation which increases at the end of the lipid peroxidation (LPO), is the breakdown product of the major chain reactions leading to the oxidation of polyunsaturated fatty acids, and thus serves as a reliable marker of oxidative stress mediated LPO in renal tissue [2, 3, 7].

Increased urinary enzyme activity is generally regarded as an indicator of renal tubular dysfunction. The lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAG) is one of the markers of tubular damage most commonly referred to, primarily because NAG assays are sensitive enough to allow dilution of the urine, overcoming any enzyme inhibition [8, 9].

Caffeic acid phenethyl ester (CAPE), a flavonoid like compound, is one of the major components of honeybee

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propolis. CAPE, unlikely to have any harmful effects on normal cells [10], has several biological properties: antioxidant [11, 12], anti-inflammatory [13], anti-carcinogenic [14], antiviral [15], and immunomodulatory [16]. CAPE was reported to inhibit lipooxygenase activities and suppress LPO [10, 12, 17]. Likewise, CAPE treatment was reported to protect the kidney from ischemia-reperfusion injury [18], this is also the case for the testis subject to torsion and detorsion [19], and diabetic renal oxidative damage [20]. Free radical formation seems to play an important role in the pathogenesis of shock wave induced renal tubular injury, therefore, our aim was to investigate the potential protective effect of CAPE on the shock wave-induced oxidative stress in rabbit kidney.

Materials and methods

Thirty adult white rabbits weighing between 1,600–2,700 g were used. They were placed in an environment maintained at 22 ± 3.0°C, isolated from noise and with a 12 h light/dark cycle. All rabbits were given food and water ad libitum.

The animals were divided into two groups, each consisting of 15 rabbits, which were exposed to 3,000 shock waves at 18 kV (Stonelith Lithotriptor PCK, Turkey, capacitor 40 nF, focus dimensions 7.7 mm axially×30 mm laterally, focal distance 135 mm, focal pressure 0–1,200 bar) under intramuscular ketamine anaesthesia (1 mg/kg).

In order to make a comparison, the animals in the first group were left as controls (ESWL without CAPE treatment), while the second group (ESWL with CAPE) were treated with CAPE (Sigma) at a dose of 10 μM ml^{-1} kg^{-1} day^{-1} CAPE intraperitoneally (i.p.) for 10 days before the daily exposure. Control rabbits were exposed to ESWL with the same environmental conditions as the exposed groups, but without exposure to ESWL (exposure device off). In addition, in control rabbits, isotonic saline solution (an equal volume of CAPE) was administered i.p.

All of the animals underwent the entire procedure, including anaesthesia and opacification of the kidneys with a contrast agent (Urografin, Schering). This was given intravenously before the procedure. The animals were placed in a supine position on the platform of the lithotriptor and fixed at the thorax and hip to allow direct entry of the waves through the abdominal wall into the right kidney. A pad was used to compress the abdomen and thus induce a dilatation of pelvis to provide better visualization.

The animals were killed when the ESWL procedure had been carried out for 10 days, and the kidneys were removed and immediately frozen. One gram of kidney tissue was homogenized in a motor-driven tissue homogenizer with phosphate buffer (pH 7.4). Unbroken cells, cell debris and nuclei were sedimented at 2,000 *g* for 10 min, and the supernatant was pipetted into plastic tubes and stored at −70°C until assayed. An automated count was used for biochemical analyses (uric acid and white cell count).

MDA levels were estimated by the double heating method of Draper and Hadley [21]. The hydrolysis of lipoperoxidases to form MDA reacts with TBA to yield a red MDA-TBA adduct. This was determined by spectrophotometry at 532 nm and the results expressed as nmole MDA/dry kidney weight.

Urine samples were obtained by putting the animal onto a plastic plate and awaiting spontaneous micturition throughout the study. The urine was collected by aspiration and centrifuged for 5 min at 1,500 rpm and the clear supernatants stored at 4°C prior to analysis. All assays were carried out on the day of collection. Urinary NAG was measured according to the method of Yakata et al. [22] at 580 nm as 3-cresol sulfonphthalein released from 3-cresol sulfonphthaleinyl β-D-glucosaminide. Creatinine in urine was measured using standard spectrophotometric methods (autoanalyzer, Abbott Aeroset Ill., USA).

The Mann-Whitney U-test was used to compare the groups because a normal distribution could not be assumed.

Results

Table 1 shows the mean levels of MDA and urine NAG activity while Table 2 shows the mean white cell counts and levels of uric acid in kidney homogenates of rabbits. There was a significant difference between the two groups: in the control group (ESWL without CAPE), shock wave exposure significantly increased the levels of MDA, urine NAG activity, uric acid and white cell count in renal tissue compared with the ESWL + CAPE treated rabbits (Fig. 1). Therefore, the comparison between the control and ESWL + CAPE demonstrated that CAPE treatment significantly prevented the increase of MDA levels, urine NAG activity, uric acid and white cell count in renal tissue.

Discussion

The protective effects of CAPE in the kidney against oxidative damage due to ischemia-reperfusion injury, also in testis subject to torsion and detorsion, and diabetic renal oxidative damage have been reported [18–20].

Table 1 Mean levels of MDA and NAG activity in kidney homogenates of rabbits (values are given as the means ± SEM)

Groups	NAG (μg^{-1} creatinine)	MDA (nM g^{-1} wet tissue)
Control ($n = 15$)	4.44 ± 0.26	46.20 ± 8.12
ESWL + CAPE ($n = 15$)	3.37 ± 0.17	23.12 ± 4.32
P	< 0.01	< 0.05

Table 2 Mean white cell counts and levels of uric acid in kidney homogenates of rabbits (values are given as the means ± SEM)

Groups	White cells ($x10^3$ μl^{-1})	Uric acid (mg dl^{-1})
Control ($n = 15$)	10.18 ± 0.32	6.30 ± 0.16
ESWL + CAPE ($n = 15$)	6.06 ± 0.44	4.15 ± 0.11
P	< 0.0001	< 0.0001

However, the effects of CAPE on ESWL exposed rabbit kidneys have not been studied extensively.

Our results demonstrate that CAPE significantly reduces the shock wave induced renal impairment resulting in increased MDA levels, urine NAG activity, uric acid and white cell count in renal tissue when compared with controls.

Free radical formation is discussed as an important mechanism of shock wave induced renal injury [1–5]. Reactive oxygen species are also important in other types of renal injury (e.g., post ischemic and toxic acute renal failure [18, 23]). By using fluorescent dyes, the generation of free radicals by shock wave induced cavitations was demonstrated directly in suspended cells [4].

The present study has shown that exposure to ESWL has a significant effect on rabbit kidney, suggesting that ROS are generated under the experimental conditions employed. A significant increase in MDA and NAG levels in the exposed group was observed. The change in uric acid and white cell count with increased MDA and NAG levels may be regarded as an indicator of increased ROS production occurring during the exposure period, and may reflect the pathophysiological process of exposure. NAG is a lysosomal enzyme present in high concentrations in the renal proximal tubular cells. NAG is also found in serum but its molecular weight does not permit glomerular filtration; thus increased urinary NAG activity is one of the most sensitive markers of renal tubular damage [8, 9, 24, 25]. To investigate the possibility of subtle renal impairment, we measured urinary NAG activity in ESWL exposed rats. The mean urinary NAG ratio was higher in the ESWL exposed group than in the control group. Increased urinary NAG levels in the animals may help to detect subtle and early alterations in kidney integrity. Thus, an impaired oxidant/antioxidant balance in the kidney might be partially responsible for the adverse renal effects of ESWL. Ischemia formation after ESWL application has been reported [26]. Sarica et al. have reported that the degree of ischemic formation at the tissue level after high-energy shock wave application occurred in a time and dose dependent manner. According to the results of their study, 7 days after ESWL ischemia disappeared with normalization of tissue antioxidant enzyme levels showing oxidative stress. Their findings on shock wave induced ischemia suggest a possible underlying mechanism of ESWL induced damage to the kidney. Moreover, a transient ischemia formation induced oxidative stress in ESWL treatment was also reported.

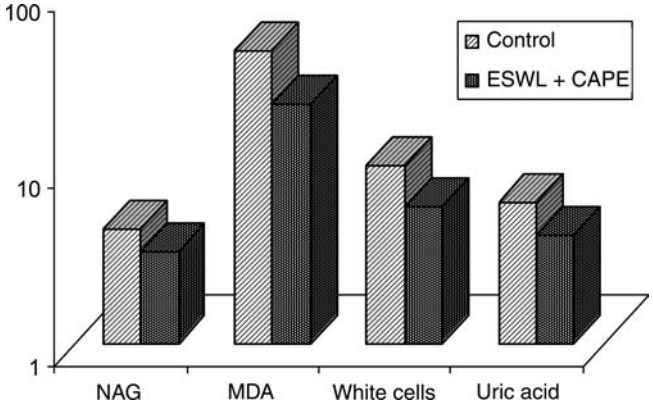

Fig. 1 Mean levels of urinary NAG and MDA activity, white cell counts and the levels of uric acid in kidney of rabbits

The preventive the effects of other agents (verapamil, alpha-tocopherol, vitamin C, selenium, melatonin) against oxidative stress in the kidney have been reported [1, 2, 7, 18, 27]: the ameliorating effect of melatonin, a powerful antioxidant, in ESWL and renal failure induced oxidative damage has been reported [7, 27]. According to these previous studies, the increase in MDA and decrease of glutathione levels (GSH) in different tissues, reflecting oxidant induced tissue damage, were reversed with a different powerful antioxidant treatment in accordance with our results. Likewise, oxidative stress after renal ischemia and its treatment with CAPE have been reported [18]. In addition, verapamil is another protective agent against shock wave induced renal tubular damage [27]. According to Yaman et al. [27], medication like verapamil, may be useful for avoiding the histopathologic and functional side effects of shock waves.

Histopathologic changes following ESWL treatment in experimental animals are well known [2, 5, 7]. Biri et al. [2] reported that the antioxidant defense potential of ESWL treated tissues was reduced, and MDA levels increased. They also suggested that vitamin (a vitamin E plus C combination) pretreatment ameliorated antioxidant defense potential in part, and prevented increases in MDA levels in the ESWL treated tissues, as well as increasing the antioxidant defense potential in the control kidney tissues. According to this study, after ESWL application a significant amount of $OH^{\cdot}$ radicals was measured in the affected tissue, and vitamin pretreatment caused a significant reduction in the OH radical concentration. In the electron microscopic investigations, they reported that significant subcellular changes, such as endothelial injury, loss of foot processes, damage of glomerular basal membrane, etc., were observed in the ESWL treated renal tissue slices. Vitamin pretreatment to a great extent prevented these subcellular changes. Likewise, in the current study, the clear biochemical changes, especially in increased NAG activity and MDA levels, suggest that the antioxidant capacity of the kidney tissue was reduced after ESWL and that the renal tissue was exposed to oxidant stress in

accordance with these previous reports [1–8, 26, 27]. Thus, in our study, treatment with a novel antioxidant, CAPE, similar to pretreatment with other antioxidant agent, exerted significant protection against the oxidant stress induced by ESWL.

In addition, this pathological process should be prevented by an efficient scavenger. Thus, a question arises as to which agent should be selected. One of them may be CAPE according to our current data since it has been used as a folk medicine for many years in the Middle East. Likewise, it is an active and natural component of honeybee propolis extracts and has no side effects [18–20]. However, Strohmaier et al. thoroughly investigating the damaging effects of ESWL treatment, reported that tubular impairment after ESWL in the majority of patients is only a transient phenomenon without clinical signs [1, 30]; the routine application of an antioxidant seems not to be justified. But, in the presence of risk factors (e.g., chronic pyelonephritis, previous lithotripsy, acute urinary tract infection) as shown by Sakamoto et al. [29, 30], prophylactic measures such as the application of an effective natural antioxidant such as CAPE may be of benefit.

In conclusion; our results supported the findings of previous studies that CAPE is a novel powerful free radical scavenger which may be useful in avoiding the side effects of ESWL applications. Further investigations are needed to determine the dose-response relationship between the damaging effects of ESWL application and its treatment with CAPE in various renal changes.

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