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Reduction of high-energy shock-wave-induced renal tubular injury by selenium

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Abstract In shock-wave-induced renal injury cavitation-generated free radicals play an important role. Using an in vitro model with Madin-Darby canine kidney (MDCK) cells, we investigated the influence of selenium, a free radical scavenger, in shock-wave-induced tubular cell injury. Suspensions of MDCK cells (33×10^6 cells/ml) were placed in small containers (volume 1.1 ml) for shock wave exposure. Two groups of 12 containers each were examined: (1) control (no medication), (2) selenium (0.4 μg/ml nutrient medium). Six containers in each group were exposed to shock waves (impulse rate 256, frequency 60 Hz, generator voltage 18 kV), while the other six containers in each group served as a control. After shock wave exposure, the concentration of cellular enzymes such as lactate dehydrogenase (LDH), *N*-acetyl-β-glucosaminidase (NAG), glutamate oxaloacetate transaminase (GOT) and glutamate lactate dehydrogenase (GLDH) in the nutrient medium was examined. Following shock wave exposure there was a significant rise in LDH, NAG, GOT and GLDH concentrations. Selenium reduced this enzyme leakage significantly. Thus we conclude that selenium protects renal tubular cells against shock-wave-induced injury. Since selenium is an essential part of glutathione peroxidase, this effect seems to be mediated by a reduction in reactive oxygen species.

Key words Free radicals · ESWL · MDCK cells · Renal injury · Shock waves · Selenium

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Introduction

Extracorporeal shock wave lithotripsy (ESWL) is a noninvasive routine treatment modality for urolithiasis. However, it is not completely free from side effects. Among others, alterations in renal tubular function (e.g., increased excretion of small molecular proteins such as β2- and a1-microglobulin and cellular enzymes as *N*-acetyl-β -glucosaminidase; decreased excretion of distal tubular Tamm-Horsfall protein) have been described [1, 21, 29, 31].

Microscopic examination of urine voided immediately after ESWL frequently demonstrates vacuolized renal tubular cells [20]. The mechanisms underlying shock-wave-induced renal tubular injury are not completely understood. Shear forces, thermal and cavitation effects and free radical formation have been discussed [6–8, 10, 11, 27]. Some years ago we established an in vitro model with Madin–Darby canine kidney (MDCK) cells to study shock-wave-induced renal tubular injury and its potential prevention [24]. Using this model, we demonstrated the protective effects of substances such as verapamil, a calcium antagonist of the phenylalkylamine type, and fosfomycin, a nephroprotective antibiotic [25, 26]. Since free radical formation seems to play an important role in the pathogenesis of shock-wave-induced renal tubular injury, we studied the influence of selenium. Selenium has been shown to reduce renal tubular toxicity of cis-platinum [2, 4, 12, 14], which is caused by an increased formation of free radicals.

Materials and methods

MDCK cells were grown in culture flasks containing Dulbecco's modified Eagle's medium and fetal calf serum. After 5–6 days, when they are forming "domes" as a sign of a certain differentiation [28], cells were trypsinated. By this procedure cell suspensions were obtained in a medium containing Dulbecco's modified Eagle's medium and fetal calf serum. The cell concentration was adjusted to 30×10^6 cells/ml. For shock wave exposure specially designed containers were used as described previously [24] (kindly supplied

by Siemens, Erlangen, Germany). To avoid acoustic air-fluid interfaces within the containers, they were filled completely (volume 1.1 ml).

Two groups of 12 containers each were examined: (1) control, (2) selenium. Selenium (Selenase, GNPharm Arneimittel, Stuttgart, Germany) was added to the culture medium immediately before shock wave exposure at a concentration of 0.4 μg/ml, which is comparable to high normal human plasma concentration after oral supplementation [16]. Six containers in each group were exposed to high-energy shock waves. For shock wave exposure a Dornier lithotripter MFL 5000 (Dornier Medizintechnik, Germering, Germany) was used. The containers were adjusted to the second focus of the ellipsoid by a specially constructed holder [24]. The impulse rate was 256 shock waves (frequency 60 Hz) at a generator voltage of 18 kV.

After shock wave exposure, the containers were emptied and the cell suspensions mixed with 1.1 ml nutrient medium. This mixture was centrifuged immediately. The supernatant was examined for lactate dehydrogenase (LDH) and *N*-acetyl-β-glucosaminidase (NAG) as membrane and cytoplasmic enzymes and glutamate oxaloacetate dehydrogenase (GOT) and glutamate lactate dehydrogenase (GLDH) as mitochondrial enzymes.

The other six containers in each group received a sham treatment: they were handled in the same way as described above but received no shock waves. Since enzyme concentrations may vary between the series, it was essential to examine sham-treated cells in each series examined.

LDH, GOT and GLDH were measured photometrically using a serum multianalyzer (Serva Feinbiochemica, Heidelberg, Germany). NAG was determined colorimetrically according to Pott et al. [18]. To obtain a better comparison of the results from the two groups, the increases in LDH, NAG, GOT and GLDH were expressed as a percentage of the sham treatment values, which were set at 100%.

Statistical analysis

All data are presented as the means ± standard deviation. The sample tests were checked for Gaussian distribution (Shapiro-Wilks test). Variance analysis was performed using the O'Brian test. In the case of Gaussian distribution and the same variance a Student's *t*-test was used; otherwise the Wilcoxon test (two-sided) was used. Differences were taken to be significant if the probability of error was less than 5% ($P < 5\%$).

Results

In the control series, shock wave exposure increased the concentrations of LDH, NAG, GOT and GLDH in the nutrient medium significantly when compared with sham treatment (Table 1). In the selenium group, there was also a significant increase in the concentrations of LDH, GOT and GLDH. The NAG concentration did not change significantly (Table 2).

The comparison between the control and the selenium groups demonstrated that selenium significantly reduced the shock-wave-induced leakage of all cellular enzymes into the nutrient medium (Fig. 1).

Discussion

Our results demonstrate that selenium significantly reduces the shock-wave-induced leakage of cellular enzymes such as LDH, NAG, GOT and GLDH in cultured MDCK cells. The difference was least pronounced for NAG. This may be due to different enzyme localizations: NAG is a lysosomal enzyme, whereas LDH occurs in the cytoplasm and cellular membrane, GOT and GLDH in the mitochondria.

Apart from shear forces, thermal and cavitation effects [6, 7, 10] free radical formation is discussed as an important mechanism of shock-wave-induced renal injury [8, 11, 27]. Reactive oxygen species are also important in other types of renal injury (e.g., postischemic and toxic acute renal failure [19]).

Using fluorescent dyes the generation of free radicals by shock-wave-induced cavitation was demonstrated directly in suspended cells [27]. Free radicals initiate lipid peroxidation of cellular membranes or oxidation of membrane protein sulfhydryl groups resulting in membrane permeability and ion pump disorders [5]. The

Table 1 Concentrations of LDH, NAG, GOT and GLDH (U/l) in the nutrient medium after shock wave exposure in the control group (*SWE* shock wave exposure, 256 impulses; *ST* sham treatment)

	SWE	ST
LDH	185.3 ± 35.6*	37.2 ± 1.2
NAG	4.43 ± 0.20*	3.38 ± 0.18
GOT	14.7 ± 3.4*	2.00 ± 0.00
GLDH	4.40 ± 1.30*	0.50 ± 0.10

*$P < 0.01$

Table 2 Concentrations of LDH, NAG, GOT and GLDH (U/l) in the nutrient medium after shock wave exposure in the selenium group (*SWE* shock wave exposure, 256 impulses; *ST* sham treatment)

	SWE	ST
LDH	121.3 ± 24.1*	34.8 ± 1.7
NAG	4.46 ± 0.26	4.22 ± 0.28
GOT	10.50 ± 3.20*	3.00 ± 10.50
GLDH	3.20 ± 1.0*	1.10 ± 0.20

*$P < 0.01$

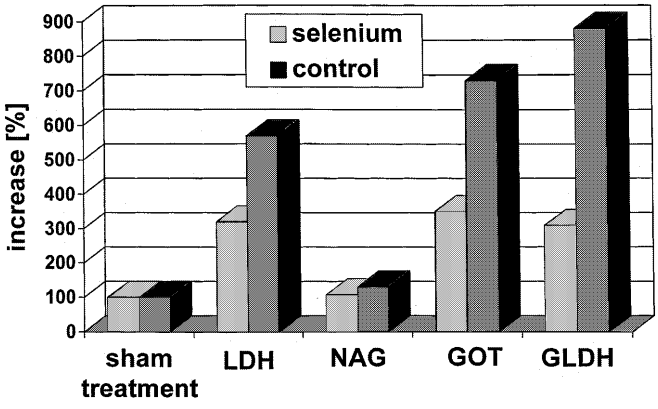

Fig. 1 Increase (%) in cellular enzyme concentrations (LDH, NAG, GOT and GLDH) after shock wave exposure in the selenium and control groups. Sham treatment = 100%. *$P < 0.01$

addition of exogenous scavengers such as cysteamine and cystamine to the suspension medium immediately before shock wave exposure limited shock-wave-induced cellular damage [27].

Selenium, an important trace element, is an essential component of glutathione peroxidase. Glutathione peroxidase is responsible for the homeostasis of oxygen-derived free radicals [13], and protects the cell against oxidative membrane damage by catalyzing the reduction of hydroxyl radicals [3] (Fig. 2). A further protective mechanism is the direct reduction of lipid hydroxyperoxides of the cell membrane [17]. In mammals, the concentration of cytosolic glutathione peroxidase is highest in the kidney [22]. The activity of glutathione peroxidase is dependent on an adequate selenium status. By supplementing selenium, the activity of glutathione peroxidase can be increased [13].

Several studies have demonstrated the protective effect of selenium against free-radical induced tissue damage (e.g., heart, liver, endothelial cells) [3, 9, 23, 30, 32]. Selenium has also been shown to reduce the renal tubular toxicity of cis-platinum [2, 4, 12, 14], which is

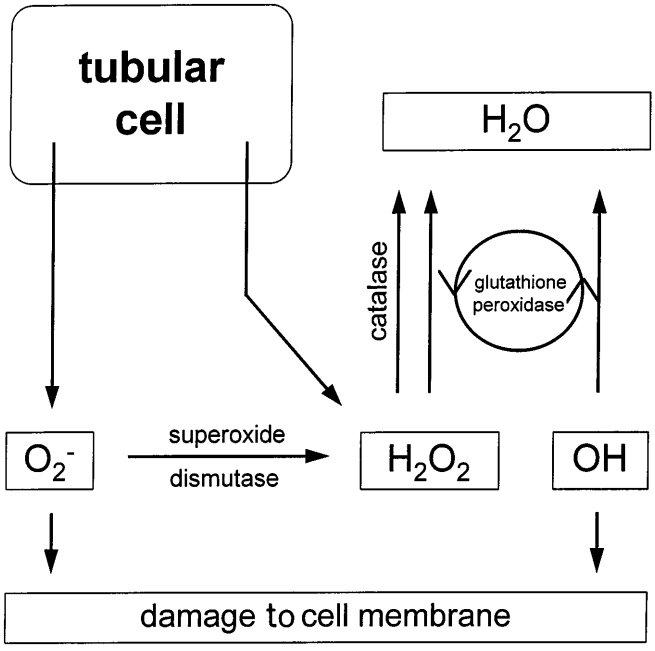

Fig. 2 Mechanism of action of glutathione peroxidase

Table 3 Increase in LDH, NAG, GOT, GLDH (%) in the nutrient medium after shock wave exposure. Sham treatment = 100% (*S* selenium group, *C* control)

	S	C
LDH	317 ± 63*	567 ± 109
NAG	106 ± 6*	131 ± 6
GOT	350 ± 107*	733 ± 169
GLDH	302 ± 97*	880 ± 250

*$P < 0.01$

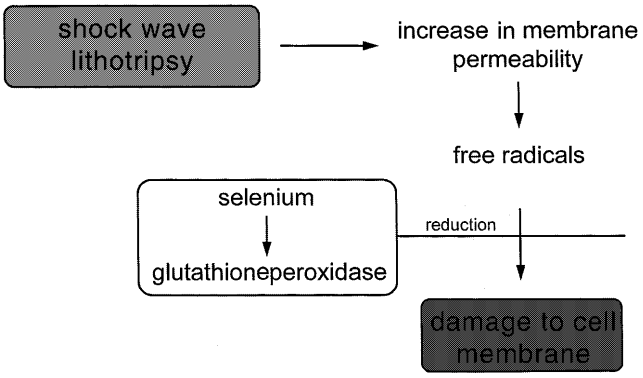

Fig. 3 Proposed mechanism of action of selenium in shock-wave-induced renal tubular injury

also caused by an increased formation of reactive oxygen species.

Since these mechanisms are important for shock-wave-induced renal tubular damage, the limitation of shock-wave-induced cellular injury by selenium, as observed in cultured MDCK cells, is probably due to an increased activity of glutathione peroxidase. By protecting the cell membranes and the mitochondria, the shock-wave-induced leakage of intracellular and membrane enzymes was reduced (Fig. 3). Glutathione peroxidase is located predominantly in the cytoplasm and the mitochondria [13]. This may explain why the effect of selenium on enzymes located there (LDH, GOT, GLDH) was better than on NAG leakage. Of course, the application of selenium could not completely prevent shock-wave-induced cellular damage. In particular, mechanical rupture of cells was not influenced by selenium. This was shown by cytological examination of the cell suspensions: the percentage of ruptured cells was identical in the selenium and control groups (14%).

Further studies are needed to investigate the influence of selenium on shock-wave-induced renal damage in vivo. Since many European countries are regions with a very low selenium status [15], these experimental findings are of potential clinical relevance. As already stated in previous papers, routine prophylaxis in all ESWL patients is not justified: the rate of severe renal complications is low [25]. However, in the presence of risk factors (e.g., chronic pyelonephritis, previous lithotripsy, acute urinary tract infection) as shown by Sakamoto et al. [21], prophylactic measures such as the application of selenium may be of benefit. This should be tested using in vivo studies.

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