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Selenium reduces high energy shock wave-induced renal injury in rats

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Abstract Using an in vitro model with Madin-Darby canine kidney (MDCK) cells, we showed that shock wave-induced renal injury could be ameliorated by selenium. We examined the influence of selenium, a free radical scavenger, in shock wave-induced tubular cell injury in vivo. Male rats were randomly assigned to three groups: 1 control ($n=18$), 2 selenium ($n=18$), 3 sham treatment ($n=4$). Groups 1 and 2 were treated with 500 shock waves on each kidney. Animals assigned to group 3 (sham treatment) received only anesthetics. Selenium (80 μg/kg per 24 h intraperitoneally) was given to the animals in group 2 for 5 days, starting 1 day before shock wave exposure. Urine was collected for 8 h on the day before and immediately, 1, 7 and 28 days after shock wave exposure (SWE) for the measurement of urine volume, N-acetyl-β-glucosaminidase (NAG), beta-2-microglobulin (β2 M), and creatinine. Blood was taken from these rats on day 1 after SWE for the determination of creatinine and the calculation of the creatinine clearance (CCr). After SWE, there was a significantly increased diuresis in group 1 and 2. The excretion of NAG and β2 M was also increased in both groups. These changes were significantly less pronounced in the selenium treated rats. CCr was higher in the selenium group than in the controls. No changes were observed in the sham treated group. These results demonstrate that selenium is able to ameliorate the damaging effects of high energy shock waves on renal tissue not only in vitro, but also in vivo.

Keywords Selenium · Shock waves · ESWL · Renal injury · Nephrotoxicity · Rats

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Introduction

Numerous studies have investigated the effects of high energy shock waves (HESW) on kidney function. These have shown that HESW predominantly compromise the tubular system as measured by urinary proteins and enzymes [1, 27, 36, 38]. Morphologically intrarenal hematomas originating from the arcuate veins, dilatation of intrarenal veins, vacuolization of the tubular epithelium and dilatation of the tubuli have been found [10, 13, 26]. Microscopic examination of urine voided immediately after extracorporeal shock wave lithotripsy (ESWL) frequently demonstrated vacuolated renal tubular cells [26]. 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 [8, 9, 11, 18, 19, 35].

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 [30]. Using this model, we showed the protective effects of substances such as verapamil, a calcium antagonist of the phenylalkylamine type, fosfomycin, a nephroprotective antibiotic and selenium, a free radical scavenger [31, 34].

This study was initiated to investigate the effects of selenium on shock wave-induced renal tubular dysfunction in vivo. For this purpose, we developed an animal model using rats [33]. Contrary to many previous animal models in which high doses of shock waves (upto 3,000 impulses) were applied on small animals, this model is more comparable to the clinical situation using only moderate doses of 500 impulses at a low generator voltage.

Material and methods

After an adaptation period of 1 week (from day –8 to –1), male Wistar rats (Interfauna Süddeutsche Versuchstierfarm, Tuttlingen,

Germany) weighing 150–200 g were randomly assigned to one of the following groups: 1. selenium ($n = 18$), 2. control ($n = 18$), 3. sham treatment ($n = 4$). Selenium was administered to the animals of group 1 from day –1 to day 3 at a dosage of 80 μg/kg per 24 h intraperitoneally. The other groups received no medication.

For shock wave exposure (SWE) (day 0), the animals were anesthetized with ketamine 50 mg/kg (Ketanest, Parke, Davis, Berlin, Germany) and dihydroxylidinothiazine 25 mg/kg (Rompun, Bayer, Leverkusen, Germany) intraperitoneally. Group 3 animals were anesthetized but were not exposed to HESW.

SWE was performed as described previously [33]. For SWE, the lithotriptor MFL 5000 (Dornier Medizintechnik, München, Germany) was used. Each kidney received 500 impulses (generator voltage 18 kV, frequency 70/min). The high-resolution fluoroscopy unit (Philips Röntgentechnik, Hamburg, Germany) integrated into the MFL 5000 allowed imaging of the rat kidneys without using contrast media, which could have influenced the results by impairing the tubular epithelium.

To collect urine, pairs of animals were placed in metabolism cages for 8 h on days –1, 0 (immediately after SWE), 1, 7 and 28. The volumes were recorded. Blood was drawn (day 1) to determine creatinine for calculation of the creatinine clearance (CCr).

The following analyses were performed on the urine: N-acetyl-β-glucosaminidase (NAG) was measured by a colorimetric assay according to Pott [24]. β-2-Microglobulin (β2 M) was assessed by an enzymatic immunoassay (Behringwerke, Marburg an der Lahn,Germany). For the determination of creatinine, the multianalyzer 550 Express (Ciba Corning, Fernwald, Germany) was used. The concentrations of NAG and β2 M were related to the creatinine concentration.

The mean maximal rise or fall of a urine parameter after SWE (%) was calculated by setting the pre-SWE level to 100%.

All data are presented as means ± SD. The sample tests were checked for normal distribution (Shapiro-Wilk test). Variance analysis was performed using the O'Brian test. In case of a normal distribution and equal variance the Student's *t*-test was used, otherwise the Wilcoxon test (two-tailed) was used. The level of significance was set at 5% ($P < 0.05$).

Results

Sham treatment (anesthesia only) did not influence any of the parameters tested. Diuresis increased significantly the day after SWE in control and selenium-treated rats (Table 1). In the control series, SWE significantly increased the concentrations of LDH, NAG, GOT and GLDH in the nutrient medium when compared to the 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 1).

The comparison between the control and the selenium group demonstrated that selenium significantly reduced the shock wave-induced leakage of all cellular enzymes into the nutrient medium (Fig. 1). The CCr (day 1 after SWE) was higher in the selenium treated animals than in the controls, the difference, however, was not statistically significant (Table 2).

Discussion

After SWE, there was a significant rise in diuresis and excretion of NAG and β2 M. These findings are signs of shock wave-induced renal tubular impairment. They are consistent with numerous previous reports [1, 12, 14, 27, 36, 38].

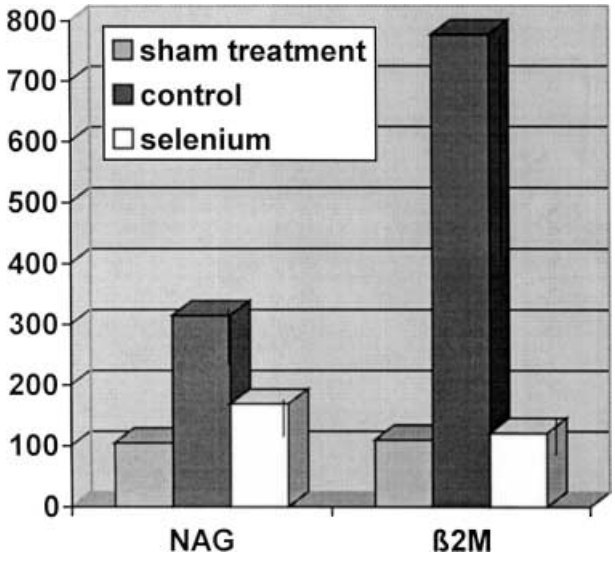

Fig. 1. Maximal change (%) after shock wave exposure (SWE) of urine volume, N-acetyl-β-glucosaminidase (NAG), β-2-microglobulin (mean ± SD). Sample –1 (before SWE) = 100%

Table 2. Mean values ± SDof the creatinine clearance (CCr) on day 1 ($n = 6$ per group). There was no significant difference

	Selenium	Control
CCr (ml/min/kg)	4.56 ± 0.52	3.62 ± 0.83

Table 1. Mean values ± SD of urine volume and the excretion of N-acetyl-β-glucosaminidase (*NAG*), β-2-microglobulin ($\beta 2$ *M*) before and after shock wave exposure (urine samples of days –1, 0, 1, 7 and 28). Significant differences of $P < 0.01$ are indicated by an asterisk

	Day –1	Day 0	Day 1	Day 7	Day 28
Volume (ml/8 h)					
Selenium	3.0 ± 2.4	11.4 ± 8.8*	3.8 ± 2.3	3.9 ± 2.9	5.0 ± 2.1
Control	2.8 ± 2.2	10.0 ± 6.0*	3.7 ± 1.7	4.4 ± 1.9	3.2 ± 1.1
NAG (U/g creat.)					
Selenium	1.30 ± 1.02	2.20 ± 2.17	0.92 ± 1.18	0.78 ± 0.88	1.54 ± 0.88
Control	1.60 ± 0.95	5.25 ± 2.58*	2.26 ± 1.34	2.09 ± 0.81	2.08 ± 0.71
β2 M (μg/g creat.)					
Selenium	0.019 ± 0.010	0.023 ± 0.030	0.016 ± 0.010	0.013 ± 0.011	0.012 ± 0.005
Control	0.018 ± 0.010	0.141 ± 0.013*	0.021 ± 0.010	0.023 ± 0.020	0.013 ± 0.011

In the selenium treated rats, the tubular dysfunction after SWE, as assessed from the biochemical markers NAG and β2 M, was significantly less pronounced than in the controls. Diuresis and CCr, however, were not significantly different from the controls.

These results are consistent with our findings in the in vitro model using MDCK cells [34]. Obviously, selenium also ameliorated shock wave-induced tubular impairment in the in vivo situation. Selenium is known to be a free radical scavenger. Free radical formation is discussed as an important mechanism in shock wave-induced renal injury [11, 18, 19, 35]. Reactive oxygen species are also important in other types of renal injury (e.g., postischemic and toxic acute renal failure [25]. Selenium is an essential part of glutathione peroxidase which is responsible for the homeostasis of oxygen-derived free radicals [21]. It protects the cell against oxidative membrane damage by catalyzing the reduction of hydroxyl radicals [3]. Another protective mechanism is the direct reduction of lipid hydroxyperoxides of the cell membrane [23]. In mammals, the concentration of cytosolic glutathione peroxidase is highest in the kidneys [28]. The activity of glutathione peroxidase is dependent on an adequate selenium status. By supplementing selenium, the activity of glutathione peroxidase can be increased [21].

Several studies also demonstrated the protective effect of selenium against free radical-induced tissue damage (e.g., heart, liver, endothelial cells) [3, 15, 29, 37, 39] in vivo. Selenium has also been shown to reduce the renal tubular toxicity of cis-platinum [2, 5, 20] which is also caused by the 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 and in rats, is probably due to the increased activity of glutathione peroxidase. By protecting the cell membranes and the mitochondria, the shock wave-induced leakage of intracellular and membrane enzymes could be reduced.

Selenium, however, did not influence the shock wave-induced changes in diuresis and CCr. This is in contrast to verapamil, a calcium antagonist. In the same animal model, verapamil reduced not only the cellular enzyme leakage but also the increase in diuresis following SWE. The shock wave-induced fall of CCr was also prevented [33]. The reasons for these differences have not been examined to date. However, we know from other studies that verapamil has not only direct but also indirect effects on renal tubular cells. Similar to selenium, verapamil could exert a membrane protective effect (protection against radical induced lipid peroxidation) and inhibit a pathologically increased influx of calcium ions [6, 7, 17, 32, 33]. In contrast to selenium, verapamil could indirectly preserve the tubular function by acting on the impaired renal hemodynamics after SWE. Calcium antagonists increase the renal blood flow in cases of pre-existing enhanced vascular resistance [4, 16]. Since the renal blood flow is decreased after SWE [12, 14], verapamil could restore the impaired renal perfusion by vasodilatation. This effect could prevent tubular ischemia and subsequent functional alterations.

Since verapamil seems to be a more potent protector against shock wave-induced renal injury, we conclude that not only do the direct effects of shock waves act on the tubular epithelium but also that the effects on intrarenal blood flow play an important role in the etiology of shock wave-induced renal tubular impairment.

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