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ORIGINAL PAPER

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Prevention of shock wave-induced renal oxidative stress by melatonin: an experimental study

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Abstract Our aim was to evaluate the effects of the potent endogenous free radical scavenger melatonin on extracorporeal-shock-wave lithotripsy (ESWL) induced renal impairment. The study was performed using 30 rabbits which were divided into two groups. Both groups were exposed to 3,000 shock waves at 18 Kv. The animals in the first group were treated with melatonin for 8 days. Controls and melatonin treated rabbits were killed a week after ESWL. MDA, uric acid and white cell counts were used as markers of oxidative stress. The mean levels of uric acid and white cell counts were significantly lower in the melatonin treated group than in the controls. The mean level of MDA was also significantly lower in the melatonin treated group compared to the controls. Our results show that melatonin may exhibit a protective effect on free radical mediated oxidative damage induced by ESWL in rabbit kidney.

Keywords Shock wave induced-renal oxidative damage · Melatonin

Introduction

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, 2, 3]. In addition to the mechanical fragmentation of the calculus, each shock wave that is generated by thermal effects of 18–24 thousand volts of electrical energy 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 [4, 5]. Because of their very high reactivities, free radicals can cause serious damage to the macromolecules in cells.

Melatonin, which is a hormone produced in the pineal gland and almost exclusively synthesised and secreted at night, is a very potent and efficient endogenous free radical scavenger. It can also protect molecules from oxidative damage by stimulating glutathione peroxidase activity which metabolises hydrogen peroxide to H_20 [6, 7].

Free radical formation has been reported during ESWL treatment. Morgan et al. [1] adapted the Fricke ferrous sulphate radiation dosimeter to examine the chemical effects of high energy shock waves and significant free radical production was documented. Suhr et al. [2] 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 hypoxantine and xantine to uric acid. Subsequent reactions generate lipid peroxides as well as cytotoxic and oxidation products, among which is malondialdehyde (MDA).

The aim of this study is to investigate the potential protective effect of melatonin against shock wave induced oxidative stress in the kidney.

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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). The animals in the first group were treated with melatonin (group I), while the second group was used as a control (group II).

Melatonin (2.5 mg) (Sigma) was dissolved in 1 ml 90% ethanol and further diluted with 100 ml 0.9% NaCl solution. The final solution contained 1% ethanol. Melatonin was given subcutaneously 50 μg/kg once daily for 8 days at 5.00 pm everyday (a day before the procedure and 7 days after shock wave treatment). The control group was exposed to ESWL but only saline was given.

All of the animals underwent the entire procedure, including anaesthesia and opacification of 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 visualisation.

The animals were killed 7 days after the ESWL procedure and the kidneys were removed and immediately frozen. One gram of kidney tissue was homogenised in a motor-driven tissue homogeniser 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).

Tissues were fixed in 10% buffered formalin and embedded in paraffin for histopathological examination. Sequential 5–6 μm sections were stained with haematoxylin and eosin and examined under a light microscope.

Another 1 g of of kidney tissue was used for MDA analysis. The levels of MDA were measured using the thiobarbutic acid (TBA) method [11] in all rabbits. 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.

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

Results

In group I rabbits, no apparent changes were seen macroscopically or microscopically following the treatment.

All of the kidneys showed moderate damage (subcapsular haemorrhage, perirenal haemorrhage) in group II after treatment. Glomerular haemorrhage and protein in Bowman's capsule was also found in this group.

Table 1 shows the mean levels of uric acid and white cell counts. The results were lower in the melatonin treated group than in the control group, and there was a significant difference between two groups ($P < 0.001$).

Table 2 shows the mean MDA levels in the kidneys of group I and II rabbits. The MDA levels were significantly less in the melatonin treated group compared to group II ($P < 0.05$)

Table 1 Mean levels of uric acid and white cell counts in kidney homogenates of rabbits (values are given as the mean ± SEM), M = melatonin

Groups	White cells ($\times 10^3/\mu l$)	Uric acid (mg/dl)	*n*
I (melantonin treated)	6.05 ± 0.42	4.55 ± 0.17	15
II (control)	11.19 ± 0.35	6.31 ± 0.11	15
P	< 0.001	< 0.001	

Table 2 The mean MDA levels in kidney homogenates of rabbits (values are given as the mean ± SEM)

Groups	MDA (nmole/dry weight)	*n*
I (melatonin treated)	0.23 ± 0.03	15
II (control)	4.62 ± 0.23	15
P	< 0.05	

Discussion

The mechanisms resulting in ESWL induced renal damage have not been elucidated in detail. Significant free radical production after ESWL has been documented in early investigation [1, 2, 3]. When free radicals are attached to cell membrane lipids, peroxidation is initiated which can ultimately cause cell death. Free radicals, produced in large amounts during oxidative stress, take part in the degradation of cellular and subcellular membrane structures. It is reasonable that pro-oxidant factors are subject to rapid changes and that a lag time exists before biological systems can adapt to them. Permanent and irreversible injury, however, occurs only if the pro-oxidant factors are chronically higher than the maintenance and repair systems of an organism. Therefore, it is of great importance that antioxidative mechanisms, such as the melatonin effect, operate throughout life. Melatonin has been shown to exert a protective effect, i.e. against radical induced lipid peroxidation [6]. Reiter et al. showed that melatonin scavenges hydroxyl radicals generated in vitro by hydrogen peroxide exposed the ultraviolet light [12]. The hydroxyl radical scavenging potency of melatonin is much greater than that of the classical hydroxyl radical scavengers like glutathione, an important endogenous radical scavenger. Melatonin is the most powerful and effective endogenous free radical scavenger detected to date. Due to its lipophillic nature, it provides on-site protection to all biomolecules [6, 13, 14]

Karalezli et al. [10] have demonstrated that macroscopic morphological changes similar to those observed in our study were closely related to the numbered of shock waves applied. They reported that all of the kidneys showed gross and microscopic morphological changes when exposed to 3,000 shock waves. Our macroscopic findings are in accordance with their findings. Newman et al. showed that gross macroscopic changes occured 7 days after rabbit kidneys were exposed to 3,000 shock waves with an electrical discharge value of 18 Kv [9]. Delius et al. showed that there were more haematomas, and diffuse haemorrhages were more extended after the application of 1,500 and 3,000 than after 500 shock waves [8].

In our study, there was no evidence of macroscopic or microscopic changes in kidneys after ESWL treatment in the melatonin group. We believe that the absence of

pathological changes in this group may be due to improved repair mechanisms rather than to protection from macroscopically visible damage. Our data are from animals killed 7 days after shock wave exposure. We are planning another study for immediately after shock wave exposure which would clarify the difference between protection or faster repair of renal damage.

The decrease in mean uric acid level and white cell count in melatonin-treated rabbits observed in our study might indicate that melatonin exerts a renal protective effect by scavenging free radical species in renal tissue. On the other hand, after melatonin treatment, the decrease of MDA level is due to inhibition of lipid peroxidation during ESWL treatment. These findings support the assumption of a protective effect of melatonin on shock wave related renal damage. Since tubular impairment after ESWL in the majority of patients is only a transient phenomenon without clinical signs, the routine application of an antioxidant seems not to be justified.

Our observations indicate that the ESWL-induced renal damage could be prevented by medication with melatonin. However, we believe that further studies of the exact role of melatonin in ESWL-induced renal damage and the potential therapeutic applications in the patients who have some risk factors for occurrence of more severe renal lesions after ESWL, including pre-existing renal diseases, urinary tract infection, and previous lithotripsies, are needed to clarify these issues.

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