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The effect of melatonin on shock wave induced renal damage

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Objective In a prospective randomized study, the effects of a potent endogenous free radical scavenger melatonin on extracorporeal-shock-wave lithotripsy (ESWL) induced renal impairment were studied.

Method An experimental study was performed in 30 rabbits. The animals were divided into two groups. Both groups were exposed to 3000 shock waves at 18 kV. The animals in the first group were treated with melatonin for eight days. Controls and melatonin treated rabbits were sacrificed a week after ESWL. The activities of the two principal antioxidant enzymes; superoxide dismutase (SOD) and glutathion peroxidase (GPx) were determined. And also, the level of malondialdehite (MDA) which is the product of lipid peroxidation was measured in the kidney tissue.

Results The mean activities of SOD and GPx were significantly lower in the melatonin treated group than in the controls. Also the mean level of MDA was significantly lower in the melatonin treated group as compared to the controls.

Conclusion Our results indicate that melatonin may exibit a protective effect on free radical-mediated oxidative damage induced by ESWL in rabbit kidney.

Key words Melatonin, free radicals, renal damage, shock wave lithotripsy

Introduction

The mechanisms by which ESWL-induced cellular damage is still controversial. One of the mechanisms discussed for tissue damage is free radical formation during ESWL (1,2). Each shock wave which is generated by thermal effects of 18 to 24 thousand volts of electrical energy at the second focus may result in biochemical events in addition to the mechanical fragmentation of the calculi. Under these conditions, a homolytic cleavege of molecules may take place leading to the formation of free radicals (3,4).

Melatonin, N-Acetyl-5-methoxytryptamine, is a hormonal product of pineal gland. Melatonin is also a very potent and efficient endogenous free radical scavenger and affords protection of molecules, especially DNA, from oxidative damage (5,6). Free radicals and the hydroxyl radical in particular, because of its very high reactivity, can be extremely damaging to macromolecules in cells. Melatonin acts as a primary non-enzymatic antioxidative against the devastating actions of the extremely reactive hydroxyl radical (7). Melatonin also stimulates glutathione peroxidase activity which metabolizes the precursor of the hydroxyl radical, hydrogen peroxide, to water (6). Melatonin is almost exclusively synthesized and secreted during darkness at night and mediates information concerning the temporal position and duration of darkness (6).

Most experimental studies with ESWL demonstrated histopathologic and ultrastructural damage of kidney cells after shock wave treatment (7-9). These studies concerning free radical formation during ESWL treatment have been reported in recent years. Suhr et al (2) presented their results of intra and extracellular in vitro measurements of free radicals and investigated cell viability after shock wave treatment. They have demonstrated an elevated concentration of intracellular free radicals during shock wave treatment in suspended cells in vitro.

In order to investigate the potential protective effect of melatonin against shock wave induced free radical formation in kidney of rabbits, we determined the activities of two principal antioxidant enzymes; SOD and GPx and the levels of MDA which is the product of lipid peroxidation measured in the rabbit kidney.

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Material and Method

Thirty adult New Zealand white rabbits weighing between 1600-2700 g were used in the study. Animals were placed in an environment maintained at 22.0±3.0 ^{0}C isolated from noise and with a 12 h light/dark cycle. All rabbits were given food and water ad lib.

The animals were divided into two groups , each consisting of 15 animals, which were exposed to 3000 shock waves at l8 kV (Stonelith lithotriptor PCK Co. Turkey, capacitor 40 nF, focus dimensions 7.7 mm axially x 30 mm laterally, focal distance l35 mm, focal pressure 0 to 1200 bar) under intramuscular ketamine anaesthesia (1mg/kg). The animals in the first group were treated with melatonin (Group I). Second group was left as control (Group II).

Melatonin (2.5 mg) (Sigma Chem. Co) was dissolved in 1ml 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 eight days at 5 pm everyday (a day before the procedure and seven days after shock wave treatment). Controls were applied ESWL and given saline only.

All of the animals underwent the entire procedure, including anesthesia and opacification of kidneys

with a contrast agent. The kidney was opacified by 5 ml 76% iodine contrast agent (Urografin Schering AG). It was given intravenously before the procedure. The animal was placed in the supine position on the platform of the lithotriptor and fixed through its thorax and hip to allow direct entry of 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 sacrificed seven days later and kidneys removed and frozen immediately. One gram tissue of kidney was homogenized in a motor-driven tissue homogenizer with phosphate buffer solution (pH 7.4). Unbroken cells, cell debris and nuclei were sedimented at 2000 x g for 10 min, and the supernatant was pipetted into plastic tubes, stoppered and stored at - 70 ^{o} C until assayed. Biochemical analyses were performed by standart spectrophotometry.

SOD and GPx activities were determined in the kidneys of both groups. SOD assay was based on its ability to inhibit the autooxidation of epinephrine at 334 nm (10). The level of enzyme that causes 50 % inhibition of epinephrine autooxidation is defined as 1 unit.

The activity of GPx was determined by measuring disappearance of NADPH at 340 nm (11). An enzyme unit was defined as 1 nmole NADPH oxidized per mg protein per minute. The purified enzyme standarts were obtained from Randox Co. (UK). Melatonin was obtained from Sigma Chemical Co.

The levels of MDA were measured with thiobarbituric acid (TBA) method (11) in all rabbits. The hydrolysis of lipoperoxidases to form MDA react with TBA to yield a red MDA-TBA adduct. The latter is determined by spectrophotometry at 532 nm and the results expressed as nmole MDA/dry kidney weight.

Kidney protein was determined with Lowry method (12). The Mann-Whitney U test was used to compare the groups because normal distribution could not be assumed.

Results

In melatonin receiving rabbits, no apparent changes were seen macroscopically or microscopicaly following the treatment.

All of the kidneys showed moderate damage (subcapsular hemorrhage, perirenal hemorrhage) in the group II after treatment. Microscopically there were glomerular hemorrhage and protein in Bowman's capsule also in this group .

Table I shows the mean catalytic activities of SOD and GPx in kidneys. The results were lower in melatonin treated group than in the control group, and there was statistically significant difference between the two groups ($p<0.05$).

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

Table I. Antioxydant enzymes in kidney homogenates of rabbits.

Groups	SOD (U/mg protein)	GPx (U/g protein)	n
I (M.treated)	7.65±0.5	375±15	15
II (Control)	10.70±1.2	526±20	15
p value	< 0.05	< 0.05	

M: Melatonin

Table II. The mean MDA levels in kidney homogenates of rabbits.

Groups	MDA (nmole/dry weight)	n
I (Melatonin treated)	0.25±0.05	15
II (Control)	4.80±0.51	15
p value	<0.05	

Discussion

The mechanisms resulting in shock wave induced impairment of renal tubular function have not been elucidated in detail. Free radical formation has been discussed in different studies. Significant free radical production after ESWL have been documented in early investigations (1,2). The antioxidant enzymes such as SOD and GPx are found in all mammalian cells. They protect the cells from the hazardous effects of free radicals. When free radicals are attached to cell membrane lipids an initiation of peroxidation occurs which can ultimately cause cell death. Increased activities of antioxidant enzymes are a protective response to free radical formation. The decrease in mean SOD and GPx activities in melatonin treated rabbits observed in our study might indicate that melatonin could exert a renal protective effect by scavenging free radical species in renal tissue.

It is reasonable that prooxidant 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 prooxidant factors are chronically higher than the maintenance and repair systems of an organism. Therefore, it is of great importance that antioxidative mechanisms, like melatonin effect, are operating throughout life. Melatonin was shown to exert a protective effect, i.e against radical induced lipid peroxidation, and to inhibit a pathologically increased influx of ions (6). Recent studies demonstrate that melatonin scavenges hydroxyl radicals generated in vitro by hydrogen peroxide exposed to ultraviolet light (13). 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, which, due to its lipophilic nature, provides on-site protection to all biomolecules (14,15)

Early investigations by Karalezli et al (9) previously demonstrated that the macroscopic morphological changes similar to that we observed in our study were closely related to the number of shock waves applied during the procedure. They reported that all of the kidneys showed gross and microscopic morphologic changes when exposed to 3000 shock waves. Our macroscopic findings are in accordance with their findings. Newman et al showed in their study that gross macroscopic changes occur 7 days after, in rabbit kidneys exposed to 3000 shock waves with an electrical discharge value of 18 kV (8).

In the melatonin receiving group, there was no evidence of macroscopic or microscopic changes in kidneys after ESWL treatment. Also the activities of SOD and GPx and the level of MDA were lower in melatonin treated group than in controls. The lower SOD and GPx activities we observed in the melatonin treated group may be related to the inhibitor effect of melatonin on free radical formation in rabbit kidney. On the other hand, after melatonin treatment, the decrease of MDA level is due to the inhibition of lipid peroxidation during ESWL treatment. These findings support the assumption of protective effect of melatonin on shock wave related renal damage.

Our results demonstrate that melatonin is a protective agent against shock wave induced renal oxidative damage. Since tubular impairment after ESWL in the majority of the patients is only a transient phenomenon without clinical signs, a routine application of an antioxidant seems not to be justified for this reason. In some studies, however, it has been reported that there are risk factors for the occurence of more severe renal lesions after ESWL, including preexisting renal diseases, urinary tract infections, previous lithotripsies and female gender (7). Furthermore, patients with a solit ary kidney are prone to more deleterious effects of ESWL. In the presence of these risk factors, the application of an antioxidant, like melatonin, could be of benefit. And also, in elderly people, if in fact free radicals play a major role in the degenerative processes after ESWL, which they seem to do, then exogenously administered melatonin may have some ameliorative effects on shock wave induced free radical damage.

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Editorial Note

Melatonin is known to have antioxidant effect. However, it is not clearly understood how it prevented the occurrance of subcapsular and perirenal hemorrhagie. Moreover, the authors stated that similiar lesions occurred in all kindeys of the control group whereas kidneys consisting these lesions were not shown in the study group.

Dr. Öner Odabaş