MELATONIN REDUCTION OF FLUORIDE-INDUCED NEPHROTOXICITY IN MICE

MV Rao, SL Chawla, N Patel
Ahmedabad, India

SUMMARY: Protective effects of melatonin (MLT) (10 mg/kg bw/day) against kidney in adult female albino mice induced by fluoride (F) (10 mg NaF/kg bw/day) are reported. Biochemical indices studied were levels of total protein, creatinine, lipid peroxidation (LPO), glutathione (GSH), and activities of enzymes including acid phosphatase (ACP), alkaline phosphatase (ALP), and succinate dehydrogenase (SDH). NaF treatment resulted in a significant decline in the activities of ACP, ALP, and SDH as well as the levels of protein and creatinine along with a reduction in kidney gravimetric data. The levels of the lipid peroxides were enhanced, accompanied by a marked decrease in GSH. These changes appear to be due to kidney damage caused by F. Pretreatment with melatonin ameliorated these marked changes, thereby confirming antioxidant effects of melatonin.

Keywords: Fluoride nephrotoxicity; Kidney enzymes; Melatonin and fluoride; Melatonin antioxidant; Mice and melatonin.

INTRODUCTION

In animals, the kidney is the most crucial organ to cope with long-term exposure to fluoride (F). Elimination of ingested F depends mainly on kidney function, and kidney malfunction can impede this excretion, thus increasing body burden of F.1,2 People with renal insufficiency have decreased ability to excrete F in urine. They have elevated plasma F levels compared with normal healthy persons and are at high risk of fluorosis even at normal recommended limits of F in drinking water.3,4 F exposure through drinking water induces alteration in kidney function of animals including man.5,6 N-acetyl-β-D-glucosaminidase (NAG) and α-glutathione-S-transferase (α-GST) are markers of renal tubular damage. Studies of F-exposed animals and humans have shown increased levels of NAG and α-GST in urine.7-9 F has also been shown to induce extensive histological and ultrastructural changes in the kidney of rabbits and mice.10-12 Recent studies in our laboratory further reported that kidney fluorosis in mice tissue is alleviated by a number of antioxidants including vitamins and herbal products.13-15

Melatonin (N-Acetyl-5-methoxytryptamine) is a secretory product of the pineal gland as well as other select organs. It has been shown to be highly effective in reducing oxidative stress at many levels. Mechanisms involved in the protective effects of melatonin against oxidative stress involve direct free radical scavenging activity16 and indirect antioxidant actions of melatonin.17 Tan et al.16 have reported that under conditions of high oxidative stress in vivo, melatonin has proven superior to vitamin C and E in reducing oxidative damage.

In view of the above reports, we have investigated the possible protective role of melatonin on fluoride-induced nephrotoxicity in mice.

---

aFor Correspondence: Professor MV Rao, Zoology Department, University School of Sciences, Gujarat University, Ahmedabad – 380 009, India. E-mail: zooldeptgu@satyam.net.in.
MATERIALS AND METHODS

Animals: The mice used in this study are the same ones as in our report on melatonin and F-induced hepatotoxicity.\textsuperscript{18} Forty healthy adult female albino Swiss-strain mice (\textit{Mus musculus}) weighing between 35–40 g were obtained from Alembic Pharmaceuticals, Vadodara, India, under the Animal Maintenance and Registration No. 167/1999/CPCSEA from the ministry of Social Justice and Empowerment, Government of India Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai, India. The mice were acclimatized for seven days prior to the commencement of the treatment and were housed in an air-conditioned animal house at 26±2°C with exposure to 10–12 hr of daylight at a relative humidity of 30–70%. They were fed a standard mouse chow (National Institute of Occupational Health (NIOH), Ahmedabad) and were given water (0.6–1.0 ppm F) \textit{ad libitum}.

Exposure: The mice were divided into four groups of 10 each with a 30-day treatment period for each group. Group I served as control; Group II mice were injected intraperitoneally with melatonin (Hi-media, Mumbai) at a dosage of 10 mg/kg bw/0.1 mL/day. Melatonin was first dissolved in ethanol and further diluted with normal saline. The final concentration of ethanol in the solution was less than 1%. Group III mice were administered sodium fluoride (NaF, Qualigens Fine Chemicals, Mumbai, 99% purity) orally (10 mg/kg bw/0.2 mL/day) with a feeding tube attached to a hypodermic syringe. Group IV mice were pretreated intraperitoneally with melatonin (10 mg/kg bw/0.1 mL/day), and, after 30 min, NaF (10 mg/kg bw/0.2 mL/day) was administered orally.

At the end of the 30-day treatments, the mice in each group were weighed and sacrificed by cervical dislocation. The kidneys were dissected out carefully, blotted free of blood and weighed to the nearest milligram and used for the estimation of protein,\textsuperscript{19} creatinine,\textsuperscript{20} lipid peroxidation (LPO),\textsuperscript{21} glutathione (GSH),\textsuperscript{22} acid phosphatase (ACP) (E.C.3.1.3.2.),\textsuperscript{23} and alkaline phosphatase (ALP) (E.C.3.1.3.1.),\textsuperscript{23} and succinate dehydrogenase (SDH) (E.C.1.3.99.1).\textsuperscript{24}

Statistical analysis: For all biochemical parameters, at least 6 to 8 replicates were performed. The data were analyzed statistically using Student’s t test and Analysis of Variance (ANOVA). A level of \textit{p}<0.05 was accepted as significant.

RESULTS

Organ weights: As shown in Figure 1, NaF treatment brought about a significant (\textit{p}<0.001) decline in the kidney weight of Group III mice as compared to the Group I controls and the mice administered melatonin alone (Group II) or with NaF (Group IV).

Protein and creatinine levels: A significant (\textit{p}<0.001) decline was observed in the levels of protein and creatinine following NaF exposure (Figure 2). SDH activity was also reduced significantly (\textit{p}<0.001) following NaF exposure (Figure 2).
Acid phosphatase (ACP) and alkaline phosphatase (ALP): The activities of ACP and ALP significantly (p<0.001, p<0.05, respectively) declined in the kidney of NaF treated mice as compared to control (Figures 3 and 4).

Lipid peroxidation (LPO) and Glutathione (GSH): A significant (p<0.001) elevation was noted in lipid peroxide levels, whereas GSH levels registered a significant (p<0.001) depletion in the kidney following NaF exposure (Figure 5).

As with the body and kidney weights, the above-mentioned parameters exhibited no significant changes in the Group II mice treated with melatonin alone. Similarly, pretreatment with melatonin of the Group IV NaF-melatonin treated
mice revealed no significant changes in these indices as compared to the controls in Group I.

**Figure 3.** Activity of ACP in kidney of control and treated groups. Data are mean ± SEM of 10 mice in each group; *p<0.001 when compared to control group (MLT=melatonin).

**Figure 4.** Activity of ALP in kidney of control and treated groups. Data are mean ± SEM of 10 mice in each group; *p<0.05 when compared to control group (MLT=melatonin).

**Figure 5.** LPO and GSH levels in kidney of control and treated groups. Data are mean ± SEM of 10 mice in each group; *p<0.001 when compared to control group (MLT=melatonin).
DISCUSSION

As described in a recent report by us, body weights of mice decreased by treatment with NaF. This treatment also brought about a marked decline in the kidney weights. This reduction in body and kidney weights correlated with protein levels. F is known to decrease protein synthesis in kidney of mice and rats. The results of the present study corroborate these findings. Dote et al. have reported a dose response relationship between F and renal tissue injury. Since the kidneys are mainly responsible for ridding the body of ingested F, impaired renal function decreases urinary F excretion, thereby increasing serum F levels.

As is well known, creatinine is an important measure of renal function. A significant decline in creatinine and F clearance in F-affected individuals has been reported. The reduction in the creatinine levels in kidney in the present study suggests impairment in glomerular functions by NaF. In agreement with our results, Chinoy and Shah have also documented reduced kidney creatinine levels from F. SDH, a mitochondrial enzyme is involved in oxidative metabolism. Decreased activity of this enzyme in the present study suggests an effect on oxidative metabolism by reducing ATP production in the Kreb’s cycle. Corroborating our data, other workers have also reported a decrease in SDH activity in the kidney of F-treated rats. Altered ACP and ALP activities in the NaF-treated mice are likewise in agreement with the data of others. The resulting inhibition in the enzyme activities might be due to alterations in lysosomal activity and membrane permeability, respectively.

The formation of lipid free radicals and lipid peroxide is considered an important feature of cell injury. Overabundance of free radicals could lead to uncontrolled chain reactions and lipid peroxidation. The enhanced levels of malondialdehyde (MDA) in the present study reveal F-induced lipid peroxidation in the kidney. Enhanced kidney MDA levels caused by F have been reported in rats.

Glutathione is the major cellular thiol. It participates in a number of cellular processes including the protection of cells against damage produced by toxic compounds, electrophiles, and reactive oxygen species due to its ability to react directly with hydrogen peroxide, superoxide anion, and hydroxyl and alkoxyl radicals. Reduced glutathione levels in the present could be due to its involvement in the mechanism of detoxification, and inhibition of lipid peroxidation. These changes could be correlated with altered histopathology of renal tissue.

Melatonin is a proven antioxidant. An added feature that possibly increases the efficiency of melatonin in reducing oxidative stress is that metabolites that are produced during the scavenging action of melatonin also seem to be efficient scavengers. For this reason, instead of scavenging a single radical, melatonin via an antioxidant cascade may neutralize a number of toxic reactants. Melatonin treatment along with NaF reduced the toxic effects of fluoride in kidney, since all the above-mentioned biochemical parameters were not appreciably altered as compared to the controls, confirming the antioxidative properties of melatonin. Thus the results obtained from the present study suggest a protective action of melatonin against F-induced nephrotoxicity due to its antioxidant properties.
ACKNOWLEDGEMENTS

MVR thanks the University Grants Commission (UGC), New Delhi for Financial assistance.

REFERENCES


