STUDY OF PROTECTIVE EFFECTS OF MELATONIN ON CISPLATIN-INDUCED NEPHROTOXICITY IN RABBITS

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ABSTRACT

Objective: To evaluate the protective effects of melatonin on cisplatin-induced nephrotoxicity in rabbits.
Study Design: Laboratory based randomized control trial.
Place and Duration of Study: Department of Pharmacology and Therapeutics in collaboration with Clinico-Pathologic Laboratory, Army Medical College, Rawalpindi, from Apr to Jun 2015.
Material and Methods: Eighteen rabbits were divided into three groups, each consisting of six rabbits. Baseline serum urea, creatinine, sodium and potassium were measured. Rabbits were weighed for dose calculation. A single dose of cisplatin 10mg/kg was given as I/P injection to the toxic group. The protective group received 5 mg/kg I/P melatonin for three days. Rabbits were sacrificed 72 hours after the cisplatin dose and both kidneys were sent for histopathology. Statistical analysis was carried out by using Microsoft Office Excel 2010 and SPSS version 21. Student's t-test and one way ANOVA, followed by 'Post Hoc Tukey' test was used for biochemical parameters, while Chi Square' test was used for histopathological comparison.
Results: Moderate nephrotoxicity (grade-II) was seen in the toxic group, with substantial elevations of serum urea and creatinine (p<0.001), and serum sodium and potassium (p<0.01). Melatonin ameliorated the renal injury.
Conclusion: The protective effects of melatonin on cisplatin-induced nephrotoxicity were due to its antioxidant properties.

Keywords: Antioxidant, Cisplatin, Melatonin, Nephrotoxicity.

INTRODUCTION

Cisplatin is an effective chemotherapeutic agent widely used for the treatment of malignant tumors including head and neck, ovarian, testicular, lung and breast cancers[1]. Despite the anti-neoplastic efficacy its optimal clinical usefulness is usually limited due to dose-related nephrotoxicity[2,3]. Acute renal injury can occur after an initial dose with about 20% of patients experiencing various degrees of renal dysfunction[4]. Cisplatin exerts its nephrotoxic effect mainly in the proximal tubular cells where it is preferentially accumulated[5]. The precise mechanisms underlying this toxicity are not fully elucidated. However, oxidative stress with increased generation of reactive oxygen species (ROS) and inflammation with increased production of pro-inflammatory cytokines seem to play a crucial role[6,7]. Cisplatin nephrotoxicity is characterized by morphological destruction of intracellular organelles, cellular necrosis, loss of microvilli, alterations in the number and size of the lysosomes and mitochondrial vacuolization, followed by functional alterations including inhibition of protein synthesis, GSH depletion, lipid peroxidation and mitochondrial damage[8].

Melatonin is synthesized and released into the circulation and especially into cerebrospinal fluid by the pineal gland in a circadian rhythm[9]. Melatonin and its metabolites possess free-radical scavenging activity[10,11]. Melatonin increases mRNA and protein levels of antioxidant enzymes through Nuclear-related factor 2 (Nrf2) activation[12,13]. Up-regulation of Nrf2 by melatonin results in an increased expression of antioxidant enzyme heme oxygenase-1[14].

The rationale of this study was to assess the extent of cisplatin-induced nephrotoxicity in rabbits and to explore the nephro protection

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offered by melatonin as an antioxidant against cisplatin-induced nephrotoxicity.

MATERIAL AND METHODS

This laboratory based randomized controlled trial was conducted in the Department of Pharmacology and Therapeutics in collaboration with Clinico-Pathological Laboratory, Army Medical College, Rawalpindi. Eighteen healthy adult male and female rabbits of mixed breed, weighing 1 to 2 kg were obtained from the local market. Rabbits younger than six months and pregnant females were excluded from the study. Standard laboratory conditions including an average temperature of 24°C and 12 hours light and dark cycle were maintained in the animal house. Before initiation of study rabbits were acclimatized for one week[15]. Rabbits were given same standard diet consisting of carrots, turnips, peas, grams and tap water *ad libitum* for drinking.

Rabbits were randomly divided into three groups, each consisting of six animals. The study period consisted of four days for all groups, in addition to the one week period of acclimatization. Rabbits were weighed before the cisplatin dose on day zero and then ahead of sacrifice on day three. Blood samples were also drawn twice; on day zero and on day three before the animals were sacrificed.

The rabbits (n=6) in distinct experimental groups were given drugs according to the following plan. Group A was the control group and received single intraperitoneal (I/P) injection of 1ml normal saline on day zero. Group B received single I/P injection of 10mg/kg cisplatin on day zero and served as toxic group. Group C received single I/P injection of 10mg/kg cisplatin on day zero along with Melatonin 5mg/kg I/P 30 minutes prior to cisplatin and every day at the same time for next two days[16].

Serum creatinine was measured based on the colorimetric Jaffe's method, before the adminis-`tration of drugs on day zero and prior to sacrifice on day three. A value of double the base line was considered significant. Serum urea was measured based on the principle of urease and

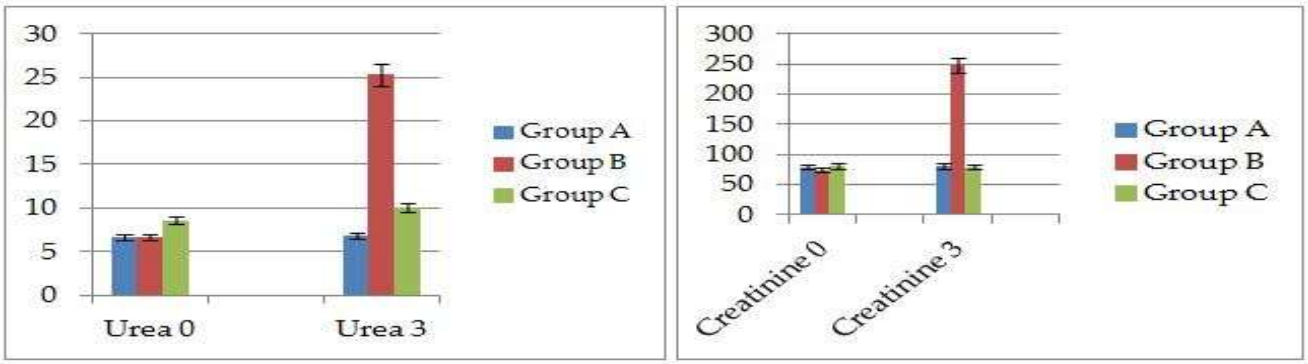

Figure-1: Serum urea (mmol/L) and creatinine (µmol/L) levels of group-A, group-B and group-C (n= 6).

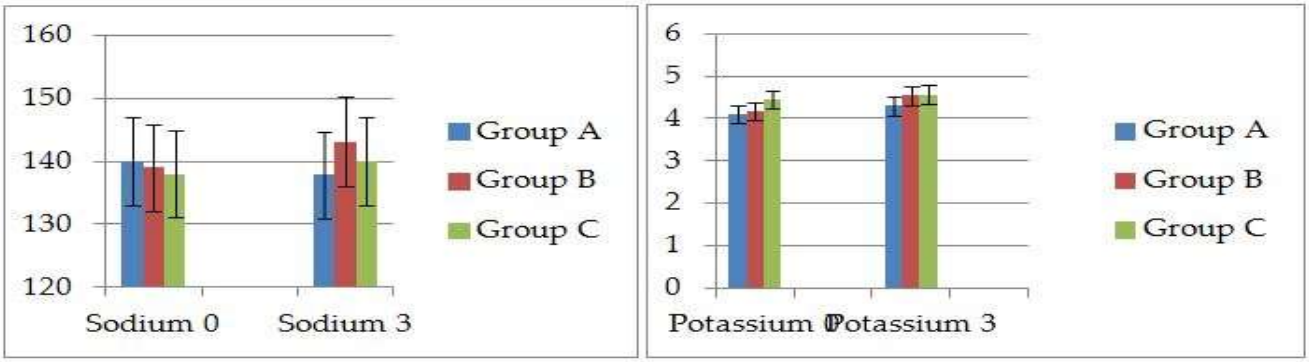

Figure-2: Serum sodium (mmol/L) and potassium (mmol/L) levels of group-A, group-B and group-C (n= 6).

glutamate dehydrogenase, before the administration of drugs on day zero and prior to sacrifice on day three.

Serum sodium and potassium levels were measured on the principal of ion selective electrodes using Easylyte before the administration of drugs on day zero and prior to sacrifice on day three. Analysis was done according to the following reference range: serum potassium 3.4-5.1 mmol/L and serum sodium 138-148 mmol/L.

Animals were sacrificed 72 hours after the cisplatin dose. Both kidneys were taken out and washed to remove excess blood. The kidney specimens were sliced sagitally and placed in 10% natural buffered formalin for 24 hours. The kidney tissue was processed for paraffin embedding. Approximately 5 micro meter thick sections were taken with a rotary microtome. Sections were mounted on glass slides and stained with hematoxylin and eosin, and were assessed for histopathological damage.

Histopathalogical abnormalities were scored according to the following criteria: 0= No cell necrosis. 1= Mild, only single cell necrosis in sparse tubules. 2= Moderate, more than one cell involved in sparse tubules. 3= Marked, tubules exhibiting total necrosis in almost every power field. 4= Massive total necrosis[17].

Data were analyzed by using SPSS version 21.0. The results of the serum analysis were expressed as Means ± S.D and were calculated on the computer using one-way analysis of variance (ANOVA). In order to find the significance of the difference between observations Post Hoc Tukey test was applied. The difference between two observations was considered as significant if the *p*-value was <0.05. The result of histopathology was analyzed using the "Chi-square test".

RESULTS

Serum analysis showed significant elevations of all the parameters after cisplatin treatment. Mean serum urea was 6.7 ± 1.01 mmol/L on day zero and 25.32 ± 1.43mmol/L on day three (p<0.001). Mean serum creatinine was 73.83 ± 6.24 µmol/L on day zero and 248 ± 84.86 µmol/L on day three (p<0.001). Mean serum sodium was 139 ± 4.34 mmol/L on day zero and 143.17 ± 2.32 mmol/L on day three (p< 0.01). Mean serum potassium was 4.17 ± 0.49 mmol/L on day zero and 4.55 ± 0.73 on day three (p<0.01). Serum analysis was insignificant for all the parameters when melatonin co-treatment was done along with cisplatin. Mean serum urea was 8.58 ± 2.74mmol/L on day zero and 10.03 ± 1.2mmol/L on day three (p<0.14). Mean serum creatinine was 80.33 ± 7.92 µmol/L on day zero and 79 ± 11.11 µmol/L on day three (p<0.4). Mean serum sodium was 138 ± 2.42 mmol/L on day zero and 140 ± 2.16 mmol/L on day three (p<0.15). Mean serum potassium was 4.45 ± 0.48 mmol/L on day zero and 4.57 ± 0.78mmol/L on day three

Table: The comparison of serum electrolyte parameters of Group A, Group B and Group C.

	Group-A (n=6)		*p*-value	Group B (n=6)		*p*-value	Group C (n=6)		*p*-value
Parameter	**Day 0**	**Day 3**		**Day 0**	**Day 3**		**Day 0**	**Day 3**	
Serum Urea (mmol/L) (Mean ± S.D)	6.7 ± 1.35	6.8 ± 1.43	0.4	6.7 ± 1.01	25.32 ± 1.43	0.001	8.58 ± 2.74	10.03 ± 1.2	0.14
Serum Creatinine (µmol/L) (Mean ± S.D)	78.8 ± 5.81	80.5 ± 7.53	0.18	73.83 ± 6.24	248 ± 84.86	0.001	80.33 ± 7.92	79 ± 11.11	0.4
Serum Sodium (mmol/L) (Mean ± S.D)	140 ± 1.55	137.83 ± 2.32	0.06	139 ± 4.34	143.17 ± 2.32	0.01	138 ± 2.42	140 ± 2.16	0.15
Serum Potassium (mmol/L) (Mean ± S.D)	4.1 ± 0.2	4.3 ± 0.32	0.12	4.17 ± 0.49	4.55 ± 0.73	0.01	4.45 ± 0.48	4.57 ± 0.78	0.26

(p<0.26).The comparative values of serum urea and creatinine (fig-1), and serum sodium and potassium (fig-2) of all the groups are given below. The Means ± SD along with the p-values of serum urea and creatinine, and serum electrolytes (sodium and potassium) of all the groups are given under as table. The kidneys from the cisplatin-treated rabbits showed moderate (grade II) toxicity, consisting of marked histological changes in the cortex and outer medulla, such as vacuolation, interstitial edema, tubular atrophy, severe tubular necrosis, and interstitial inflammation (fig-3(ii)). Melatonin treatment decreased the cisplatin-induced tubular necrosis and most of the changes caused by cisplatin treatment (fig-3(iii)). Consequently, kidneys from the melatonin-treated rabbits were almost normal (grade 0).

Melatonin was given in a dose of 5mg/kg 30 minutes prior to cisplatin administration and then daily at the same time for the next two days. On comparison with the toxic group, significant nephroprotection was seen with melatonin, which was evident from a significant decrease in serum urea and creatinine (p<0.001), and amelioration of the histopathological damage (p<0.02). Histopathology showed almost normal renal architecture. Moreover, there was also less weight loss (p<0.03), as compared to the toxic group.

Melatonin has the ability to donate electrons to free radicals, there by diminishing their reactivity. Consequently melatonin protects against neurodegenerative disorders, ischemia/ reperfusion injury, acts as a radio-protector, and attenuates herbicidal and metal toxicity. At the intracellular level, melatonin decreases electron escape from the respiratory chain complexes of mitochondria. Simultaneously, it scavenges free radicals produced in the cytosol and the nucleus. Comparative studies conducted with other naturally occurring antioxidants like vitamin-E, vitamin C, mannitol, glutathione, N-acetylcysteine and 2-lipoic acid show an equal or greater efficacy of melatonin in neutralizing highly toxic oxygen and nitrogen-reactants. Additionally, its metabolites can also detoxify free radicals. The whole process is known as the anti-oxidative cascade. Hence, melatonin may be viewed as a pro-drug for a series of molecules having ability of reducing oxidative/ nitrosative stress[20].

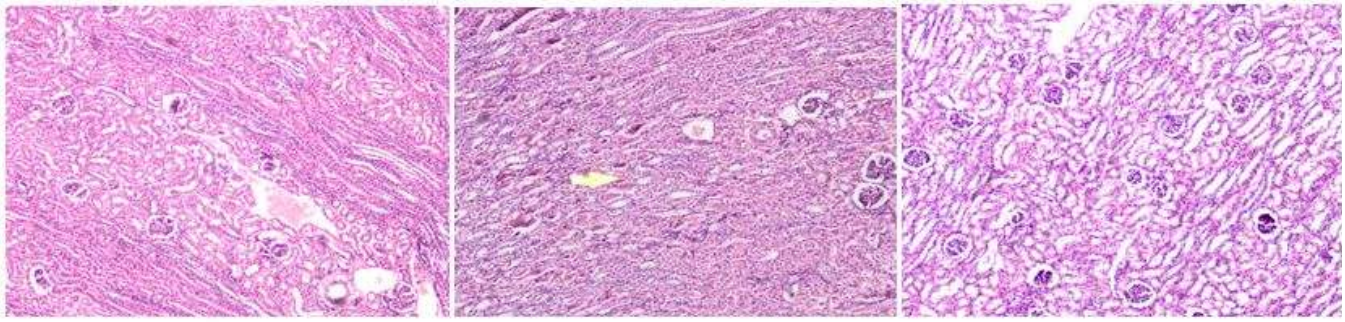

Figure-3: (i) Essentially normal renal architecture (Group-A), (ii) Amorphous tubular casts with mild inflammation (Group-B),(iii) Almost normal renal morphology with mild inflammation (Group-C).

DISCUSSION

Various mechanisms contributing towards cisplatin-induced nephrotoxicity have been described in previous studies. Tubular apoptosis and cell-cycle changes, mitochondrial injury, oxidative stress, and local inflammation have been regarded as the crucial occurrences leading towards renal failure[18,19]. Among these processes, increased oxidative stress was found to be, one of the earliest features. This was later accompanied by a marked infiltration of the inflammatory cells and a secondary wave of ROS generation. Considering the crucial role played by ROS in the pathogenesis of cisplatin-induced nephrotoxicity, we decided to use melatonin for its attenuation.

The role of melatonin as an antioxidant has been investigated in different models of nephrotoxicity. These include nephrotoxicity associated with anthracyclin antibiotics; danorubicin and doxorubicin[21], gentamicin-induced nephrotoxicity[22], colistin-induced nephrotoxicity[23], and mercury chloride induced acute renal failure and oxidative stress[24], in addition to cisplatin-induced nephrotoxicity. Consequently, many studies have regarded melatonin as an efficient free radical scavenger[25].

CONCLUSION

The protective effects of melatonin on cisplatin-induced nephrotoxicity were due to its antioxidant properties.

It is a powerful free radical scavenger. Hence, melatonin may be employed to broaden the therapeutic window of cisplatin.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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