Original Article

Protective Role of Melatonin on Streptozotocin induced Renal Damage in Albino Rats

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ABSTRACT

Aims and Objectives: This study was designed to evaluate the protective role of melatonin on the morphology of proximal convoluted tubules (PCT) of albino rats made nephrotoxic by a chemotherapeutic drug like streptozotocin (STZ).

Study Design: Prospective experimental study.

Place and Duration of Study: This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Post graduate Medical Centre (JMPC), Karachi, for 6 weeks from March to April, 2012.

Materials and Methods: 60 male albino rats were divided into 4 groups, containing 15 animals each. Group A was treated as control, groups B & C received 37 mg/kg STZ Intraperitoneally (I/P) once at the start of experiment, whereas group C additionally received 10mg/100 ml of melatonin (MEL) 3-days prior to STZ administration, and group D received only MEL at the same dose. Serum glucose was measured weekly. The kidneys were processed for histological examination and periodic Acid Schiff Haematoxylin (PAS-H) stained sections were viewed under the light microscope for detailed morphological examination of the proximal convoluted tubules.

Results: The microscopic examination revealed marked epithelial, cytoplasmic and nuclear changes in the P.C.T. of STZ treated group B & a significant reduction in the severity of these changes in MEL treated group C. Serum glucose was significantly increased in both group B and C.

Conclusion: The results of the investigation indicated that MEL administration suppressed the progression of renal injury induced by nephrotoxic drugs like STZ. It could not decrease STZ induced hyperglycaemia, but it did prevent the histopathological damage of the P.C.T. So dietary supplementation of MEL could be an easy and inexpensive method of protecting cancer patients from renal damage caused by chemotherapy induced oxidative stress.

Key Words: Streptozotocin, Melatonin, Nephrotoxicity, Oxidative stress, Oxygen Free Radicals, Reactive Oxygen species, proximal convoluted tubules.

INTRODUCTION

Medications commonly used in patients with cancer are notoriously nephrotoxic. The major groups of agents causing acute tubular toxicity are antibiotics, NSAIDS and chemotherapeutic agents. The proximal renal tubular cells vulnerability to the direct toxic action of chemicals is largely due to the role played by this portion of the nephron in absorption and secretion[1].

Nephrotoxicity is intrinsic to the pharmacological effect of certain anticancer drugs. Because antineoplastic agents have a narrow therapeutic index, the amount of drug required to significantly reduce tumour burden usually induces significantnephrotoxicity. Philosophically, greater toxicity is acceptable for curative therapy as opposed to palliative therapy[2].STZ is amongst one of the most nephrotoxic chemotherapeutic compounds in frequent use for the treatment of pancreatic islet cell carcinoma and carcinoid tumors[3]. The effects of STZ on different organs have been extensively studied. It is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration[4].

Renal toxicity is the major dose limiting side effect of STZ[5]. The site of injury involves both the glomerulus and tubules, based on histologic changes[5]. By producing hyperglycaemia and hypoinsulinemia, STZ alters various metabolic and enzymatic functions of kidney, resulting in various pathologic lesions[3]. Formation of reactive oxygen species (ROS) is thought to be a mediator of the cytotoxic actions of STZ[6]. Organisms have developed several defence mechanisms to protect their cells against ROS. Such mechanisms include use of antioxidant enzymes and antioxidant molecules such as vitamin C, E and flavonoids.

Antioxidants are compounds that either reduce the formation of free radicals or react with and neutralize them. However, when a condition of oxidative stress establishes, the balance between free radicals production and the level of antioxidant molecules tilts towards excess of free radicals, and the defence capacities against ROS become insufficient.Melatonin (N-accetyl-5 methoxytryptamine), the chief secretary product of the pineal gland, is a multi-facetted free radical scavenger and a strong antioxidant[8]. It breaks down many free radicals, such as highly toxic hydroxyl and peroxyl radicals and oxygen free radicals (OFR). Melatonin can penetrate all the morphophysiological barriers in the human body due to its lipophilic and hydrophilic characteristics[9]. Thus MEL can effectively protect cell walls, organelles and nuclei from damage by free radicals. MEL functions as a modulator of

sleep, sexual behaviour, immune functions and circadian rhythm. Moreover, MEL has a potent ROS scavenger activity chiefly because of its capacity to act as an electron donor[10]. It decreases inflammation and impedes the progress of tissue edema[9]. It inhibits the accumulation of neutrophils in the damaged renal tissue[11].

In the light of the proceeding statements, this study was designed to study the protective role of MEL on the morphology of P.C.T. under the light microscope in albino rats made nephrotoxic by STZ. The effects of these drugs on serum glucose levels were also monitored.

MATERIALS AND METHODS

This study was conducted in the department of Anatomy, BMSI, JPMC,Karachi for a period of 6 weeks. In this study, 60 healthy male albino rats, 90-120 days old, weighting around 250-290 gm were obtained from the animal house of BMSI and divided into 4 groups, each group containing 15 animals.They were kept in propylene cages, equipped with drinking water bottles and wood chip floor bedding under laboratory environment. Serum glucose of all the animals was determined by a glucometer from the tail vein.

Group A was taken as control. The animals of group B and C were fasted overnight and administered STZ I/P in a dose of 37 mg/kg[12]dissolved in 1 ml of citrate buffer at 4 PH, only on the first day of the experiment. Group C additionally received 10 mg/100 ml[13] of MEL.Group D received the same amount of MEL in drinking water. The water bottles were covered with aluminium foil to prevent degradation of MEL by sunlight. Clean water bottles and freshly prepared MEL solutions were provided each day. Serum glucose of Group B and C animals was closely monitored throughout the experimental period. They were sacrificed at the end of treatment period the abdomen was opened by a midline Incision. Both the kidneys were exposed, dissected out and they were fixed in buffered neutral formalin for 24 hours. After thatthey were kept in 70% alcohol overnight, dehydration of the tissues was done with ascending strength of alcohol, cleared in xylene and infiltrated with paraffin at 59 degree. 5microns thick paraffin embedded longitudinal sections were made and stained with PAS-H for a detailed morphological examination of the P.C.T underlight microscope.A minimum of 10 fields of each kidney slide were examined and scored semi quantitatively for severity of changes. The scoring was done as none (-) mild (+), moderate (++) and severe (+++).

RESULTS

Group A: The lining epithelial cells of the P.C.T were regularly arranged on an intact and well defined basement membranes and distinct brush borders (fig-1). The mean values of serum glucose were 88.06± 5mg/dl (Table-1) and no lesions were observed upon Pathological grading (Table-2).

Group B: Most of the P.C.T. showed dilatation with severe sloughing and degeneration, while others showed shrinkage in size with necrotic changes (Fig-2) Most of the cells showed vacuolated appearance obscuring cytoplasmic details. The brush borders and basement membranes were highly discontinuous and distorted (Fig-2). The mean values of serum glucose were 379.12±15mg/dl, which were highly significant as compared to control (Table-1) Diffuse lesions were observed upon grading of the tubular damage (Table-2).

Group-C: There was an overall improvement and preservation of the morphology of P.C.T. as compared to group B(Fig-3). Most of the cells had well defined and intact brush borders and basement membranes. The mean values of serum glucose (360.18±7 mg/dl) were highly significant as compared to control group, high lighting the insignificant effect of MEL on serum glucose. The extension of tubular injury was significantly reduced by MEL (Table-2).

Table No.1: Mean*Serum glucose levels in different experimental groups of albino rat.

Groups	Serum Glucose (mg/dl)
A (Control)	88.06 ± 5.12
B (STZ)	379.12 ± 15.29**
C (STZ + MEL)	360.18 ± 7.36**
D (MEL)	84.02 ± 5.02

Each value is mean ± S.E.M. for 15 rats in each group
* Significant P < 0.01
** Highly Significant P < 0.05as compared to control.

Table No.2: Grading ofhistological changes ofP.C.T in different groups of albino rats

S.No	Lesions of P.C.T.	GROUPS			
		A	B	C	D
1	Degeneration of Tubular Epithelium	-	+++	+	-
2	Tubular Dilatation	-	++	+	-
3	Cytoplasmic Vacuoles	-	+++	+	-
4	Distortion of brush border membrane	-	+++	+	-
5	Distortion of basement membrane	-	+++	+	-
6	Interstitial inflammation	-	++	+	-

Key to Scores:
- No Lesion observed
+ Mild lesion observed
++ Moderate lesion observed
+++ Severe lesion observed

Group-D: Normal morphology of the P.C.T. was observed (Fig-4), same as control group (Fig-1). The mean values of serum glucose were similar to the

control group (table-1) and no lesions were observed upon grading of the tubular damage (table-2).

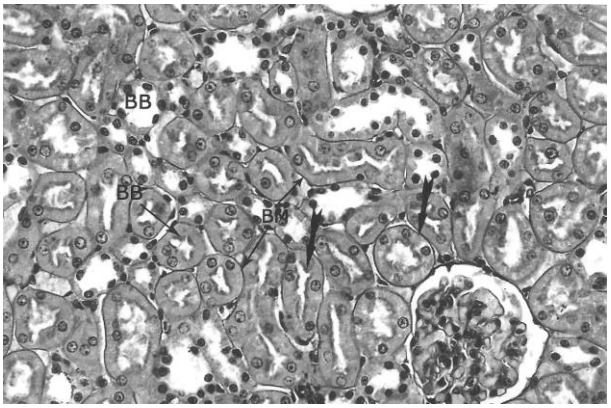

Figure No.1: Photomicrograph of microns thick PAS-H stained section from cortex of kidney in Group A (control) rat showing normal architecture of proximal tubules with intact Brush Borders (BB) and Basement Membranes (BM) X400.

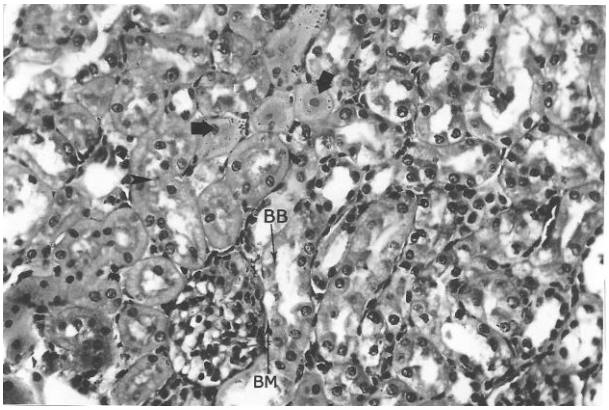

Figure No.2: Photomicrograph of 5 microns thick PAS-H stained section from cortex of kidney in STZ treated group B showing disturbed architecture with indistinct BB and BM, nuclear and epithelial debris in the lumina and tubules showing necrotic changes.x400.

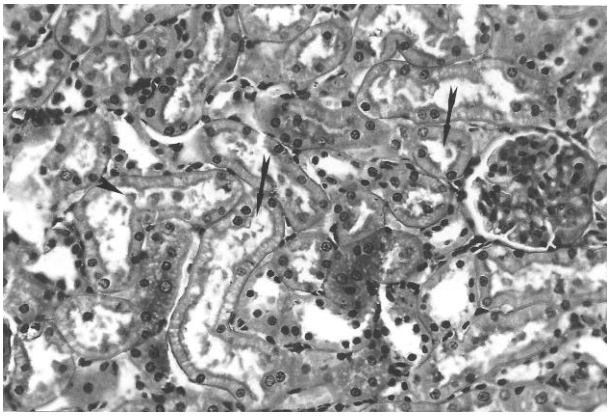

Figure No.3:PAS-H stained 5 micron thick longitudinal section of kidney from STZ and MEL treated group C showing dilated proximal tubules with epithelial casts in the lumina.x400.

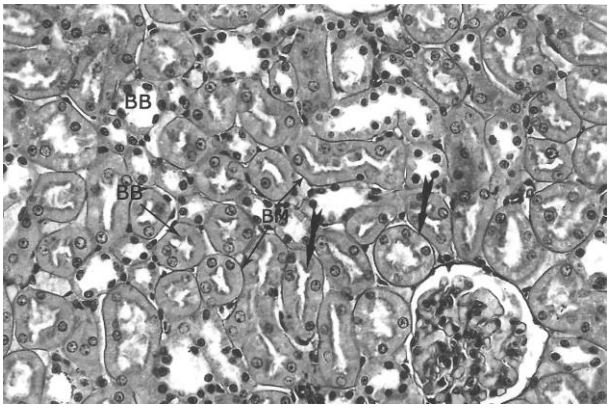

Figure No.4: Photomicrograph of 5 microns thick PAS-H stained section from cortex of kidney in Group D (MEL Treated) rat showing no change in proximal tubules with intact BB and BM.

DISCUSSION

The present study demonstrated the significance of MEL in reducing the severity of renal damage in animals exposed to a nephrotoxic drug like STZ. Since the kidney is highly susceptible to the toxic injury by a multitude of different drugs, it is not surprising that several antineoplastic agents may exert potent nephrotoxicity.STZ generates ROS which contributes to DNA fragmentation and evoke other deleterious changes in the cells[13].Petzold and Swenberg(1978)[14] demonstrated that a single I.V. dose of STZ induces strand breaks in kidneys and liver of rats.Alejandro D and Martha S(2002)[7] stated that STZ induces cell death by apoptosis and necrosis, which are in agreement to our results. Rodriguez et al in 2004 stated that oxidative stress and its constant companion inflammation play a major role in the pathogenesis of the progression of renal injury.MEL has potent antioxidant and anti-inflammatory properties and its production is impaired in chronic renal failure.

The favourable results seen with melatonin administration in our experiment are likely related to the antioxidant and anti-inflammatory properties of this compound resulting from its strong ROS scavenger properties.MEL has been shown to ameliorate inflammation by blocking transcriptional factors and tumour necrosis factor alpha[15] .In those situations where free radical production is enhanced, MEL has demonstrated to be more effective than other antioxidants with the advantage that lower doses are needed[10].It has been proved earlier that MEL could effectively neutralize the impaired anti oxidative status in rats with STZ induced diabetes.MEL has been shown to be effective in protecting against severe free radical mediated toxicity in a variety of conditions including chemotherapy[16], ischemia reperfusion injury[17] ,acute renal failure caused by mercuric chloride and gentamycin[18] In a study conducted by Naqvi A[12] in 1992, STZ at the dose of 37mg/kg for 6 weeks

produced marked hyperglycaemia in albino rats which is in agreement with our results. In the present study, STZ resulted in significant hyperglycaemia and MEL supplementation did not affect this parameter. In a similar study conducted by Sudnikovich et al in 2007[19], STZ administration to albino rats for 25 days resulted in significant hyperglycaemia, increased levels of glycated hemoglobin and retarded growth of animals, whereas melatonin administration did not effect these parameters.

Present experimental study reveals that the renal injury caused by STZ is not mainly due to hyperglycaemia, but due to its direct toxic effects on the morphology of the kidneys.M.Akmali in 2010[7] stated that STZ forms ROS which is responsible for its cytotoxicity. The present study further reveals that MEL preserves the morphology of the kidneys without producing a significant effect on blood glucose levels.

CONCLUSION

In conclusion, this study demonstrates that MEL administration suppresses the progression of renal injury induced by nephrotoxic drug like STZ.MEL could not decrease the hyperglycaemia in STZ treated animals, but it did prevent the histopathological damage of the proximal convoluted tubules caused by free radicals generated by STZ .So dietary supplementation of MEL could be an easy and inexpensive method of protecting cancer patients from renal damage caused by oxidative stress.

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