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Original Article

ROLE OF EBSELEN, A SELENOORGANO COMPOUND IN CISPLATIN INDUCED NEPHROTOXICITY IN WISTAR RATS

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ABSTRACT

Objective: Nephrotoxicity is encountered worldwide, irrespective of several factors, but drug induced nephrotoxicity is a complication that is attributed to the high dose and even low dose of drugs. Cisplatin, a platinum complex used in the chemotherapy of several solid tumors was found to have a chief dose limiting side effect namely nephrotoxicity, which occurred due to lipid peroxidation and formation of reactive oxygen species. Ebselen, a eleno organo compound, a glutathione peroxidase mimic with anti-oxidant activity was used in our study to evaluate its nephro protective potential.

Methods: Male wistar rats, 6-8 wks old, weighing 180-200 grams were used for the study, which was carried out for a period of 7 wks. Animals were divided into five groups; each group consisting of 6 animals. Group I served as control. Group IV & V received the test drug Ebselen in doses of 10 mg/kg & 20 mg/kg respectively. Group III received Amifostine at 50 mg/kg. The drugs were administered once a week intraperitoneally for 5 wks. Nephrotoxicity was induced at a dose of 5 mg/kg single dose for groups II to V in the 6th wk and the drugs in group III, IV & V continued for 5 days post induction. In the 7th wk blood samples were collected for biochemical analysis and kidney tissues for histopathological study.

Results: The serum urea, creatinine levels were significantly increased in Cisplatin group compared to other groups. The estimation of antioxidant levels (catalase, superoxide dismutase and glutathione peroxidise) was significantly decreased in cisplatin group and increased in other groups. The estimation of Malondialdehyde an indicator of lipid peroxidation was significantly increased in group II and decreased in drug treated group. Histopathology results of animals treated with Cisplatin showed inflammatory changes such as tubular degeneration, edema, and necrosis, infiltration of cells in tubular interstitum, mild intertubular hemorrhage and atrophy of glomeruli which was severe in group II. Some changes were also observed in Group III, IV and V animals but with less severity.

Conclusion: The result of our study effectively proves the antioxidant potential of Ebselen in ameliorating Cisplatin induced nephrotoxicity.

Keywords: Cisplatin, Ebselen, Nephrotoxicity, Malondialdehye, Amifostine.

INTRODUCTION

Cisplatin (Cis-diamminedichloroplatinum II; CDDP) is a potent antitumor agent for solid tumours including various types of cancer. Cisplatin-induced nephrotoxicity is a complex process that comprises of acute cytotoxic effects on tubular epithelial cells, resulting in loss of tubular cells by necrosis and apoptosis followed by inflammatory cell in filteration and fibroproliferative changes [1]. Hannerman and Baumann 1988; Sodhi *et al.* 1988 reported that pathogenesis of kidney injury caused by cisplatin was in part due to oxidative damage by free radical production. Cisplatin has been reported to induce the expression of inflammatory cytokines, such as interleukin (IL-1) and (IL-6). [2] Increased renal expression of tumour necrosis factor-α, transforming growth factor (TGF-β). Macrophage inflammatory protein-2, IL-1 and intracellular adhesion molecule-1 has been detected in kidneys of cisplatin-treated animals [3]. Ebselen (2-phenyl-1, 2-benzisoselenazol-3(2H)-one) a seleno organic compound exhibits glutathione peroxidase like activity *in vitro*, reducing hydroperoxides in the presence of a thiol in a catalytic reaction. [4-6] Ebselen has been investigated for the treatment of various diseases such as arthritis, stroke, organ transplantation, antiasthmatic, ozone toxicity, diabetes related atherosclerosis and neuropathy, and also as an inhibitor of the neuronal demyelination [7, 8]. Ebselen also induces DNA damage in yeast (Saccharomyces cerevisiae) leading to activation of the check point kinase proteins and derepression of DNA damage response genes [9]. Ebselen proved to be effective in in-vitro techniques due to its antioxidant potential [10]. Hence the objective of our present study was to evaluate the effect of Ebselen in preventing cisplatin-induced nephro toxicity in male wistar rats.

Groups	Pre-induction	Induction	Post-induction
Group I	Normal saline+DMSO intraperitoneally once a week for 5 weeks	-	Continued
Group II	-	Cisplatin(5 mg/kg) single dose intraperitoneally in 6th week	-
Group III	Amifostine(50 mg/kg) intraperitoneally once a week for 5 weeks	Cisplatin(5 mg/kg) single dose intraperitoneally in 6th week	Amifostine(50 mg/kg) intraperitoneally twice a day for 5 consecutive days
Group IV	Ebselen(10 mg/kg)+DMSO intraperitoneally once a week for 5 weeks	Cisplatin(5 mg/kg) single dose intraperitoneally in 6th week	Ebselen (10 mg/kg)+DMSO intraperitoneally twice a day for 5 consecutive days
Group V	Ebselen(20 mg/kg)+DMSO intraperitoneally once a week for 5 weeks	Cisplatin (5 mg/kg) single dose intraperitoneally in 6th week	Ebselen (20 mg/kg)+DMSO intraperitoneally twice a day for 5 consecutive days

MATERIALS AND METHODS

Healthy adult male wistar strain rats 6-8 wks old weighing 180-200 grams were purchased from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India. The animals were housed in well ventilated rooms (temperature 23±2 degree Celcius, humidity 65-70% and 12h light/dark cycles) and provided standard pellet feeds and water *ad*

libitum. All studies were conducted in accordance with the committee for the purpose of control and supervision on experiments in animals (CPCSEA) and the National Institute of Health guidelines "Guide for the care and use of Laboratory Animals. The study was approved by IAEC (proposal no 1066/dated 29/11/13)

Study design

The male wistar rats were divided into 5 groups. Each group consists of 6 animals.

Method of sample collection

Urine samples were collected for 24 hr following Cisplatin administration from each group keeping animals separately in special metabolic cages. 50th percentile animals in group III to V were sacrificed at the end of 3rd day of Cisplatin administration. The blood samples were collected for biochemical analysis of serum parameters & tissue samples sent for Histopathological analysis. The rest of the animals were observed for a period of 10 days post induction for morbidity & mortality.

Tissue sampling

The left kidney was divided into 3 parts, and each was immediately homogenized in the specified buffered solutions for different biochemical assays. Kidney homogenate was analysed for Catalase, Superoxide dismutase, Glutathione peroxidase & MDA levels. The right kidney was sectioned in blocks and fixed in 10% formalin, then dehydrated in graded concentrations of alcohols, and embedded in paraffin.

Histopathological staining

For histopathological evaluation of kidney damage, Kidney tissue was fixed in 10% neutral paraformaldehyde, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E).

Biochemical analysis

Blood Urea was estimated by Urease method and Serum Creatinine was estimated by the modified Jaffe's method. Anti-oxidants enzymes were estimated in the serum. Superoxide dismutase (SOD) in the tissues was assayed by the method of Kakkar *et al.*, and activity of Catalse (CAT) in the tissues was determined by the method of Sinha [11]. Glutathione peroxidase (Gpx) in the tissues was determined by the method of Wendel, A. (1980) [12]

Statistical analysis

Values of biochemical analyses and antioxidant enzyme levels were expressed as means±S. D for six rats in each group. The data were analysed by Duncan's Multiple Range Test (DMRT), using SPSS software version 17.0 (SPSS, Inc., Chicago, Illinois). Values not sharing a common superscript differ significantly at $p<0.05$.

RESULTS

The mortality was>95% in Cisplatin treated group, within in 72 hrs (3 days) post induction. No mortality in group III, IV & V was observed till the 10th day post induction.

Blood urea and creatinine estimation

Blood Urea and Creatinine estimation showed a significant increase in cisplatin treated group II as compared to group I reflecting nephrotoxicity of cisplatin. Blood Urea and Creatinine values in Ebselen treated group IV and group V was significantly lower and values in group 5 were comparable with Amifostine group.

Anti-oxidant level estimation

Cisplatin resulted in a decrease in glutathione peroxidase (>50%) but treatment with Ebselen in groups IV & V and Amifostine in group III showed a remarkable increase in glutathione peroxidase activity similar to normal rats. SOD &CAT values in the drug treated group were also significantly elevated. Levels of malondialdehyde a marker of lipid peroxidation were found to be highest in Cisplatin group but were comparatively reduced in group III to group V.

Renal histological examination

The histopathological examination of kidney specimen in Cisplatin treated group showed moderate to severe histological damage in the form of tubular degeneration, Necrosis, Edema, Interstitial cell Infilteration, Haemorrhage and Glomerular atrophy, Suggestive of extensive tubule-interstitial nephrotoxicity. The histopathological examination of kidney of ebselen treated group IV & V, and Amifostine treated group III was comparatively very minimal. The degree of damage and the results are tabulated below.

Results of biochemical analysis

Test variable	Serum urea [mg/dl]	Serum creatinine [mg/dl]
Group 1	27.00±0.63 ^{e}	0.28±0.07 ^{e}
Group 2	89.98±0.63 ^{a}	5.25±0.08 ^{a}
Group 3	37.65±0.39 ^{d}	1.14±0.01 ^{d}
Group 4	49.56±0.79 ^{b}	1.79±0.01 ^{b}
Group 5	39.78±o.39 ^{c}	1.18±0.01^{c}

Values are expressed as means±SD for six in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)

Results of antioxidant estimation

Test Variable	Catalase [U/mg Protein]	Glutathione peroxidase [U/mg Protein]	Superoxide dismutase [U/mg Protein]	Malandialdehyde [U/mg Protein]
Group 1	17.18±.07 ^{a}	18.48±.07^{a}	24.16±.10 ^{a}	55.50±.14 ^{e}
Group 2	6.36±0.10 ^{e}	5.01±0.14 ^{e}	7.75±0.10 ^{e}	98.15±0.10 ^{a}
Group 3	16.18±0.07 ^{b}	16.46±0.10 ^{b}	22.25±0.10^{b}	65.26±0.15 ^{c}
Group 4	15.43±0.12 ^{d}	14.18±0.07 ^{d}	18.55±0.10 ^{d}	70.16±0.10 ^{b}
Group 5	16.11±0.13 ^{c}	15.15±0.10 ^{c}	19.45±0.10 ^{c}	62.35±0.10 ^{d}

Values are expressed as means±SD for six in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)

Grading of the histopathological changes in kidney sections of rats treated with Ebselen in Cisplatin Induced Nephrotoxicity

Groups	Tubular degeneration	Necrosis	Edema	Interstitial cell infiltration	Hemorrhage	Glomerular atrophy
GI	-	-	-	-	-	-
GII	+++	++	++	++	++	+++
GIII	+	-	-	+	-	+
GIV	+	-	+	+	-	++
GV	+	-	+	-	-	+

(-)-None; (+)-Mild; (++)–Moderate; (+++)–Severe

DISCUSSION

In this study, we observed that the CDDP (5 mg/kg ip) induced renal damage was characterised by significant increase in mortality. Serum Urea and Creatinine levels in the Cisplatin treated group was substantially elevated with reduction in urine volume suggestive of acute renal failure. [13] The serum parameters correlated well with the morphologic alterations observed in the kidneys revealing selective and severe proximal tubular damage, moderate degree of necrosis, haemorrhage and proteinaceous casts. Also, CDDP administration resulted in a decrease in GSH content and superoxide dismutase (SOD) activity. Although several mechanisms of CDDP induced nephrotoxicity has been postulated, major evidence points towards lipid peroxidation and free radical formation. Chemoprotection by various agents is being evaluated against Cisplatin which are in trials in clinical treatment protocols. [14]. Therefore this study categorically implies the protective effects of Ebselen, a glutathione peroxidase mimic in alleviating Cisplatin nephrotoxicity in rats. Pretreatment with Ebselen followed by continuation of therapy for 5 days post induction helped in reducing the mortality in drug treated group. Its antioxidant potential was furthur substantiated by biochemical parameters. Data indicates that Ebselen supplementation replenishes glutathione stores as evident by the increase in levels of glutathione (GSH) in drug treated group to near normal values. This effect was found to be comparable to Amifostine. It has been reported that glutathione (GSH) depletion by BSO (Bluthionine sulfoximine) resulted in potentiating Cisplatin nephrotoxicity [15]. Our results also agree with other reports pertaining to Cisplatin induced renal GSH depletion. The MDA levels which were found to be reduced in kidney tissues of rats pretreated with ebselen. This indicate nephroprotective potential of the drug, which helps in ameliorating Cisplatin-induced oxidative damage. Although protection with several thiols have been reported, it is unclear whether protection is an extracellular or intracellular event. Sparing of renal glutathione (GSH) levels in Ebselen pretreated rats is suggestive of a role for intracellular GSH in mitigating the CDDP induced nephrotoxicity. [16] Amifostine, a thiol compound was used as a reference standard in our study, since it was found to be nephroprotective, as reported in several studies [17]. The antioxidant action of Ebselen and histopathological analysis was comparable to Amifostine. This is suggestive of its role in supplementing/scavenging the free radicals formed in cisplatin induced nephrotoxicity. Mitochondrial dysfunction reported to occur in rat renal cortical slices exposed to Cisplatin following depletion of cytosolic GSH resulted in an increase in lipid peroxidation [18]. Our present study of renal tissues following CDDP administration showed depletion of GSH, SOD & CAT which is in accordance with reports of (Sugiharen *et al.*). Platinum compounds tend to deplete renal GSH stores thereby inducing lipid peroxidation. Evidence has also shown that decreased in SOD activity can cause initiation and propagation of lipid peroxidation. In addition, levels of Cu & Zn, which are elements for enzymatic activity were decreased in kidney tissues following cisplatin administration as demonstrated by Sinet *et al.* Reduction in CAT activity & GSH levels reduces the tissue redox potential in scavenging the toxic H_2O_2 and lipid peroxides formed [19]. Several in-vitro studies of Cisplatin induced nephrotoxicity have recognised apoptosis as an important mode of cell death in normal and pathologic states. The restoration of the antioxidant SOD, CAT & GSH with Ebselen pretreatment could go a long way in rejuvanating the renal tissues by scavenging free radical and/or supplementing depleted enzymes. Since lipid peroxidation following cisplatin administration is a consequence of GSH depletion and impaired antioxidant enzyme activity, the addition of Ebselen to supplement these deficiencies would also help in selectively suppressing components of nephrotoxicity [20].

CONCLUSION

In conclusion, Ebselen a novel selenoorganic compound with anti-oxidant potential was found to ameliorate CDDP induced nephrotoxicity at dose of 10 mg/kg and more effectively at 20 mg/kg. Ebselen replenished the depleted glutathione stores in the thioredoxin reductase system, inhibiting lipid peroxidation and mitigating the nephrotoxic effects. It can be conjectured that nephroprotection offered by Ebselen is related at least, in part to preservation of renal anti-oxidant system. Furthur studies in human are yet to be done to substantiate its clinical significance.

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CONFLICT OF INTERESTS

Declared None

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