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Effect of Cisplatin on Total Protein in Rat Kidney Cortical Slices

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Incubation of rat renal cortical slices with 3.3 mM cisplatin produced concentration - dependent decrease in total protein. The reduction in protein content was significant only after 30 min. To find out the role of reactive oxygen species (ROS), incubation was carried out in the presence of antioxidants. The water soluble analog of Vitamin E, trolox, protected the cortical slices against the cisplatin effect but ascorbic acid failed to produce any effect. Similarly, catalase and hydroxyl radical scavengers like dimethyl sulphoxide (DMSO), mannitol produced protection but superoxide dismutase (SOD) did not show protection. In conclusion, this study indicates that cisplatin causes decrease in the protein content in renal cortical slices and this decrease may be mediated through reactive oxygen species.

CISPLATIN is a potent anticancer drug used in the treatment of solid tumors of the testes, ovary, breast, lung and bladder[1]. Its clinical use is limited due to its ability to induce nephrotoxicity[2]. The nephrotoxicity is dose-dependent[3]. Although the molecular mechanism of the nephrotoxicity induced by cisplatin is still not clear, it has been suggested that free radicals play an important role. Incubating renal cortical slices with cisplatin results in increased lipid peroxidation[4]. Many antioxidants such as SOD[5], sodium selenite[6] and hydroxyethyl rutoside[7] have been reported to reduce the nephrotoxicity caused by cisplatin. Interaction of cisplatin with DNA resulted in the generation of superoxide and hydroxyl radicals in cell free system[8]. It has also been suggested that electrophilic species from cisplatin binds to macromolecules which may account for the persistent retention of cisplatin in kidney[3].

Free radicals are known to degrade protein by various mechanisms[9]. Cisplatin also increases cytosolic calcium which may activate proteases[10]. Since reactive oxygen species are implicated in cispaltin toxicity and the reactive oxygen species are known to degrade proteins, we found it interesting to study the effect of cisplatin on the total protein content in renal cortical slices.

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MATERIALS AND METHODS

Cisplatin, Trolox, SOD, Catalase and Bicinchoninic acid were obtained from (Sigma, St. Louis, Mo, USA). Ascorbic acid, Mannitol and DMSO were obtained from (Ranbaxy Lab. Ltd. Punjab). All other chemicals are of high purity were obtained from E-merck (India) Ltd. Bombay.

Inbred male Wistar rats, weighing 220 ± 20 g, kept on a standard laboratory diet (Brooke Bond Lipton's India Limited, Calcutta) were used for the experiments and received water *ad libitum*. Rats were anaesthetised with pentobarbitone sodium (50 mg/kg, i.p.), the right kidney was perfused with SOD and catalase in phosphate buffered saline for 20 minutes in separate experiments. Left kidney was perfused only with PBS and served as a control. Immediately after perfusion, rats were decapitated, the kidneys were removed, cooled and decapsulated in ice cold saline. Renal cortical slices were prepared using a razor blade in a kidney holder device to achieve the slice thickness of about 0.3-0.5 mm[4]. Kidney slices of about 100 mg/sample were incubated in a buffer consisting of 97 mM

Table 1 : Effect cisplatin on total protein in rat kidney cortical slices

Cisplatin (mM)	Lowry's method		BCA method	
	Total protein* (mg/g tissue)	Decrease %	Total protein (mg/g tissue)	Decrease %
0.0	15.1 ± 1.4	-	14.6 ± 0.7	-
0.5	13.3 ± 1.2	11.30	ND	-
1.0	11.7 ± 0.8[a]	22.50	10.6 ± 0.5[a]	27.40
2.0	10.2 ± 1.1[a]	32.50	9.6 ± 0.7[a]	34.20
3.3	9.7 ± 0.6[a]	35.80	8.5 ± 0.6[a]	41.80

Rat kidney cortical slices were incubated with cisplatin in buffer, washed and homogenised. After centrifuging at 800 g, the supernatant was estimated for protein content by either Lowry's method or bicinchoninic acid (BCA) method.

* - Mean ± standard deviations (SD) of five experiments. ND - not determined.

a - $p < 0.05$, significantly different from control.

NaCl, 40 mM KCl, 7.4 mM sodium phosphate buffer, pH 7.4 and 0.74 mM $CaCl_2$[11]. The medium was supplemented with 10 mM lactic acid.

To study the effect of cisplatin on degradation on tissue protein, the kidney slices were incubated with 0.0, 0.5, 1.0, 2.0 and 3.3 mM cisplatin for 120 min in a sample volume of 4.0 mL. The effect of various antioxidants on cisplatin - induced degradation of total proteins was studied. The kidney slices were incubated with cisplatin 2.0 mM in the presence or absence of trolox, ascorbic acid, dimethyl sulphoxide (DMSO) and mannitol at different concentration for 120 min. Incubations (n=5) were carried out in a metabolic shaker at 37°, pH 7.4 in an atmosphere of 95% O_2, 5% CO_2. Controls were incubated under identical conditions but without addition of cisplatin. At the end of incubation, the renal cortical slices were immediately removed, blotted, weighed and homogenised in 5.0 mL sodium phosphate buffer 7.4 mM at 2°. After centrifugation at 800 g, the protein concentration of the samples were determined by Lowry's method[12] as well as BCA method[13].

Statistical Evaluation

Mean ± standard deviation (SD) were calculated followed by ANOVA. The student's Newman Keuls test was used to compare between group data. A 'P' value of less than 0.05 was considered to be significant.

RESULTS

Effect of cisplatin on total protein

Incubating cortical slices with cisplatin at various concentrations resulted in significant decrease in protein levels compared to control (Table 1). The decrease was found to be concentration dependent. Incubation with 0.5 mM cisplatin resulted in 11.3 % decrease and at 3.3 mM concentration the decrease was 35.8% when the protein was estimated by Lowry's method. Similar results were obtained by BCA method also.

Effect of antioxidants

Incubating cortical slices with Trolox before the addition of cisplatin resulted in significant protection against decrease in protein. However, ascorbic acid failed to protect at the concentrations tested. Incubating with hydroxyl radical scavengers like DMSO or mannitol also resulted in protection. SOD dismutase, which scavenges superoxide, did not show protection. To rule out the possibility of difficulty in transport across membrane, kidney was perfused with PBS containing SOD for 20 min. before removing. Followed by preparation of slices which were incubated with cisplatin. In this procedure also no protection was observed. The effect of catalase was studied in a similar manner by perfusing kidney with catalase before preparation of slices. Catalase at 140 and 280 U showed protection.

Table 2 : Effect of antioxidants on degradation of total protein by cisplatin in rat kidney slices

Treatment	Total protein* (mg/g tissue)
Control	15.10 ± 1.26
Cisplatin	
2.0 mM	10.20 ± 1.08[a]
+ trolox	
0.1 mM	12.05 ± 0.50[b]
0.5 mM	13.15 ± 0.35[b]
1.0 mM	13.90 ± 0.71[b]
+ ascorbic acid	
1.0 µM	10.95 ± 0.92[a]
10.0 µM	10.15 ± 0.78[a]
0.1 mM	11.00 ± 0.42[a]
+ DMSO	
0.1 mM	11.15 ± 0.92[a]
0.5 mM	12.85 ± 0.36[b]
1.0 mM	13.70 ± 0.85[b]
+ mannitol	
0.5 mM	11.10 ± 0.29[a]
2.5 mM	13.15 ± 0.35[a,b]
5.0 mM	13.90 ± 0.71[b]
+ SOD**	
12.0 U	10.50 ± 0.57[a]
48.0 U	11.40 ± 0.34[a]
96.0 U	10.50 ± 0.42[a]
480.0 U	11.65 ±0.35[a]
+ catalase**	
28.0 U	10.95 ± 0.50[a]
48.0 U	11.40 ± 0.57[a]
96.0 U	13.10 ± 0.42[b]

Protein estimation was done by BCA method

* - Mean ± standard deviation (SD) of five experiments.

a - Significantly different from control ($P < 0.05$)

b - Significantly different from cisplatin, 2.0 mM ($P < 0.05$)

** - Rat kidneys were perfused with SOD or catalase in phosphate buffered saline for 20 minutes.

DISCUSSION

The present study shows that the total protein in rat cortical slices decreases when incubated with cisplatin. This effect is partially reversed in the presence of catalase and hydroxyl radical scavengers like DMSO and mannitol suggests the involvement of hydrogen peroxide and hydroxyl radicals in the cisplatin action. The involvement of superoxide anion may be excluded since SOD did not show protection. Among the antioxidants, trolox, which is a water soluble analog of vitamin E, showed protection but ascorbic acid failed to protect. It is reported that ascorbic acid does not protect protein against oxidative damage caused by cigarette smoke[14]. Also, in *in vitro* conditions, it behaved as prooxidant[14]. Thus the observed reduction in protein concentration when incubated with cisplatin may be mediated through ROS like peroxide and hydroxyl radicals. Cisplatin is also known to decrease the activity of many other enzymes like SOD, glutathione-S-transferase and catalase[15]. Some of these effects may also be mediated through ROS. Proteins are known to undergo oxidation, fragmentation, crosslinking with other biopolymers, in the presence of ROS[9]. Oxidised protein undergo rapid degradation by intracellular proteases stimulated by oxidant stress[16]. Calcium dependent proteases are activated by oxygen free radicals and cisplatin[10]. Treatment with cisplatin results in increased number and size of lysosomes in renal tissues[17]. Repture of lysosomes will result in the release of proteolytic enzymes. Thus, the observed decrease in protein in cortical slices incubated with cisplatin may be mediated through ROS either directly or indirectly by proteolytic degradation. Further studies are in progress to find out whether the effect is direct or indirect also to know what kind of proteins are affected.

In summary, cisplatin causes decrease in total protein when incubated with renal cortical slices. Trolox, DMSO, mannitol and catalase offers partial protection suggesting that ROS may be involved in the cisplatin-induced effect on renal cortical slices. In conclusion, the study shows that cisplatin causes reduction in the protein content in renal cortical slices which may be mediated through reactive oxygen species.

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