CHAPTER - 4

EFFECT OF CARBOPLATIN ON RENAL METABOLIC ACTIVITIES IN RAT

Platinum compounds are in case world wide as antineoplastic agents. The antineoplastic activity of Platinum (Pt) compounds appears to be related to the chemical nature of the species which are bound to the central Platinum atom and relative position on ligands to one another. Active Platinum compounds have two sites at which they can interact with intracellular targets. Cisplatin (CDDP) is the most potent antitumour agent amongst all the Platinum complexes. Other compounds of this class are Carboplatin (CBDCA), Iproplatin, Tetraplatin etc. Cisplatin, though widely used has chronic side effects, especially hyperglycemia and renal toxicity. Hence hindrances are met with prolonged usage in treatment. Other side effects observed are nausea, vomitting, hypocellularity, haemorrhagic enterocolitis etc. Studies by various investigators (Foster et al., 1985; Reece et al., 1987) suggest that CBDCA is a less toxic agent which could replace Cisplatin.

Ozols and colleagues (1987) reported that patients with ovarian cancer can safely receive 800 mg of Carboplatin in two doses. Peak levels of DNA binding by Carboplatin was 6-12 hours later than that by Cisplatin. Carboplatin is 45 times less toxic than Cisplatin (Micetich et al., 1985). When plasmid DNA was treated with drug, a 100 fold larger dose of CBDCA was needed to produce levels of DNA binding equivalent to that produced by Cisplatin (Roberts et al., 1982). Ca^{+}-ATPase and Na^{+}-K^{+}-ATPase were adversely affected following CDDP treatment (Batzer and Agarwal, 1986). They studied comparative effects of various Platinum compounds in two different strains of rats. The study addressed two main issues. First and

foremost was to test nephrotoxicity. Particularly this was appropriate because one of the most severe side effects on CDDP chemotherapy was nephrotoxicity (Goldstein et al., 1981; Schueppl et al., 1972). Second was to analyse the physiologic response to drug compromise, since CBDCA was thought to have less toxicity and low metabolic derangements.

Studies were conducted on rats by Batzer and Agarwal (1986) using CBDCA and CDDP on various phosphatases. Acid and Alkaline phosphatases showed higher levels with CDDP at 5 mg and 9 mg/kg body weight, while CBDCA administration of 50 mg/kg body weight did not evoke such high response. Urine volumes measured after CDDP treatment showed higher levels, where after CBDCA injections urine volumes were found reduced than that of the CDDP injected animals. Since CBDCA is reported to be comparatively free of neural and nephrotoxic derangements, it may not cause inhibition of autonomic system as is met with CDDP treated animals (Chapters 2,3). Hence, this study was initiated to fathom the involvement of CBDCA in the renal metabolic activities of rat.

Methods and Materials

Adult male rats of Charles foster strain weighing about 250-300 grams were used for the study. They were acclimatized to laboratory conditions atleast three weeks prior to experimentation. They were divided into two groups of six each. One group received injections (ip) of Carboplatin (CBDCA) at a dose of 50 mg/kg body weight in sucrose (0.5%). The second group treated with saline alone served the controls. Food and water was

provided ad libitum. All the rats were sacrificed under mild anaesthetia by exsanguination. Kidneys from both sides were removed and weighed. Known portion of the tissue was transferred to alcoholic KOH for estimating glycogen content. Enzymes such as glycogen synthetase, glucose-6-phosphatase, phosphorylase, aldolase, LDH, SDH, pyruvate carboxylase, transaminases and various phosphatases (AcPase, AlkPase, Na^+-K^+-ATPase) were also analysed along with protein content (see Chapter 1 for methods).

Statistical Analysis :

All the data are expressed as mean ± SEM. Difference between the means were analysed statistically by students 't' - test. The 0.05 level of probability was used as the criterion of significance.

Results

Carboplatin administration caused a considerable decrease in the glycogen content of the kidney and the reduction was as much as 71%. Phosphorylase activity showed only a non-significant reduction in the activity when compared to that of controls. G-6-Pase however showed a 73% reduction in the activity after the drug administration. Glycogen synthetase on the other hand showed an increase in the kidney. Tremendous increase in aldolase and LDH activities were observed, whereas, SDH and pyruvate carboxylase exhibited a decrease. Of the two transaminases GOT showed an increase but GPT showed no significant change from that of control. All phosphatases studied (Na^+-K^+-ATPase, AcPase, AlkPase) showed a slight but significant increase after CBDCA treatment.

TABLE 1 : EFFECT OF CARBOPLATIN ON RAT KIDNEY METABOLISM

GLYCOGEN	0.015± 0.0008	0.0043±**** 0.0007
GLYCOGEN-SYNTHETASE	0.264± 0.009	0.856±**** 0.035
G-6-PASE	0.058± 0.007	0.0152±**** 0.012
PHOSPHORYLASE	115.60± 2.873	109.704±** 1.69
ALDOLASE	0.0005± 0.00004	0.0020±**** 0.0002
LDH	15.423± 0.5427	32.1079± *** 2.021
SDH	52.624± 1.410	37.3493±**** 1.580
PYRUVATE CARBOXYLASE	0.3639± 0.0019	0.2390±**** 0.0016
GPT	25.413± 0.698	24.485±** 0.465
GOT	13.516± 1.44	24.61±**** 1.05
Na^+-K^+-ATPase	3.099± 0.623	8.950±**** 0.302
ALKALINE PHOSPHATASE	2.224± 0.050	2.475±* 0.067
ACID PHOSPHATASE	2.217± 0.050	2.448±** 0.091
PROTEIN	12.361± 0.175	12.473±** 0.271

$P \leqslant 0.02^{*}$, $P \leqslant 0.05^{**}$, $P \leqslant 0.01^{***}$, $P \leqslant 0.001^{****}$

TABLE 2 : EFFECT OF CARBOPLATIN ON RAT KIDNEY METABOLISM - IN TERMS OF PERCENTAGE CHANGES

GLYCOGEN	71.3	↓
GLYCOGEN-SYNTHETASE	224.2	↑
G-6-PASE	73.7	↓
PHOSPHORYLASE	5.1	↓
ALDOLASE	300	↑
LDH	108	↑
SDH	29.02	↓
PYRUVATE CARBOXYLASE	34.3	↓
GPT	3.6	↓
GOT	82	↑
Na^+-K^+-ATPase	8.8	↑
ALKALINE PHOSPHATASE	11.2	↑
ACID PHOSPHATASE	10.4	↑
PROTEIN	0.9	↑

(% is corrected to nearest whole number, expressed as increase [↑] decrease [↓] in value of the group in parenthesis compared to its adjoining group)

$P \leq 0.02^{*}$, $P \leq 0.05^{**}$, $P \leq 0.01^{***}$, $P \leq 0.001^{****}$.

EXPLANATION TO FIGURES

Effect of Carboplatin treatment with respect to:

Fig (1) : Glycogen content and activities of glycogen synthetase, glucose-6-phosphatase, phosphorylase in the kidney.

Fig (2) : Activities of aldolase, LDH, SDH, pyruvate carboxylase in the kidney.

Fig (3) : Protein content and activities of GPT, GOT, Na^+-K^+-ATPase, acid and alkaline phosphatases in the kidney.

Con

CBDCA

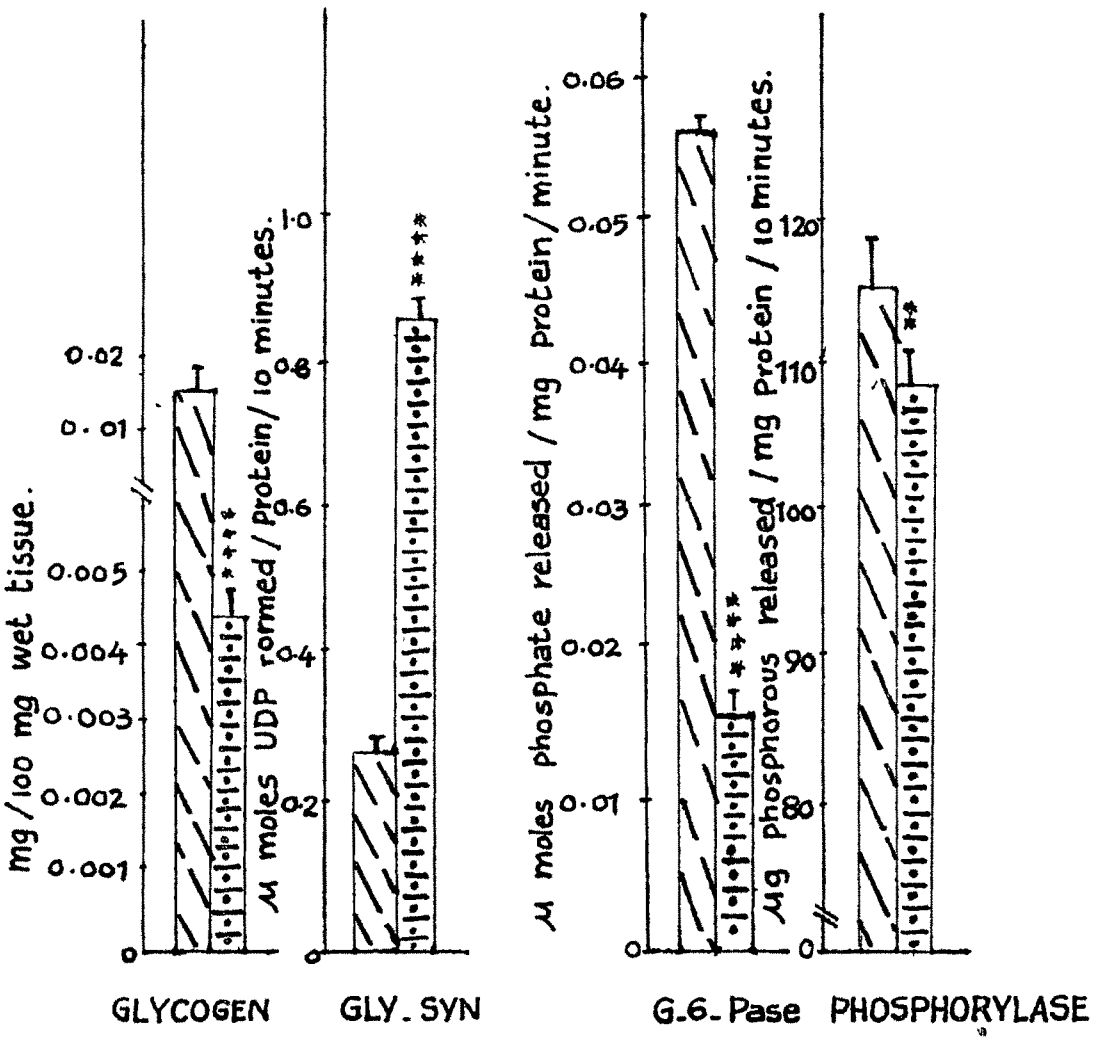
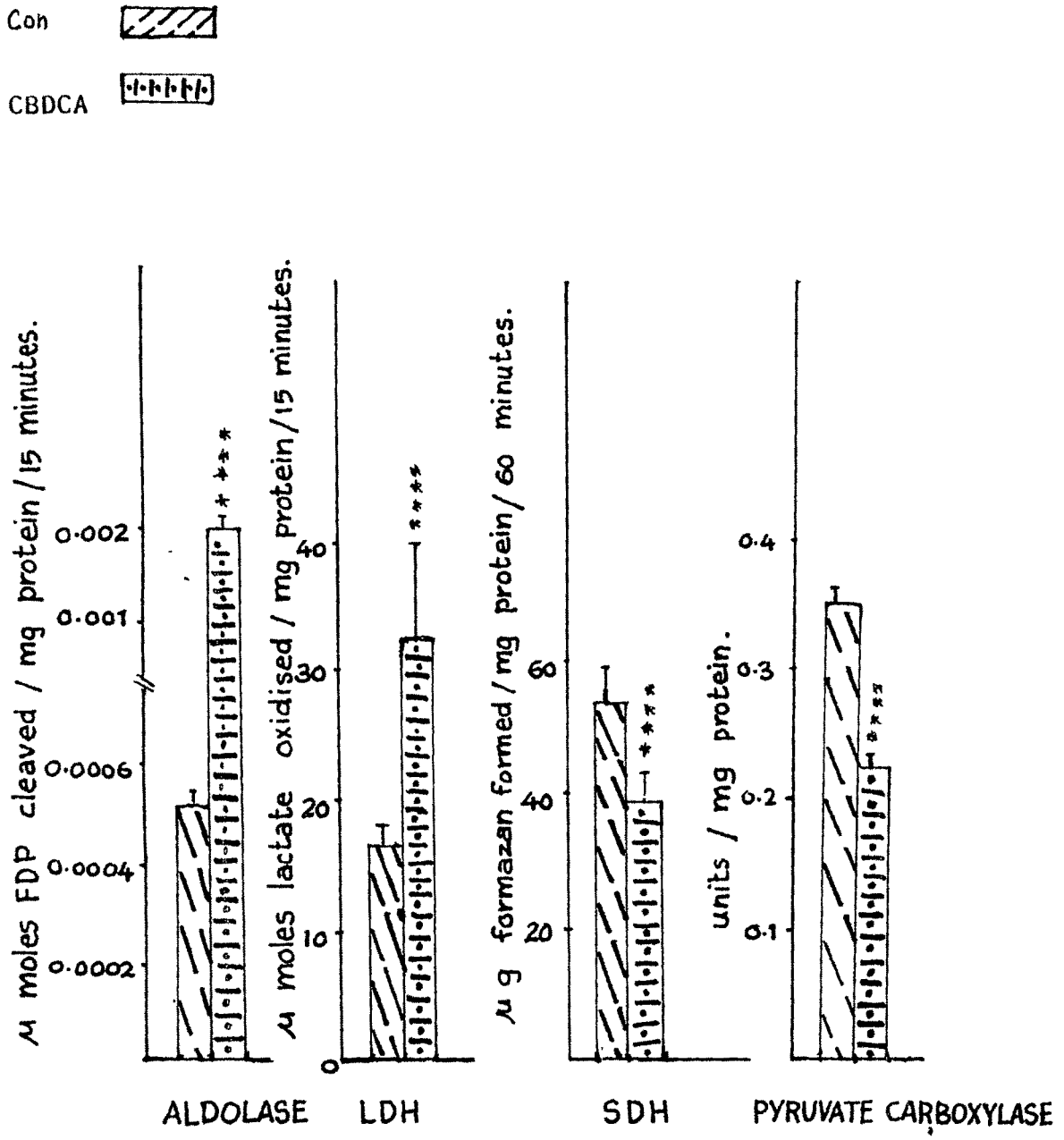


Fig : 1 Results given as mean ± SEM of six experiments.
$P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$



Fig·2 Results given as mean ± SEM of six experiments.
$P < 0.02^{*}$, $P < 0.05^{**}$, $P < 0.01^{***}$, $P < 0.001^{****}$

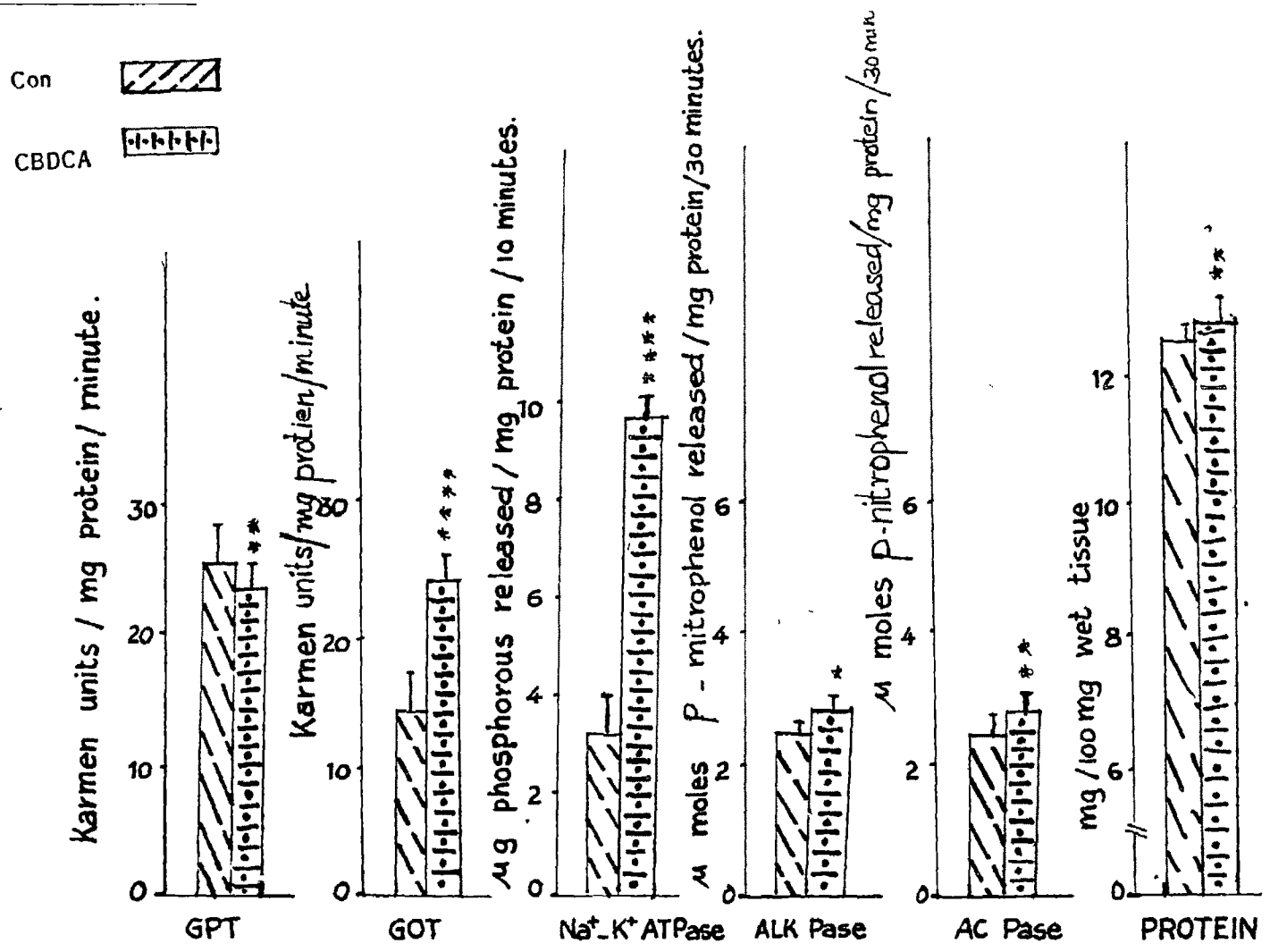


Fig: 2 Results given as mean ± SEM of six experiments.
$P < 0.02^{*}$, $P < 0.05^{**}$, $P < 0.01^{***}$, $P < 0.001^{****}$

Discussion

The biological actions of Platinum (Pt) complexes are such that they become stably bound to DNA, RNA or proteins. Carboplatin and other cytotoxic analogues form lesions at the DNA sites (Micetich et al., 1985). A comparative study made by Batzer and Aggarwal (1986) on the toxic effect of various Platinum drugs revealed that CBDCA was less toxic when compared to other Platinum cased drugs. Carboplatin administration has been shown to produce hyperglycemia (Oomen, 1992). The cause of hyperglycemia could be mainly due to two reasons: (1) decreased uptake of glucose by tissues and organs and (2) increased release of glucose by tissues and organs. Normally the increased uptake of glucose by liver, kidney, muscle and other tissues will lead to increased deposition of glycogen. On the other hand increased glucose release is the result of increased glycogenolysis, as well as gluconeogenesis.

In the kidney of carboplatin treated rat there was a decrease in glycogen content. There was no accompanying increase in phosphorylase and G-6-Pase. It could be reasoned that reduced glycogen content in the kidney was not due to increased glycogenolysis. In fact glycogen synthetase activity was found to increase in the kidney. Aldolase was found to be more active than that of control. As LDH level also remained high, it could be assumed that lactate production was very high in CBDCA treated rat kidney. Since aldolase and LDH were high, glycolytic pathway might have remained pronounced for glucose release. Decreased pyruvate carboxylase, SDH and GPT indicated decreased gluconeogenic activity. However, GOT levels remained high.

Appreciable enhancement in the activities were met with the various phosphatases. Na^+-K^+-ATPase, involved in transport of ions and flow-coupled transport of metabolites such as glucose was also found to increase in the CBDCA treatment. Probably the glucose uptake in rats was not sufficient to decrease the blood sugar level or to deposit more glycogen in the kidney. Amongst the non-specific phosphatases acid and alkaline phosphatase showed higher levels. Batzer and Aggarwal (1986) (in vivo studies) viewed the reaction densities through light microscopy and further confirmed the higher levels by measuring urinary alkaline and acid pase after CBDCA treatment in rats.

On the whole, carboplatin produced only an increase in the glycolytic pathway of kidney and probably raised the lactate production. The hyperglycemia found in CBDCA treated rat could be due to the failure of insulin or acetylecholine mediated uptake of glucose. In conclusion it could be reasoned that in the CBDCA treatment, the metabolic derangements will be far too less since neurotoxicity and nephrotoxicity is minimized.