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Evaluation of Biochemical and Pathological Parameters at Different Doses of Cisplatin in Experimental Animal Model: Toxicological Study on an Antineoplastic Drug

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Abstract

Background: This study aimed to assess the effect of cisplatin-induced toxicities on biochemical and pathological parameters such as body, liver, and kidney weights, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), and blood cells (RBCs and WBCs) in white Swiss albino mice.

Methods: Cisplatin's potential toxic effects on body, liver, and kidney weights were evaluated using standard laboratory methods. Blood biochemical levels such as BUN, creatinine, and ALT levels were determined by an auto-hemolyzer using commercial diagnostic kits. Blood cells (RBCs and WBCs) were counted under a microscope by a hemocytometer.

Results: Cisplatin's potential toxic effects on body, liver, and kidney weights were evaluated using standard laboratory methods. Blood biochemical levels such as BUN, creatinine, and ALT levels were determined by an auto-hemolyzer using commercial diagnostic kits. Blood cells (RBCs and WBCs) were counted under a microscope by a hemocytometer.

Conclusions: This study suggested to increase caution when using cisplatin, particularly at high doses. Further investigation shall be performed to minimize its toxic effect and optimize its use.

Keywords: alanine aminotransferase, biochemical parameters, blood urea nitrogen, cisplatin, mice

INTRODUCTION

Cisplatin is an efficacious antineoplastic agent that is widely and commonly used against several types of cancer, such as ovarian, lung, bladder, and testicular. This drug is used as a single dose or in combination with other antineoplastic drugs.[1] Its significant antitumor activity is often hampered by the development of various toxic effects. Neurotoxicity, ototoxicity, nephrotoxicity, nausea, and vomiting are the most common toxic effects of this drug and thus became the major limiting factors for its use.

The most important toxic effect of cisplatin is dose-dependent nephrotoxicity and hepatotoxicity.[2] In general, its efficacy is reduced due to these undesirable side effects. This drug may induce acute nephrotoxicity by initiating apoptosis and DNA damage and accelerating inflammation on the proximal and distal convoluted tubules of the kidney.[3] In this organ, cisplatin may gather in the proximal convoluted tubule and activate various signaling pathways responsible for tubular cellular death.[4] Cisplatin (single dose) administration exhibits toxicity in nephrons in a considerable number of patients (around 32% to 38%).[5] At post cisplatin administration, higher platinum concentration can be found in renal tissues than in other organs.[3] According to a molecular level study, significant expression of copper transporter receptor 1 and inorganic cation receptor 2 can accelerate the damage of proximal tubules in S3 segment, resulting in cisplatin transport to the kidney tubule and remarkable platinum accumulation in renal tissues. Nephrotoxicity is the prompt loss of kidney function and is elucidated by the high serum concentrations of creatinine and blood urea nitrogen (BUN) that result in kidney failure.[3]

Although cisplatin-associated nephrotoxicity is common in clinical and animal studies, hepatotoxicity is another major dose-dependent adverse effect of cisplatin-based chemotherapy[6] and therefore must be considered. One of the most important causative factors for cisplatin-

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induce hepatotoxicity is oxidative stress. Metallothionein is one of the most important protective protein for the treatment of cisplatin-induced liver injury.[7] Vitamin E and selenium reduce the cisplatin-induced oxidative damage to the liver.[8, 9] Heme oxygenase and catalase exhibit significant defensive activities against cisplatin-induced liver toxicity.[10] Elevated GSH and GST levels are an important marker for CDDP-induced hepatotoxicity.[11] Many studies reported that patients treated with platinum combinations such as cisplatin have a great possibility of developing severe anemia.[12, 13]

Cisplatin or chemotherapy combinations containing this agent are also responsible for severe myelosuppression. This drug induces leukocytopenia, thrombocytopenia, and severe anemia, an unusual term to use for patients treated with combination therapy containing cytotoxic agents that are not only cisplatin. For hemolysis and erythropoiesis, anemia is the secondary consideration. However, thorough investigation has not been conducted to determine the mechanisms. A previous work evaluated the cisplatin effects on hemopoiesis, especially on hemopoietic stem cells.[14]

No previous single study employed different cisplatin doses and evaluated all these parameters at a specific time. Therefore, we designed the current research to examine the effect of different specific doses of cisplatin on these parameters in an experimental animal model.

MATERIALS AND METHODS

Materials

Cisplatin injection was purchased from Beacon Pharmaceutical Ltd., Dhaka, Bangladesh. Urea, creatinine, and ALT was provided by Randox Laboratories Ltd., UK. Ketamine HCl (10 ml), xylazine HCl (30 ml), 10% formalin, and distilled water were obtained from Gonoshasthaya Pharmaceuticals Ltd., Dhaka, Bangladesh. Reagents and other chemicals were of analytical grade, and all dilutions were prepared using high-purity deionized water.

Animal model and housing

Healthy female white Swiss albino mice (6–8 weeks of 18–22 g) were purchased from the Department of Pharmacy, Jahangirnagar University, Savar-1342, Bangladesh and were kept in plastic (polypropylene plastic) cages of 30 × 20 × 13 cm dimension. Soft wood shavings were used as bedding in the cages. Animal feeding was performed with standard laboratory pellet diet and water at libitum, and the animals were exposed to alternate cycle of 12 h dark and light at temperature 25 ± 2 °C and relative humidity 55% ± 10%. All the experimental animals were allowed to adjust to the laboratory environment for 7 days before the experiment. Ethical approval was obtained from the institutional animal ethical committee.

Experimental design

Forty-eight healthy mice were randomly selected and divided into four groups of five (n = 5): Group 1, control group; Group 2, mice treated with cisplatin at 10 mg/kg body weight; Group 3, mice treated with cisplatin at 20 mg/kg body weight; and Group 4, mice treated with cisplatin at 40 mg/kg body weight.

Cisplatin was administered intraperitoneally (i.p.) at a single dose to the respected groups, which were then sacrificed 6 h, 72 h, and 7 days later. Mouse initial and final body weights were documented prior to the treatments and before sacrifice. Body organs (kidney and liver) and blood samples were collected to analyze the toxic effect of cisplatin. The protocol was approved by the institutional research committee and was adopted in this study with minor modification.

Blood biochemical analysis

The blood samples were centrifuged for 15 min at 3000 rpm (Digisystem Laboratory instruments Inc., China, DSC-200T). After separation, the serum was collected carefully and stored in the refrigerator at −80 °C for analysis. All biochemical parameters were assessed within 24 h of sample collection using respective analysis kits, e.g., Urea kit (CAT# CS 611, Crescent Diagnostics, Saudi Arabia), Creatinine kit (CAT # CR510, Randox Laboratories, UK), and ALT kit (CAT# CS 601/602, Randox Laboratories Ltd., UK) in accordance with the manufacturer's instructions. BUN, creatinine, and ALT levels were measured by a semi-automatic biochemistry analyzer using commercial diagnostic kits (CHEM-7, Erba Diagnostics Mannheim, Germany).[15] Blood cells (RBCs and WBCs) were counted under a microscope by a hemocytometer.[16]

Pathological examination

After the experimental animals were sacrificed, the organs of interest were separated and preserved in normal saline for 24 h. After being collected and dried, each organ was weighed, and the average weights were compared for statistical evaluation.

Statistical analysis

All data were expressed as mean ± standard error mean (SEM). Paired t-test was performed to evaluate statistically significant differences between experimental and control groups (SPSS software, version 20.0). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ were considered as statistically significant.

Ethical approval

This study was approved by the ethical committee of Noakhali Science and Technology University, Noakhali, Bangladesh (Application no: 91).

RESULTS

Clinical observation

The growth function and body weight of the experimental animal were monitored. After cisplatin administration, the body weight of the mice depleted remarkably ($p < 0.0001$) compared with that in the control group as shown in Table 1.

Organ weight

The average kidney and liver weights of the mice decreased in the cisplatin-treated groups ($p < 0.05$) than in the control group. For different doses (10, 20, and 40 mg/kg), the average liver and kidney weights were reduced. Statistically significant decrease in the average liver and kidney weights was observed in the high cisplatin dose (40 mg/kg) group compared with that in the other group. All these values are listed in Table 2.

Biochemical examination

Tables 3, 4, 5, and 6 show the different biochemical parameters tested in this study. A statistically significant increase in BUN, creatinine, and ALT levels was found in the mice treated with cisplatin. The mice treated with 40 mg/kg cisplatin dose showed statistically significant ($p < 0.05$) increase in BUN, creatinine, and ALT levels compared with the other groups including the control group. Meanwhile, RBC and WBC counts decreased in all the experimental groups, but this change was significant in group 4 ($p < 0.01$).

TABLE 1. Changes in the body weight of the mice after 6 h, 72 h, and 1 week

	Body Weight (g)			
Treatment	6 h	72 h	1 week	% of Body Weight Changes
Group 1 (Control)	22.00 ± 0.58	22.89 ± 0.11	23.83 ± 0.44	13.47%
Group 2 (10 mg/kg body)	22.06 ± 1.06	18.00 ± 0.10	19.00 ± 0.19	-13.87%
Group 3 (20 mg/kg body)	22.03 ± 0.22	15.00 ± 0.29	17.50 ± 0.58	-20.56%
Group 4 (40 mg/kg body)	20.00 ± 0.58	12.00 ± 0.30	16.00 ± 0.59	-20%

Values are represented as mean ± standard error mean (n = 5)

TABLE 2. Effect of cisplatin on the average kidney weight of mice

Treatment	Kidney weight (g)		
	6 h	72 h	1 week
Group 1 (Control)	0.35 ± 0.01	0.45 ± 0.04	0.48 ± 0.02
Group 2 (10 mg/kg body)	0.39 ± 0.03	0.21 ± 0.002**	0.22 ± 0.001*
Group 3 (20 mg/kg body)	0.34 ± 0.05	0.21 ± 0.002**	0.21 ± 0.001*
Group 4 (40 mg/kg body)	0.32 ± 0.021	0.11 ± 0.002**	0.16 ± 0.030**

Values are represented as mean ± standard error mean (n = 5); *$p < 0.05$, **$p < 0.01$ versus control

TABLE 3. Effect of cisplatin on the average liver weight of mice

Treatment	Liver weight (g)		
	6 h	72 h	1 week
Group 1 (Control)	1.294 ± 0.104	1.39 ± 0.058	1.464 ± 0.049
Group 2 (10 mg/kg body)	1.552 ± 0.102	0.612 ± 0.112**	1.188 ± 0.120*
Group 3 (20 mg/kg body)	1.438 ± 0.102	1.184 ± 0.025**	1.249 ± 0.017**
Group 4 (40 mg/kg body)	1.248 ± 0.031	0.271 ± 0.132**	0.862 ± 0.218*

Values are represented as mean ± standard error mean (n = 5); *$p < 0.05$, **$p < 0.01$ versus control

TABLE 4. Effect of cisplatin on the average liver weight of mice

Treatment	BUN (mg/dL)		
	6 h	72 h	1 week
Group 1 (Control)	2.317 ± 0.607	24.02 ± 0.489	24.433 ± 0.361
Group 2 (10 mg/kg body)	18.403 ± 0.226***	74.613 ± 0.576***	43.393 ± 0.636***
Group 3 (20 mg/kg body)	25.367 ± 0.610**	129.447 ± 0.448***	60.603 ± 0.473***
Group 4 (40 mg/kg body)	31.04 ± 0.774***	180.91 ± 0.692***	97.583 ± 0.468***

Values are represented as mean ± SEM (n = 5); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control.

TABLE 5. Effect of cisplatin on creatinine

Treatment	Creatinine (mg/dL)		
	6 h	72 h	1 week
Group 1 (Control)	0.5 ± 0.058	0.307 ± 0.012	0.567 ± 0.033
Group 2 (10 mg/kg body)	0.373 ± 0.037	1.5 ± 0.058***	1.4 ± 0.251**
Group 3 (20 mg/kg body)	0.667 ± 0.033	4.067 ± 0.088***	1.4 ± 0.058***
Group 4 (40 mg/kg body)	0.767 ± 0.033**	5.397 ± 0.232***	2.59 ± 0.006***

Values are represented as mean ± standard error mean (n = 5); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control.

DISCUSSION

In this study, cisplatin toxicity was examined at specific doses and time intervals. The most common toxic effect of cisplatin is acute renal failure (ARF), and its high prevalence is increasing rapidly in developed and developing countries and is responsible for severe mortality and morbidity.[3, 17] Around 20% of cisplatin-induced ARF cases occur in hospitalized patients, and a third of these patients may experience renal injury within 10 days.[3] The cisplatin doses used for this experiment were 10,[18] 20,[4] and 40 mg/kg,[11] which were reported to alter renal and hepatic functions. During the whole treatment, no death and abnormal clinical signs were observed in the experimental mice.

Proper growth in animals or humans can be assessed by monitoring their body weight. Some researchers considered body weight loss as a reliable sensitive indicator for toxicity.[19-21] In our study, a reduction in body weight was observed in the treated mice compared with that in the control group. The cytotoxic effect of cisplatin may hamper the eating behavior, and the renal tubular injury may affect water reabsorption and cause dehydration and inflammation; both of which are responsible for the significant body weight loss.[22] Similar results were previously reported.[18] Therefore, this finding indicated that cisplatin potentially alters growth. In addition to its role in growth, the effect of cisplatin on different organ weights was studied. Cisplatin decrease the weight of vital mouse organs such as the liver and kidney.[23]

Cisplatin-induced nephrotoxicity and hepatotoxicity remain poorly understood. For the determination of kidney function, serum creatinine and BUN levels were examined. High levels of these parameters were found in the cisplatin-treated groups, especially in group 4 after 7 days of experiment. Elevated serum creatinine and BUN are indicators of renal dysfunction.[24, 25] The kidney is affected by cisplatin through the necrosis of tube, the dislocation of tubular cells from the membrane of basement, and the presence of proteinaceous casts in the lumen of tube.[26] This drug affects the proximal and distal convoluted tubules of kidney by inducing apoptosis, DNA damage, and oxidative stress that increases reactive oxygen species and inflammation.[27] These results indicated that cisplatin is responsible for renal dysfunction.

For hepatic activity study, the mice treated with cisplatin showed depleted liver weight and elevated serum ALT, the markers of liver damage.[11] In this experiment, three doses were applied to perform the same function but in different rates. The results indicated that cisplatin suppressed liver function by the increasing serum ALT in the mice, particularly in group 4 after 7 days of observation. Comparison between groups revealed that cisplatin caused alterations in liver parenchyma such as hepatic lobule disorientation, hepatocyte swelling, inflammatory cell infiltration, bile duct proliferation, and necrotic hepatocytes.[23]

These data are similar to previous study. However, our research adopted a minor modification in methods. In a previous study, high blood urea and creatinine concentrations were observed in the cisplatin-treated group because of the marked depletion of creatinine clearance compared with that in the control group.[3]

Moreover, cisplatin has adverse effects on hematological parameters such as RBCs and WBCs. Approximately 9%–40% of patients experience cisplatin-related anemia, a well-known side effect. One possible explanation for this symptom is the failure to produce erythropoietin due to ARF.[28-31] Cisplatin chemotherapy can also reduce the number of infection-fighting WBCs in the body because the oxidative stress caused by cisplatin in lymphocytes may hamper their life expectancy and initiate apoptosis. As a result, the number of these cells may decrease, the immune system may weaken, and the risk for certain types of infection may increase.[32]

On the basis of the above consideration, cisplatin should be used carefully because of its dose-dependent toxicity. The results of this study are important because of the dose variation outcome of study parameters. Despite its importance, this work has some limitations. Interventions on the study parameters by nutritional supplements or other medications that are used along with cisplatin were not considered. Future research may focus on determining whether nutritional supplement or other medication changes the study parameters.

TABLE 6. Effect of cisplatin on SGPT, RBC, and WBC

Treatment	SGPT (U/L)			RBC (millions/cmm)			WBC (T/cmm)		
	6 h	72 h	1 week	6 h	72 h	1 week	6 h	72 h	1 week
Group 1 (Control)	18.230 ± 0.302	21.760 ± 0.291	24.910 ± 0.447	8.247 ± 0.009	8.207 ± 0.035	8.203 ± 0.008	7.267± 0.088	8.300 ± 0.058	8.567 ± 0.033
Group 2 (10 mg/kg body)	22.370 ± 0.451**	123.120 ± 0.400***	83.087 ± 0.216***	8.450 ± 0.281	6.300 ± 0.058***	7.647 ± 0.037***	8.200 ± 0.058***	6.600 ± 0.058***	7.597 ± 0.032***
Group 3 (20 mg/kg body)	30.417 ± 0.485	193.537 ± 0.462***	95.287 ± 0.294***	7.577 ± 0.038***	5.200 ± 0.058***	6.290 ± 0.021***	7.567 ± 0.240	5.167 ± 0.176***	6.340 ± 0.133***
Group 4 (40 mg/kg body)	25.327 ± 0.548**	271.413 ± 0.560***	167.793±1.226***	7.843 ± 0.075**	3.367 ± 0.088***	5.627 ± 0.058***	8.283 ± 0.059***	3.300 ± 0.208***	5.567 ± 0.046***

Values are represented as mean ± standard error mean (n = 5); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control.

CONCLUSIONS

Cisplatin chemotherapy causes many problems in the physiological and biochemical parameters on mice particularly at high doses. Long-term observation also supported this result. Although cisplatin is a well-established chemotherapy drug and is extremely effective, it should be used carefully and cautiously for cancer treatment.

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CONFLICT OF INTEREST

None.

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ABBREVIATIONS

BUN: Blood urea nitrogen; RBC: Red blood cell; WBC: White blood cell; ALT: Alanine aminotransferase.

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