

Toxic Effects of Cisplatin on Hepatocytes and Liver Enzymes of Rats

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Article info:

Received: 15 Mar. 2015

Accepted: 21 Sep. 2015

Key Words:

Cisplatin, Liver, Enzymes, Sinusoidal diameter, Rat

ABSTRACT

Introduction: Cisplatin is an antineoplastic drug, which is widely used for the treatment of solid tumors. However, its clinical usage is limited because of its side effects such as hepatotoxicity. This study aimed to identify toxic effects of cisplatin on hepatocytes of rats.

Methods: A total of 45 adult Sprague Dawley rats weighing 200±30 g were randomly divided into experimental (n=30) and control (n=15) groups. Rats of experimental groups were divided into 2 subgroups; subgroup 1 received 2 consecutive 2.5mg/kg dose of cisplatin, intraperitoneally in the beginning of first and fifth weeks of the study. Subgroup 2 also received 2 consecutive 5mg/kg dose of cisplatin in the same manner of subgroup 1. After 8 weeks, rats of both groups were anesthetized and killed. Then, their blood and tissue samples were taken. Prepared sections were stained by HE method. Collected data from microscopic slides and blood samples were analyzed by SPSS using analysis of variance (ANOVA) and Tukey test.

Results: Statistical analysis showed significant differences in the activity of enzymes (ALT, AST, ALP) between control and experimental groups (P<0.001). Analysis of sinusoidal diameter also showed a significant difference between studied groups (P<0.001) too.

Conclusion: Cisplatin disorganizes the architecture of hepatic lobules and increases sinusoidal diameter in rat liver.

1. Introduction

Platinum-based drugs have revolutionized treatment of solid tumors of organs such as ovarian, head and neck, lung, brain, and testis [1]. This compound stops mitosis

and induces apoptosis by oxidative stress and crosslinking with DNA [2,3]. The main mechanism of antitumor activity of platinum-based agents is interference with purine base in DNA [4]. Cisplatin (Cis-diamine dichloroplatinum II) is one of the platinum-based drugs, which is commonly used clinically (1). Cisplatin is thought to kill

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cancer cells primarily by forming DNA adducts, causing G2 cell cycle arrest, and finally triggering apoptosis [5].

However, because of the various side effects of the cisplatin, its use is restricted to the treatment of patients with cancer. Some of the most important side effects of cisplatin are nephrotoxicity, hepatotoxicity, ototoxicity, neurotoxicity, and cardiotoxicity. As cisplatin is metabolized mainly through kidney and liver, hepatotoxicity and nephrotoxicity are associated with cisplatin treatment [5,6]. Hepatotoxic side effects of cisplatin is less known than other organs, because treatment protocol with higher dose or repeated low dose could cause hepatotoxicity and alter the clinical situation of patients [5,7,8].

Little information is known about the underlying mechanism of hepatotoxicity induced by cisplatin, although reportedly cisplatin may interfere with the tissue antioxidant defense system and generates highly reactive oxygen species (ROS). Thus, cisplatin can cause oxidative damage to the liver [5,9,10]. In addition to functional and structural mitochondrial injury, apoptosis, perturbation Ca^{2+} homeostasis, involvement of pro-inflammatory genes such as COX-2, and inducible nitric oxide synthase (iNOS) may play some important role in the mechanism of cisplatin hepatotoxicity [5]. ROS are highly reactive molecules, which are mainly composed of superoxide radical (O_2^-), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2). ROS could damage biological molecules such as lipid, protein, DNA, and eventually impair cell integrity [9,11]. Cisplatin could also increase lipid peroxidation, and presumably this is the main mechanism for cisplatin hepatotoxicity [11,12].

There are other molecules, which play antioxidant role in hepatocytes [9]. Superoxide dismutase (SOD) is an antioxidant enzyme that is electron donor and reacts with the free radicals to form harmless products such as water [9,11]. Catalase is the other enzymatic antioxidant agent responsible for the protection of cell damage induced by ROS via converting H_2O_2 into water and oxygen [9]. Reduced activities of enzymatic antioxidant lead to decreased activities against ROS, finally damage cell [5,11]. Several studies have shown that cisplatin injection alters weight, histology, and vascular structure of the liver [6,11,13]. Clinical evidence of cisplatin-induced liver damage has been demonstrated by increasing activity of serum enzyme, bilirubin rise, and development of jaundice [5,6]. The aim of the present study was to evaluate changes induced by intraperitoneal injection of 2 consecutive doses of cisplatin (2.5 and 5 mg/kg) on rat hepatocytes as an experimental model.

2. Materials and Methods

A total of 45 adult male Sprague-Dawley rats weighing 200 ± 30 g obtained from Laboratory Animal Research Center of Zahedan University of Medical Sciences. Then, they were randomly divided into 2 groups; control ($n=15$) and experimental ($n=30$). They were kept under identical conditions (12:12 h light/dark, $22^\circ\text{C} \pm 2^\circ\text{C}$ temperature and 45%-50% humidity) and received normal amount of water and food. In the next step, rats of the experimental group were divided into 2 subgroups. Subgroup 1 received 2 consecutive 2.5 mg/kg dose of cisplatin intraperitoneally (purchased from MYLAN Co, U.S.A.) in the first and fifth weeks of the experiment. Subgroup 2 received 5mg/kg of cisplatin in the same manner of subgroup 1. Rats were coded in groups one per cage and weighed before and after the experiment.

At the end of the experiment (end of the eighth week), rats of both groups (control and experiment) were given ether anesthesia and killed. Blood samples were collected from their heart directly, and their livers were removed and weighed. Then, their blood samples were sent to the laboratory for measuring serum enzymes (ALT, AST, ALP). In addition, liver weight and liver to body weight ratio were also measured. Tissue samples were processed routinely and prepared 5- μm sections were stained with HE method. After calibration of the light microscope (Motic, Las Ez, version 2), histological sections were blindly studied to determine changes in sinusoidal diameter and photomicrography was taken. Collected data were analyzed using SPSS with ANOVA and Tukey test.

3. Results

Statistical analysis of blood samples showed significant increase in the average activity of serum enzymes (ALT, AST, ALP) in both experimental subgroups in comparison to the control group ($P < 0.001$) (Table 1). Analysis of sinusoidal diameter showed a significant increase in the diameter of sinusoid in experimental subgroups in comparison to the control group ($P < 0.001$). Analysis of data for liver weight to body weight ratio showed a significant difference between experimental and control groups ($P < 0.001$). Furthermore, statistical analysis of weight differences (final weight minus the beginning weight of rat) showed a significant difference between experimental groups in comparison to control group ($P < 0.001$) (Table 2). Evaluation of histological slides prepared from the subgroup 1 stained with HE showed sinusoidal dilation, congestion, and inflammation in liver. Some cells had pyknotic nucleus and their disorganized architecture

Table 1. Comparison of liver enzymes after IP injection of 2 consecutive doses of cisplatin in rats.

Groups	Variables	Subgroup 1	Subgroup 2	Control group	P-value
	ALT, U/l	45.93±9.7	55.2±13.65	61.6±11.15	<0.001
	AST, U/l	92±8.95	106.27±14.48	142.86±52.56	
	ALP, U/l	106.73±12.19	151.27±76.68	154.93±32.79	

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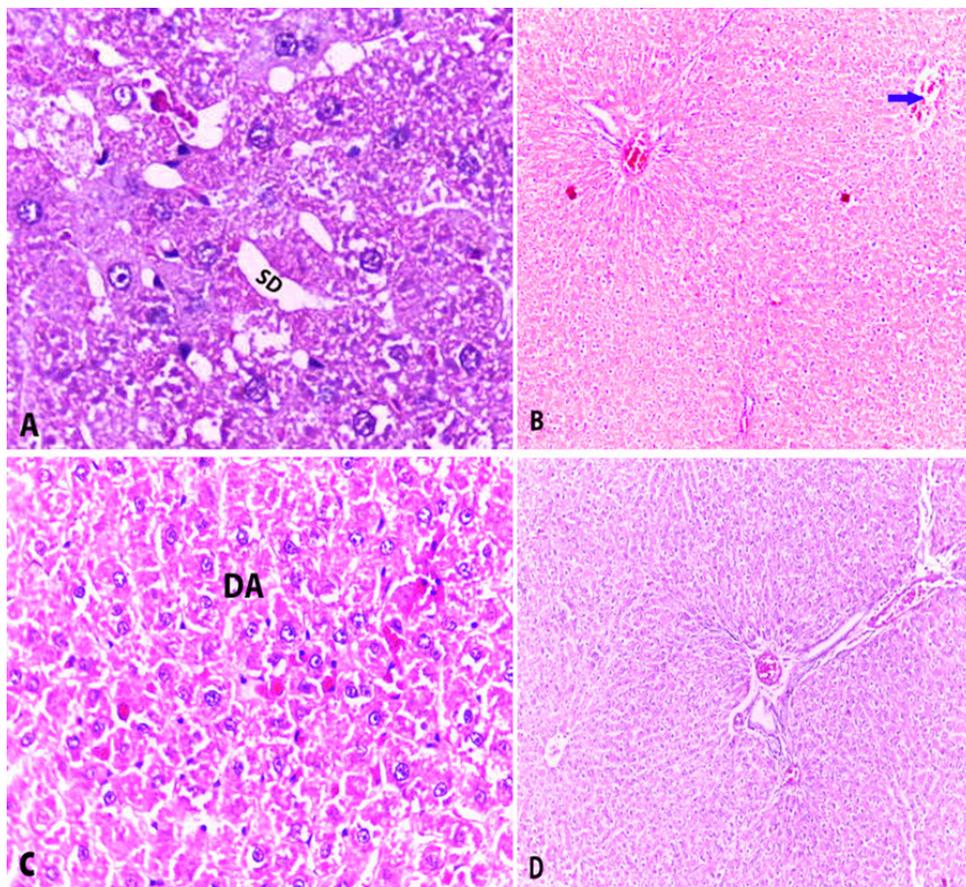
Table 2. Comparison of sinusoidal diameter, liver weight, and liver to body weight ratio after IP injection of 2 consecutive doses of cisplatin in rats.

Groups	Variables	Subgroup 1	Subgroup 2	Control group	P-value
	Sinusoidal diameter, μm	4.24±0.14	4.56±0.12	3.88±0.23	<0.001
	Liver weight, g	37.28±3.33	41.33±3.03	46.80±7.21	<0.001
	Liver/body weight ratio	41.33±3.03	37.28±3.33	46.80±7.21	<0.001

ANATOMICAL SCIENCES

was prominent. In evaluation of sections prepared from subgroup 2, sinusoidal dilation and congestion was more clear in comparison to subgroup 1 (Figure 1). In sinusoi-

dal wall, endothelial cells with typical appearance were not seen. The number of cells with pyknotic nucleus was



ANATOMICAL SCIENCES

Figure 1. A: Prominent sinusoidal dilation in experimental group 1 (SD). B: Congestion in experimental group 2 (thick arrow). C: Prominent disorganized architecture in experimental group 2, (DA). D: Normal architecture of hepatid lobule in control group.

elevated in subgroup 2 in comparison to subgroup 1 and the control group.

4. Discussion

Hepatotoxicity induced by cisplatin is recognized by the alteration of the biochemical, histological, and molecular parameters [6,11,13]. It has been well documented that cisplatin chemotherapy induces many clinical signs such as changes in hair follicle, bone marrow, gastrointestinal tract, testis, and ovary [10]. Also hepatotoxicity can be recognized by certain liver enzymes. Since the enzymes activities of liver are about 1000 times more than that of serum, if only %1 of hepatocytes necrotize, the enzymes activities of serum will be doubled. As ALT exists mainly in the liver cell cytoplasm and mitochondria, it is one of the most sensitive parameters for liver cell function test as recommended by WHO. AST is more in cardiac muscle than liver cells. AST has 2 isoenzymes, ASTs and ASTm, respectively. In the normal serum, AST exists mainly as ASTs, and when necrosis occurs, ASTm is released from liver mitochondria, and its level in the blood serum increases. ALT and AST are the key indexes measuring the level of liver cell injury [7].

The result of our study showed that activities of serum enzymes (ALT, AST, ALP) in both experimental subgroups increased in comparison with the control group. ALT, AST, and ALP enzymes levels increase in serum confirmed hepatocytes cell membrane damage and enzymes leak from the hepatocytes. Our results are in agreement with studies of Karadiniz et al. and Attyeh et al. [6,11]. They showed that increase in ALT, AST, ALP activities in the experimental group was related to liver cell damage and many other changes in the hepatic function.

Apoptosis is a gene-regulated event related to special morphological changes such as shrinkage of cell, chromatin condensation, and DNA damage. Many factors participate in apoptotic mechanism, that are believed to act through 2 main families of proteins, including cysteine protease called caspase enzyme (especially caspase 3, 8, 9) and Bcl-2 family. Caspase 3 is the most important member of caspase family, which is responsible for many biochemical manifestation of apoptosis that lead to cleavage of nuclear and cytosolic substrates, chromatin condensation, fragmentation of DNA, and apoptotic body [11].

Our result showed that cisplatin makes dose-dependent changes in liver structure: some of these changes impair hepatocytes arrangement, liver lobulation, and

necrosis. These findings are in agreement with study of the Ez Din et al. [5]. The hepatocytes are known to accumulate significant amounts of cisplatin, so hepatotoxicity is attributed to cisplatin storage in hepatocytes [13]. Our result showed an increase in sinusoidal diameter in both experimental subgroups, which confirm sinusoidal dilation. These results are in agreement with the study of Ashan et al. [12], which showed vascular dilation in liver.

This study also confirmed that addition of some antioxidant foods may have preventive effects on cisplatin-induced hepatotoxicity. Our finding showed that liver weight to body weight ratio had a significant difference. It seems that increasing metabolism and low intake of nutrient is the cause of this result. The difference in weight of animals after the experiment showed a significant difference between control and experimental group. Apparently, cisplatin induces many biochemical and histological changes in hepatocytes in a dose-dependent manner, which is the basis for increase in ALT, AST, ALP enzymes.

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