

Histopathology of Small Intestine Induced by Cisplatin in Male Wistar Rats

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Abstract

Cisplatin, *cis*-diamminedichloroplatinum (II) is a highly effective drug used to treat several cancers. Unfortunately, it has various clinical side effects affecting many tissues. However, small intestine toxicity induced by cisplatin has been rare reports to date. This study aims to investigate the pathology of small intestine upon cisplatin treatment at various doses using histological techniques. Male Wistar rats were divided into 5 groups: group 1 was intraperitoneally (IP) injected with normal saline; group 2, 3, 4 and 5 were IP injected with cisplatin at doses of 25, 50, 75 and 100 mg/kg body weight (BW), respectively. On day 3, rats were weighed and small intestines were collected. This study found that cisplatin significantly reduces BW at all concentrations compared to saline group. Histological analysis of small intestine induced by cisplatin illustrating various pathologies includes hemodynamic change (hemorrhage), reversible injuries (distortion of mucosal architecture, development of subepithelial space and lifting of epithelial layer from the lamina propria) and irreversible injuries (degenerative changes of villi, sloughing of necrotic villi into intestinal lumina and loss of villi). Additionally, cellular adaptations were also elicited including hyperplasia of lamina propria and columnar epithelium lining villi and atrophy of villi. In conclusion, cisplatin administrations lead to pathologies of small intestine, consequently causing weight loss whose severity depends on its concentrations.

Keywords: Pathology, toxicology, small intestine, cisplatin

Introduction

Cisplatin {*cis*-diamminedichloroplatinum (II)} is a water-soluble anti-cancer drug formed when a platinum atom is surrounded by chloride and ammonium atoms in the *cis* position of a horizontal plane [1]. The mechanism of cisplatin action is the interaction of the activated platinum complex with DNA to form both intrastrand and interstrand cross-links, resulting in replication and transcription inhibition, single- and double-stranded breaks and miscoding and induction of apoptosis [2]. Despite its effectiveness, cisplatin can cause deleterious effects including hepatotoxicity, nephrotoxicity and ototoxicity [3-5]. Toxicity to the small intestine has also been reported [6,7].

Normally the small intestine plays a key role in several functions including digestion and absorption of carbohydrates, protein and fat, absorption and secretion of water and electrolytes and absorption of vitamins and minerals. Moreover, it acts as a barrier and has an immune function known as gut-associated lymphoid tissue. Additionally, it is recognized as the largest hormone-producing organ in the body [8]. Cisplatin treatment causes various alterations in the small intestine including increased oxidative stress biomarkers, especially lipid peroxidation, changed activities of several enzymatic antioxidants, induced apoptosis of epithelial cells and impaired fluid and electrolyte absorption [9-11]. Although cisplatin has

been shown to have toxicity to the small intestine, only a limited number of characteristic patterns of cisplatin-induced pathology in the small intestine have been investigated. Histological analysis stained by hematoxylin and eosin (H&E) is a sensitive parameter which is crucial for evaluating the pathological changes generated by toxins in target organs. This study aims to determine the pathology of the small intestine upon cisplatin treatment at various doses using histological techniques.

Materials and methods

Chemicals

Cisplatin and 10 % neutral buffered formalin solution were purchased from Sigma-Aldrich Chemical Company (USA). All other chemicals were analytical grade.

Animals

Fifteen male Wistar rats (*Rattus norvegicus*) aged 5 weeks ranking 150 - 170 g were obtained from the Division of Animal House, Faculty of Science, Prince of Songkla University, Thailand. All animal procedures were reviewed and approved by the Animal Ethics Committee, Walailak University (Protocol number: 004/2012). Rats were kept in stainless-steel cages under constant conditions of temperature (23 ± 2 °C), relative humidity (50 - 60 %) and lighting (12 h light/dark cycles). Animals were provided with a standard commercial rat diet and distilled water. Animals were acclimatized and closely cared under laboratory conditions for 1 week before the experiment.

Experimental design

Male Wistar rats were divided into 5 groups. Group 1 (control) was IP injected with 1 ml of normal saline. Groups 2, 3, 4 and 5 were IP injected with single doses of cisplatin at concentrations of 25, 50, 75 and 100 mg/kg body weight (BW), respectively. At 72 h, rats were weighed and then anesthetized with an overdose of thiopental sodium administered by IP injection (100 mg/kg BW). The abdominal cavity of each rat was opened and the small intestine immediately collected and washed in cold normal saline.

Histological examination and semi-quantitative evaluation

The small intestines were preserved in 10 % neutral buffered formalin solution for 24 h and washed with 70 % ethanol. The tissue was then placed in small metal caskets, stirred by a magnetic stirrer, dehydrated using 70 to 100 % alcohol series and embedded in paraffin using an embedding machine. Each paraffin block was sectioned using a rotary microtome and distributed onto glass slides and dried overnight at room temperature. After staining with H&E dyes and mounting, the slides were observed under a light microscope.

Statistical analysis

Data at 0 and 72 h of each group were compared by using one-way analysis of variance (ANOVA) which were expressed as mean $\pm$ standard deviation (SD). P-value < 0.05 was considered significant.

Results

The effect of cisplatin on body weight

Cisplatin reduced BW of Wistar rats as illustrated in **Figure 1**. There were not significant differences in BW at 0 h compared with at 72 h in control group (normal saline). At 72 h, groups administered cisplatin at doses of 25, 50, 75 and 100 mg/kg BW significantly ($P < 0.05$) reduced BW compared with BW at 0 h.

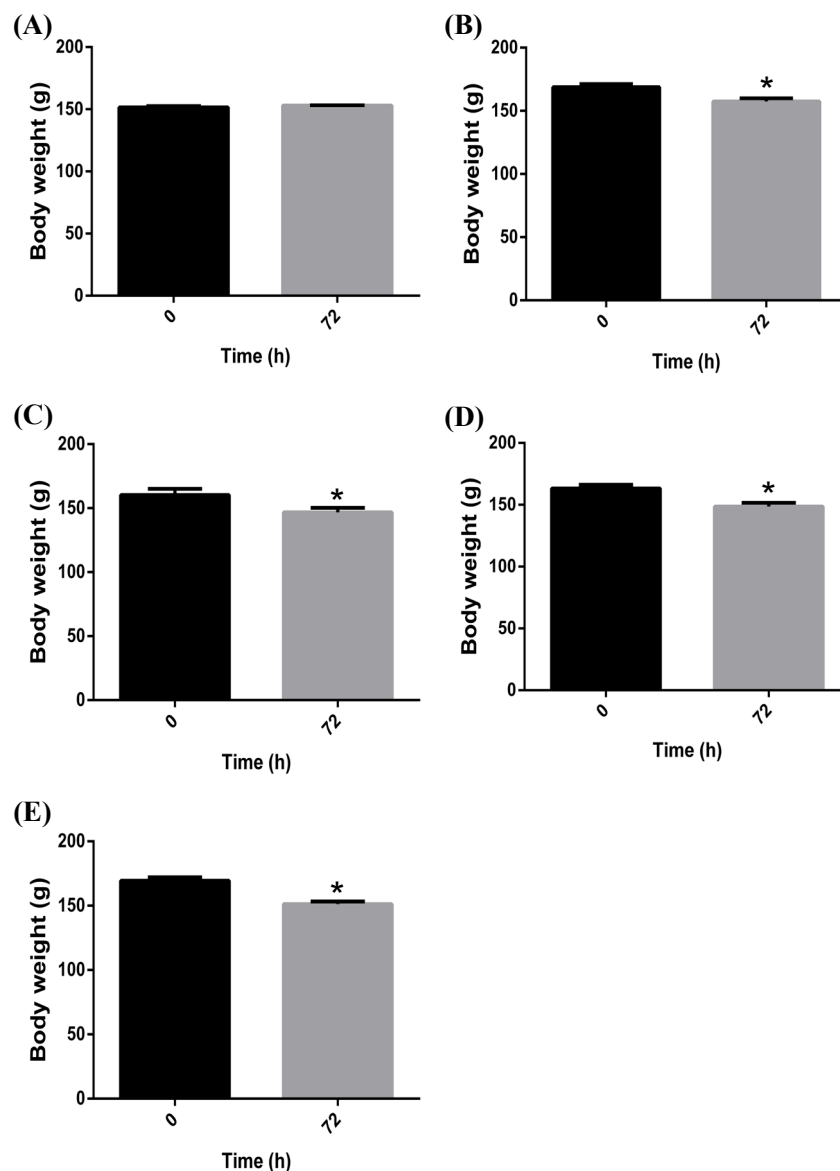


Figure 1 Body weight of Wistar rats: (A) control group; (B, C, D, E) administration of cisplatin at doses of 25, 50, 75 and 100 mg/kg BW, respectively. *Indicates significant difference at $P < 0.05$ between 0 and 72 h.

Histopathologic effect of cisplatin on small intestine

Normally, the small intestine comprises 4 distinct layers; the mucosa, submucosa, muscularis externa and serosa. The mucosa consists of 3 layers, including the epithelium, lamina propria and muscularis mucosae, and is organized into villi and crypts (crypts of Lieberkühn). Villi are finger-like projections of the epithelium and underlying lamina propria which contain blood and lymphatic vessels that extend into the intestinal lumen [8]. Cisplatin can cause various pathologies to the small intestine as shown in **Figure 2** and **Table 1**. Cisplatin administered at 25 mg/kg BW can lead to mild development of

subepithelial space (**Figures 2D-F**), distortion of mucosal architecture, lifting of the epithelial layer from the lamina propria, mild hemorrhage and moderate hyperplasia of the lamina propria, hyperplasia of columnar epithelium lining villi and atrophy of villi. Cisplatin administered at 50 mg/kg BW led to mild hemorrhage (**Figures 2H and 2I**), moderate distortion of mucosal architecture and development of subepithelial space, and marked hyperplasia of lamina propria, hyperplasia of columnar epithelium lining villi, atrophy of villi and lifting of the epithelial layer from the lamina propria. Cisplatin administered at 75 mg/kg BW instigated mild hyperplasia of lamina propria, hyperplasia of columnar epithelium lining villi, degenerative changes of villi, moderate hemorrhage (**Figures 2K and 2L**), sloughing of necrotic villi into intestinal lumen (**Figures 2K and 2L**), loss of villi, severe atrophy of villi and distortion of mucosal architecture (**Figures 2J and 2K**). Moreover, cisplatin administered at 100 mg/kg BW induced moderate hemorrhage (**Figure 2O**) and severe atrophy of villi, distortion of mucosal architecture (**Figures 2M and 2N**), degenerative changes of villi (**Figure 2N**), sloughing of necrotic villi into intestinal lumen and loss of villi. We suggest that high doses of cisplatin caused more pathologic severity than low doses associated with BW reduction.

Discussion

Although it is highly effective for treatment of cancers, cisplatin cause various deleterious effects in target organs, especially the liver, kidneys and small intestine. In the present study, intraperitoneal administration of cisplatin at doses of 25, 50, 75 and 100 mg/kg significantly reduced BW, and caused a dose-related histopathology of the small intestine of Wistar rats. Previously, Arivarasu *et al.* (2007) illustrated that cisplatin causes toxicity in the small intestine by reduced activities of brush border enzymes including alkaline phosphatase, leucine aminopeptidase, γ -glutamyl transferase and sucrose [7]. Additionally, cisplatin can reduce fluid and electrolyte absorption, which may be related to presentation of diarrhea during cisplatin treatment [11]. The present study indicates that cisplatin causes various pathologies of the small intestine including hemorrhage, hyperplasia of lamina propria, hyperplasia of columnar epithelium lining villi, atrophy of villi, distortion of mucosal architecture, development of subepithelial space, lifting of the epithelial layer from the lamina propria, degenerative changes of villi, sloughing of necrotic villi into intestinal lumina and loss of villi, consequently causing reduction in body weight. Ultimately, cisplatin may cause malabsorption, maldigestion and motility alterations.

Hemorrhage or bleeding is instigated by hemodynamic alterations. Anti-coagulant and anti-platelet drugs also usually present occult intestinal bleeding depending on dose, clotting parameters, concomitant medications and presence of co-morbid conditions [12]. Cellular adaptations found in the small intestine include hyperplasia of lamina propria and columnar epithelium and atrophy of villi, indicating pathologic response against cisplatin at low concentrations. Atrophy may result in diminished function of villi, which has also been reported in the jejunal mucosa upon treatment with oral fluorouracil anti-cancer drugs [13]. Moreover, villous atrophy has been well documented with non-steroidal anti-inflammatory drugs such as mefenamic acid, aminoglycoside antibiotics such as neomycin, progestational steroids and colchicines [14]. Irreversible injury induced by cisplatin administered in high doses may be caused by necrosis or apoptosis. In 2002, Chang *et al.* demonstrated that apoptosis is one of the most significant pathways causing degeneration of epithelial cells lining the small intestine as assessed by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay [10]. Anti-apoptotic Bcl-2 has been reported to block cisplatin-induced apoptosis through inhibition of ERK-mediated p53 signaling pathway in B104 cells. Coagulative necrosis has been found in kidneys induced by cisplatin, particularly in distal convoluted tubules and collecting ducts [15].

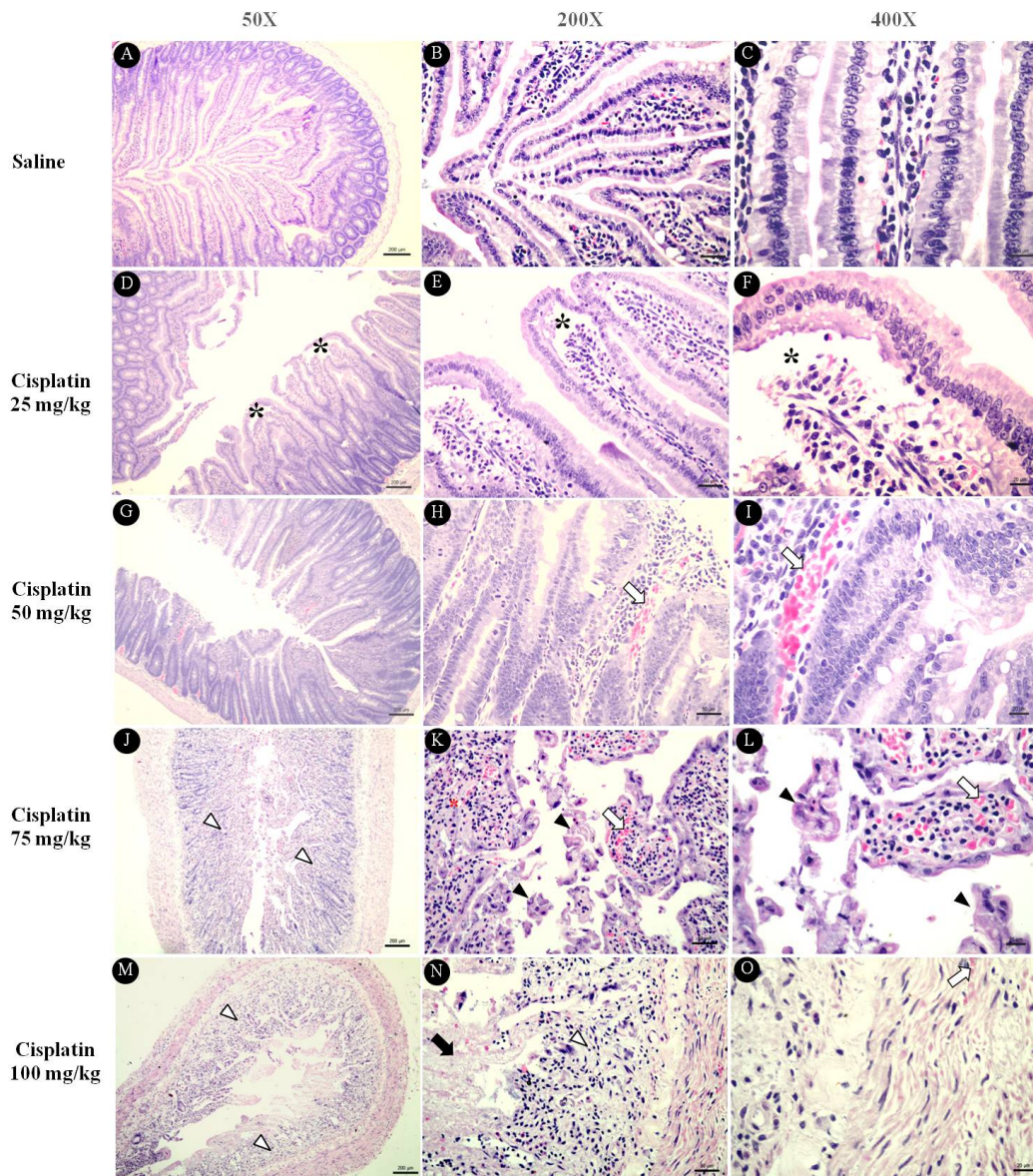


Figure 2 Histology of the small intestine at magnification 50X, 200X and 400X (H&E staining): (A, B, C) photographs of the small intestine of the control group; (D, E, F) photographs of the small intestine induced by 25 mg/kg BW of cisplatin; (G, H, I) photographs of the small intestine induced by 50 mg/kg BW of cisplatin; (J, K, L) photographs of the small intestine induced by 75 mg/kg BW of cisplatin; (M, N, O) photographs of the small intestine induced by 100 mg/kg BW of cisplatin. Black asterisk indicates development of subepithelial space, white arrowhead indicates distortion of mucosal architecture, black arrowhead indicates sloughing of necrotic villi into intestinal lumen, white arrow indicates hemorrhage, black arrow indicates degenerative changes of villi. Scale bar: magnification of 50X = 200 μ m, 200X = 50 μ m and 400X = 20 μ m.

Table 1 Histological evaluation of the small intestine induced by cisplatin.

Histological alteration	Cisplatin (mg/kg BW)			
	25 (n = 3)	50 (n = 3)	75 (n = 3)	100 (n = 3)
1 Hemorrhage	1+ (2/3)	1+ (3/3)	2+ (3/3)	2+ (3/3)
2 Hyperplasia of lamina propria	2+ (3/3)	3+ (3/3)	1+ (1/3)	0
3 Hyperplasia of columnar epithelium lining villi	2+ (3/3)	3+ (3/3)	0	0
4 Atrophy of villi	2+ (2/3)	3+ (3/3)	3+ (3/3)	3+ (3/3)
5 Distortion of mucosal architecture	1+ (2/3)	2+ (2/3)	3+ (3/3)	3+ (3/3)
6 Development of subepithelial space	1+ (3/3)	2+ (3/3)	0	0
7 Lifting of epithelial layer from the lamina propria	1+ (3/3)	3+ (3/3)	0	0
8 Degenerative changes of villi	0	0	1+ (2/3)	3+ (3/3)
9 Sloughing of necrotic villi into intestinal lumina	0	0	2+ (3/3)	3+ (3/3)
10 Loss of villi	0	0	2+ (3/3)	3+ (3/3)

n = number of Wistar rats, and the number in parenthesis means number of animals affected by cisplatin over total animals. Severity scores: 0 = Not found; 1+ = Mild; 2+ = Moderate; 3+ = Severe.

Oxidative stress is believed to be a crucial mechanism of cisplatin-caused pathologies. Oxidative stress is caused by an imbalance between oxidants and antioxidants. Cisplatin treatment-induced oxidative stress through increased oxidation of lipids called lipid peroxidation. Lipid peroxidation alters the antioxidant status by decreased activities of catalase, Cu-Zn superoxide dismutase, glucose 6-phosphate dehydrogenase and glutathione reductase and increased activities of glutathione S-transferase and thioredoxin reductase [7]. Exogenous antioxidants including caffeic acid and L-carnitine were able to ameliorate cisplatin-induced oxidative stress [9,10]. Thus, cisplatin treatment is limited by its side effects, whose severity depends on drug dose. Future studies should investigate the main underlying mechanism of cisplatin-induced pathology in the small intestine for effective management and prevention strategies of drug toxicity.

Conclusions

Cisplatin administration causes various pathologies of the small intestine including hemodynamic change (hemorrhage), reversible injuries (distortion of mucosal architecture, development of subepithelial space and lifting of the epithelial layer from the lamina propria) and irreversible injuries (degenerative changes of villi, sloughing of necrotic villi into intestinal lumina, and loss of villi). Additionally, cellular adaptations are elicited including hyperplasia of lamina propria and columnar epithelium lining villi and atrophy of villi, consequently causing weight loss, the severity of which depends on concentrations of the drug.

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