Research Paper

Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats

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Abstract

Cisplatin, doxorubicin and fluorouracil (5-FU), drugs belonging to different chemical classes, have been extensively used for chemotherapy of various cancers. Despite extensive investigations into their hepatotoxicity, there is very limited information on their effects on the structure and ultra-structure of liver cells *in vivo*. Here, we demonstrate for the first time, the effects of these three anticancer drugs on rat liver toxicity using both light and electron microscopy. Light microscopic observations revealed that higher doses of cisplatin and doxorubicin caused massive hepatotoxicity compared to 5-FU treatment, including dissolution of hepatic cords, focal inflammation and necrotic tissues. Interestingly, low doses also exhibited abnormal changes, including periportal fibrosis, degeneration of hepatic cords and increased apoptosis. These changes were confirmed at ultrastructural level, including vesiculated rough endoplasmic reticulum and atrophied mitochondria with ill-differentiated cisternae, dense collection of macrophages and lymphocytes as well as fibrocytes with collagenous fibrils manifesting early sign of fibrosis, especially in response to cisplatin and doxorubicin -treatment. Our results provide *in vivo* evidence, at ultrastructural level, of direct hepatotoxicity caused by cisplatin, doxorubicin and 5-FU at both light and electron microscopi. These results can guide the design of appropriate treatment regimen to reduce the hepatotoxic effects of these anticancer drugs.

Key words: Cisplatin, doxorubicin, 5-FU, Hepatotoxicity, Rat liver, Chemotherapy.

Introduction

Chemotherapy involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor. However, it does not distinguish between a cancer and normal cells, and eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body, including, hair and blood cells. More than half of all people diagnosed with cancer receive chemotherapy regimen, that usually include drugs to treat cancer as well as drugs to help support the completion of the cancer treatment at the full dose on schedule (1, 2).

Cisplatin, doxorubicin and 5-FU have been extensively used for chemotherapy of various cancers, including that of the liver (3-5). However, while they generate acceptable outcome in chemotherapy of some cancers, they also exhibit severe toxicity and undesirable side effects (6-8). Extensive investigations have been conducted on the hepatotoxicity as well as general organ toxicity of these three anticancer drugs (9-10). These include light and electron microscopic studies of various organs and biochemical studies of liver enzymes (11-14). However, there is very limited information on the effects of these drugs on histopathology and ultrastructure of liver cells. Further, a variety of agents including anti-oxidants have been shown to attenuate the hepatotoxicity of these three compounds (15-17). Also, analogues of these compounds have been synthesized and shown to be much less toxic (18).

In the present study, we investigated the effects of Cisplatin, doxorubicin and 5-FU belonging to different chemical classes on rat liver toxicity. We showed that the drugs induced drastic abnormalities with respect to body and liver weight changes as well as the histology and the ultrastructure of liver tissue. We further identified pathological features at both structural and ultrastructural levels, which could be used to adjust the dose and duration of treatment.

Materials and methods

Animals studies and drug treatment

All of the procedures involving animals in this study were approved by the institution's animal welfare regulatory committee. Thirty five adult fertile inbred male albino rats were divided into seven groups (n=5). The first and second group of rats was treated intraperitoneally with low (0.2mg/kg) or therapeutic (1mg/kg) doses of cisplatin, respectively. The third and the fourth group of rats were treated intraperitoneally with low (0.2mg/kg) therapeutic (1mg/kg) doses of doxorubicin alone, respectively. The fifth and sixth group of rats were treated intraperitoneally with low (10mg/kg) or therapeutic (20mg/kg) doses of 5-FU alone, respectively. The remaining group of rats (n=5) received vehicle (PBS) alone. Body weights were measured twice weekly to determine weight changes during the course of treatment.

Histological studies

At the end of treatment both control and experimental rats were euthanized using carbon dioxide. The livers were collected from all the groups, fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast at 58-62°C. Five micron sections were obtained, stained with Harris Hematoxylin & Eosin and evaluated for any structural changes under a bright field microscope.

Electron microscopy analysis

Liver tissue from both control and treated groups were immediately fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). After rinsing in 0.1 M cacodylate buffer, samples were post fixed in a buffered solution of 1% osmium tetroxide at 4°C for 1.5 hour. This was followed by dehydration in ascending grades of ethyl alcohol (30%, 50%, 70%, 90%, and absolute) and embedded in epoxy-resin. Ultrathin sections were obtained with a diamond knife on a LKB microtome and mounted on formvar-coated grids, stained with uranyl acetate and lead citrate and evaluated for structural abnormalities with a Joel Transmission electron microscope.

Statistical analysis

Means and standard deviations were calculated from the five replicates per each group. Student's t-test was performed and the differences were considered significant when $P < 0.05$.

Results

Body weight

Body weights were measured twice weekly at 1, 4, 8, 12, 16 and 20 days post-treatment. The results demonstrate that treatments with both low and therapeutic (Fig. 1) doses of cisplatin, doxorubicin and 5-FU caused inhibition in the percentage of weight gain as compared to controls, indicating retardation in growth. 5-FU treatment exhibited the least adverse effects (Fig. 1). The results of low doses were quite similar to those of therapeutic treatment doses.

Histological effects

Light microscopic observation revealed that the control hepatic tissue showed normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells (Fig. 2A). In contrast, groups receiving cisplatin or doxorubicin at doses of 0.2 mg/kg or 5-5-FU at 10mg/kg on alternate days for 20 days showed massive hepatotoxicity, particularly with therapeutic doses. However, cisplatin and doxorubicin exhibited increased hepatotoxicity in comparison to 5-FU treatment. The most pronounced histopathological abnormalities observed in rats treated with 1mg/kg body weight involved dissolu-

tion of hepatic cords, which appeared as empty vacuoles aligned by strands of necrotic hepatocytes (Fig. 2B). The hepatic tissues showed the presence of dense focal inflammatory cells or necrotic tissues (Fig. 2C and 2D). Low dose-treatment of the drugs resulted in common histo-pathological alterations including perivascular round cell infiltration, associated with membrane changes of endothelial lining cells mani-

festing periportal fibrosis, marked degeneration of hepatic cords, increased incidence of vacuolar degeneration and apoptotic cell death (Fig. 2C-H). Doxorubicin treatment showed higher tendency for liver fibrosis manifested by the presence of many spots of focal cellular granulomatous lesions (Figs. 2E and 2F).
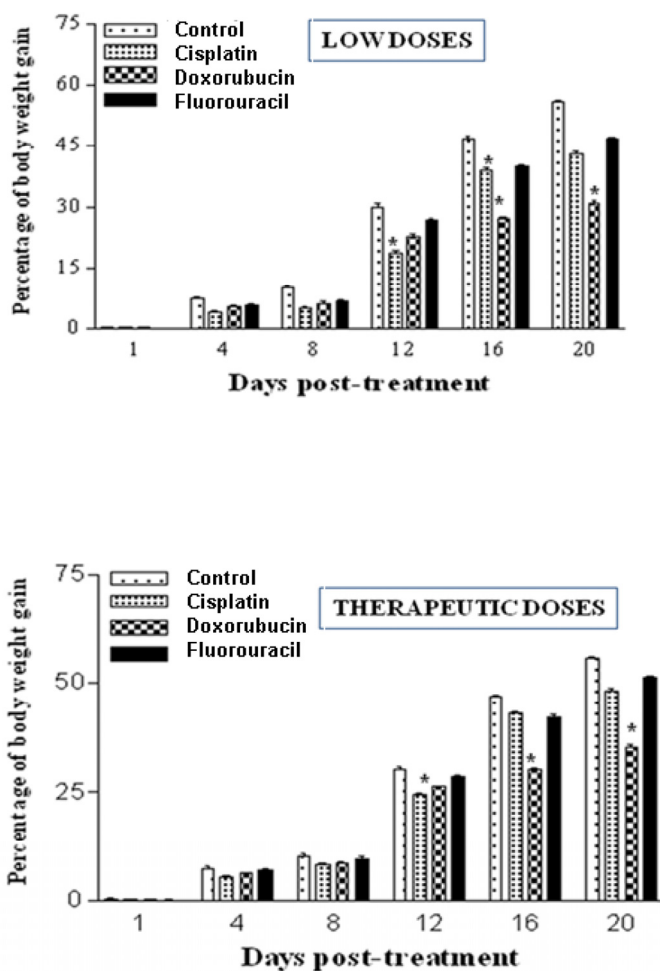

Figure 1. Chemotherapeutic treatment with cisplatin, doxorubicin and 5-FU influences the percentage of body weight gain in the rat. **A:** Percentages of body weight gain post treatment with low doses of cisplatin (0.2 mg/Kg), doxorubicin (0.2 mg/Kg) and fluorouracil (10 mg/Kg). **B:** Percentages of body weight gain post treatment with either cisplatin (1 mg/Kg), doxorubicin (1 mg/Kg) and fluorouracil (20 mg/Kg). Body weights were measured twice weekly at 1, 4, 8, 12, 16 and 20 days post-treatment. Body weight gains during the course of the treatment of both control and experimental groups were determined. (*$P < 0.05$).
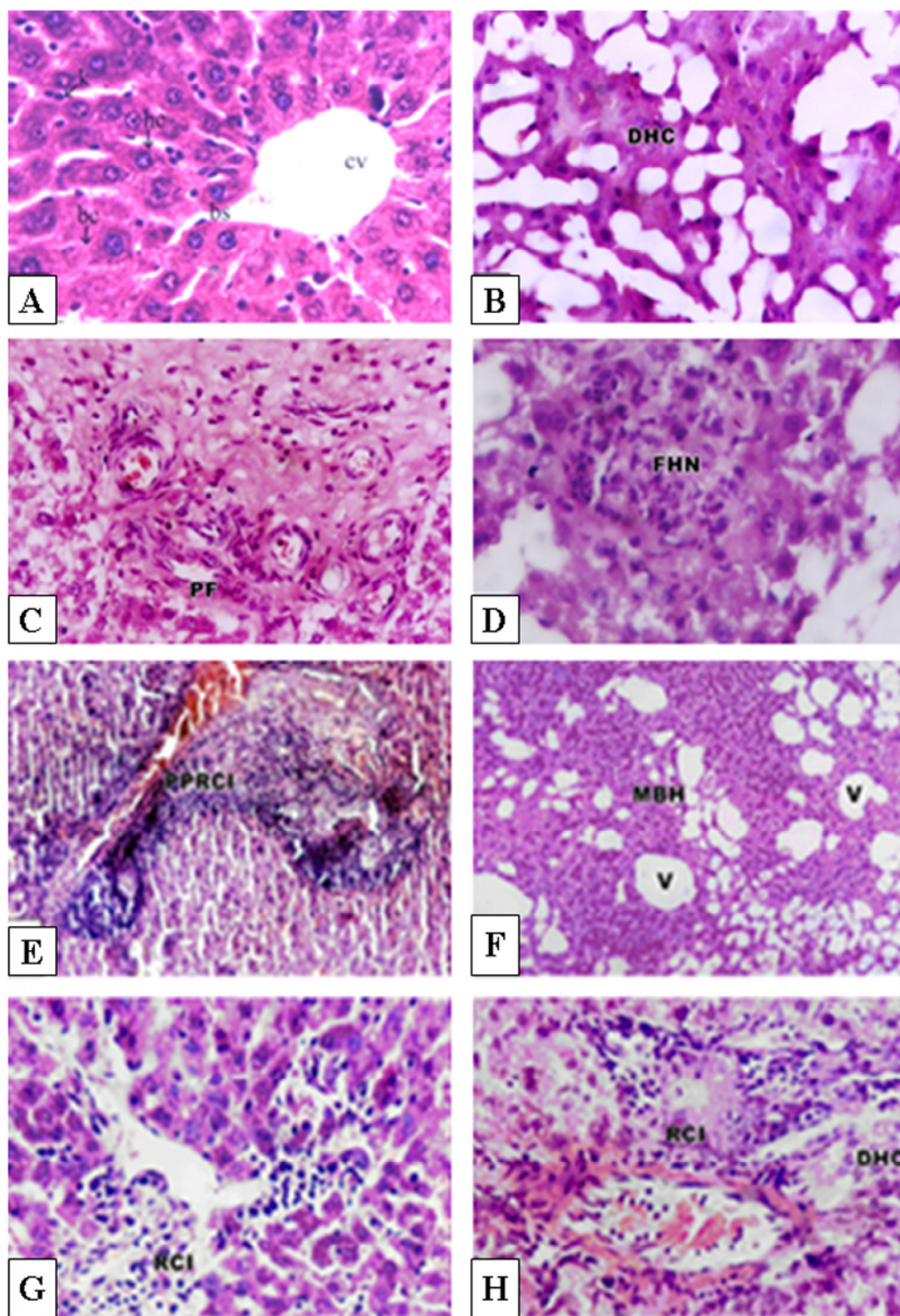
Figure 2. Histopathological effects of cisplatin, doxorubicin and 5-FU in rat liver. **A:** Histology of normal control rat liver. **B-D:** Pronounced histopathological abnormalities seen in rats treated with cisplatin (1.0 mg/kg body weight). **E-F:** Doxorubicin-treated rat liver showing higher tendency for liver fibrosis manifested by the presence of many spots of focal collected cellular granulomatous lesions. **G:** Low dose treatment with 5-FU (10 mg/kg body weight) showed lower density of periportal inflammatory cells. **H:** Higher dose (20 mg/kg body weight) showed focal collection of inflammatory cells and loss of hepatic tissue structural pattern. CV, Central vein; BS, Blood sinusoids; DHC, Degenerated hepatic cord ; PF, Periportal fibrosis; FHN, focal hepatic necrosis; PPRCI, Periportal round cell infiltration; MBH, Massive breakdown hepatocytes; V, vacuole; RCI, round cell infiltration. Magnification: A&F, X400; B-E, X250, CX160.

Control hepatocytes were normal polygonal with oval-shaped nuclei, cytoplasm crowded with organelles, particularly rough endoplasmic reticulum, smooth endoplasmic reticulum, golgi apparatus, ribosomes, mitochondria and glycogen particles (Figs. 3A and 3B). In contrast, the hepatocytes of liver from group treated with cisplatin showed pyknotic nuclei with irregular nuclear membrane, the cytoplasm contained vesiculated rough endoplasmic reticulum and atrophied mitochondria with ill-differentiated cisternae (Figs. 3C-F). The hepatic tissue showed numerous spots of densely collected inflammatory cells composed mainly of macrophages and lymphocytes at the center and a number of fibrocytes at the periphery; the collagenous fibrils appeared markedly distributed in the necrotic foci (Figs. 3C-F). Doxorubicin (Figs. 4A and 4B) and 5-FU (Figs. 4C and 4D) treatments revealed similar cytological alterations as in cisplatin treatment.
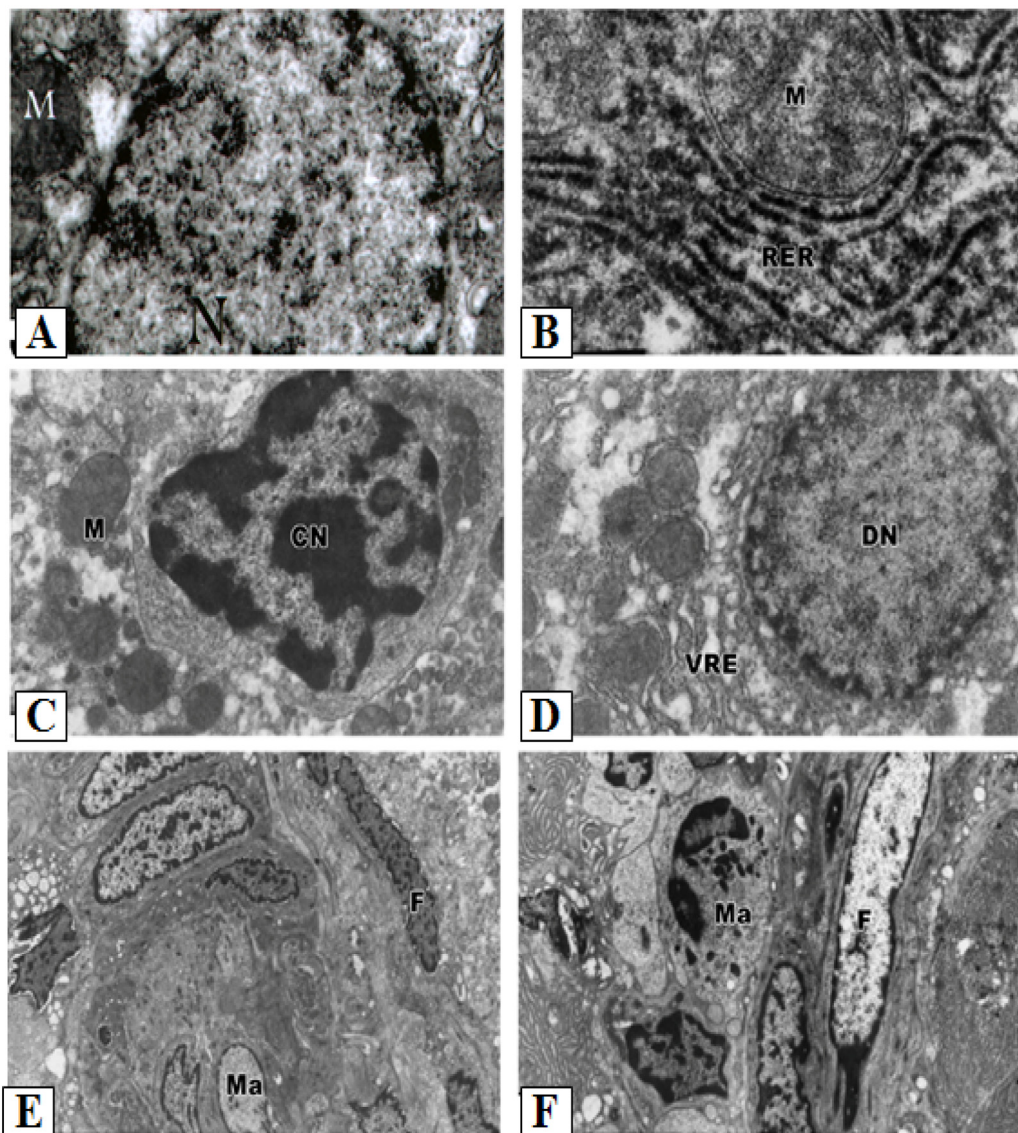

Figure 3. Ultrastructural pathological effects of cisplatin in rat liver. **A- B:** Hepatic tissue of the control normal liver. **C-F:** Section of rat liver treated with cisplatin, showing dense collection of inflammatory cells including macrophages (M) and fibrocytes (F) forming pattern of cirrhotic liver (Lead citrate and uranyl acetate X 7500). The cytoplasm also contained vesiculated rough endoplasmic reticulum (VER) and atrophied mitochondria (Ma) with ill-differentiated cisternae.
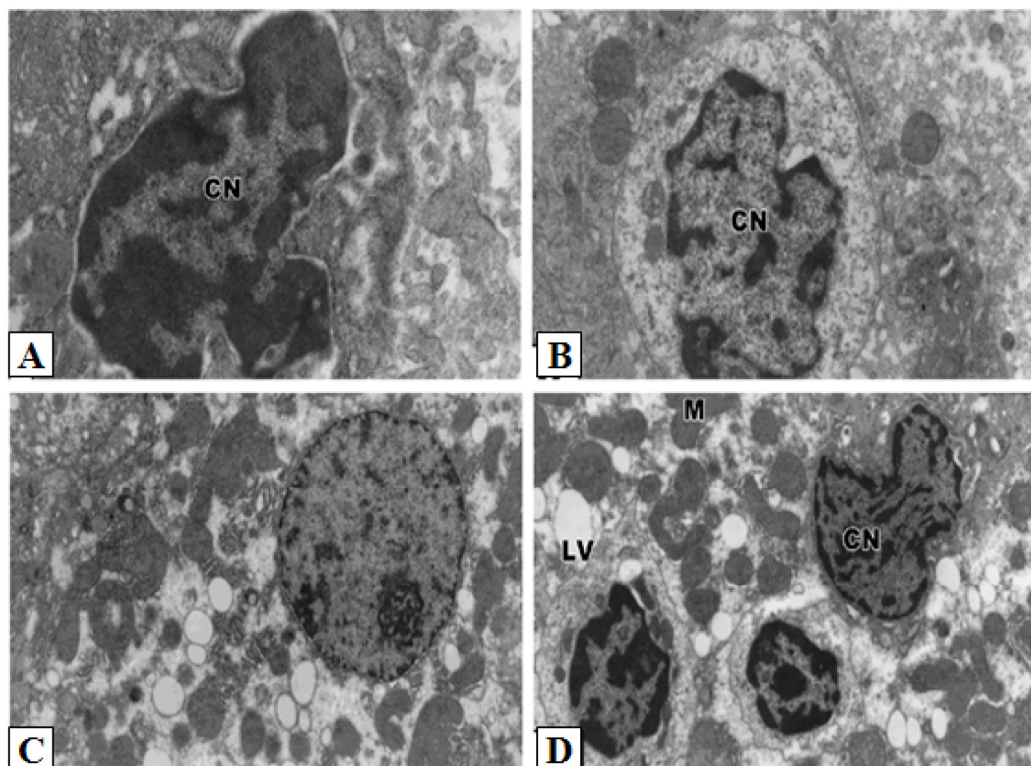
Figure 4. Ultrastructural pathological effects of doxorubicin and 5-FU in rat liver. Doxorubicin (**A-B**) and 5-FU (C-D) treatments revealed similar cytological alterations as in cisplatin treatement (Lead citrate and uranyl acetate X 7500).

Discussion

Although a number of studies have demonstrated some side-effects of the chemotherapeutic drugs cisplatin, doxorubicin and 5-FU, the present study is the first comprehensive *in vivo* study revealing drastic abnormalities of these drugs both at cellular and ultrastructural levels. Rats treated with the anticancer drugs showed a significant decrease in body weight gain, suggesting that hepatotoxicity might have contributed to this loss as previously reported (19). Dissection of these animals indicated that the loss of the body weights were due to loss of skeletal muscles and adipose tissue as previously suggested by Devlin *et al* (20). Moreover, the reduction in body weight of the animals in this study correlate with the decreased food intake observed during the experimental period.

The present investigation showed many histopathological and ultrastructural abnormalities in the liver including inflammatory infiltration, hyperplasia, periportal fibrosis, marked disruption of hepatic cords and dilated blood sinusoids. Many hepatocytes showed karyomegaly and pyknotic nuclei indicating apoptosis. The liver is known to accumulate signifi-

cant amounts of cisplatin, second only to the kidney (21), thus hepatotoxicity can be associated with cisplatin treatment (22). Clinical evidence of cisplatin-induced liver injury has been demonstrated by elevated activities of serum enzymes and bilirubin levels, and the development of jaundice (23). Cell death can result from naturally occurring apoptosis (physiological apoptosis) or from irreparable cell injury (pathological apoptosis) as described by Farber (1994) (24). Apoptosis is a common feature of hepatotoxicity induced by many chemicals; it may precede necrosis, as in the hepatotoxicity induced by thioacetamide (25), or it may occur concurrently with necrosis as in hepatotoxicity associated with acetaminophen (26). Cisplatin is thought to kill cells primarily by forming DNA adducts, causing G_2 arrest in the cell cycle, triggering apoptosis (27).

Doxorubicin is firmly established as a major therapeutic agent in the treatment of a wide variety of tumors. Although the precise mode of antitumor action of this drug is not well established, it is thought to involve the interference with the synthesis of macromolecules, covalent DNA binding and DNA cross-linking, inhibition of topoisomerase II, arrest of tumor cell cycle progression in G_2 phase, induction of

apoptosis and generation of reactive oxygen radicals (28). Two of the listed phenomena require enzymatic activation, including covalent modification of macromolecules and redox cycling with reactive oxygen species and both these effects can cause cytotoxicity (29). A number of studies indicate that enzyme activation of doxorubicin begins with the drug conversion to a semiquinone free radical *via* one-electron reduction, and such a reaction is catalyzed by several enzymes, including P-450 reductase (30). In the present study inflammatory cells forming granulomatous lesions and periportal fibrosis were detected after doxorubicin administration. Doxorubicin has been shown to induce accumulation of inflammatory cells (31), associated with increased activities of tissue aminotransferases, LDH and ALP, indicating hepatic damage (32).

5-fluorouracil has been used in the treatment of breast cancer, head and neck cancer, and gastrointestinal cancers. When given intravenously, it is metabolized in tissues to its active form, 5-fluoro-deoxyuridine-monophosphate, which inhibits thymidylate synthase. The drug is also catabolized primarily in the liver, as dihydrouracil, and the reduced compound is then cleaved to α-fluoro-β-alanine, ammonia, urea, and carbon dioxide. Both the toxicity and antitumor effect are potentiated if the catabolism is blocked by inhibiting dihydrouracil dehydrogenase. Although the liver plays a key role in its catabolism, 5-FU has not been reported to cause liver damage when given orally, and few reports have indicated its hepatotoxicity when given intravenously (33). Our study showed many histopathological and ultrastructural abnormalities in the liver after intraperitoneal administration of 5-FU including apoptotic cell death, appearance of numerous areas of inflammatory cells, and the cytoplasmic organelles were markedly affected with collagenous fibrils in a number of necrotic cells. 5-FU was found to produce liver toxicity associated with a number of abnormalities (34).

In conclusion chemotherapeutic agents such as cisplatin, doxorubicin, and 5-FU cause direct hepatic toxicity. Appropriate protective measures must be applied with anticancer treatment for improving liver function. Our results provide *in vivo* evidence, at light microscopic and ultrastructural levels, of direct chemotherapeutic hepatotoxicity caused by cisplatin, doxorubicin and 5-FU. Furthermore, this study identified pathological features at both structural and ultrastructural levels, which could be used as the basis for determining the appropriate dose of these drugs to reduce their hepatotoxic effects.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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