CISPLATIN (CDDP)-INDUCED ACUTE TOXICITY IN AN EXPERIMENTAL MODEL OF HEPATIC FIBROSIS

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(Received March 8, 2007; Accepted April 9, 2007)

ABSTRACT — Cisplatin (CDDP)-induced acute toxicity was investigated in an experimental model of liver fibrosis produced through repeated intraperitoneal injections of swine serum in rats. A significant increase in level of hepatic markers, such as plasma ASAT, LDH, glucose, total cholesterol and bile acid levels, and a significant decrease in the plasma triacylglycerol level were observed. Slight histological changes, such as necrosis, vacuolar degeneration, and the proliferation of bile ducts were observed as compared with the control fibrotic rats. On the other hand, a significant increase in levels of renal markers, such as plasma BUN and creatinine levels as well as more remarkable tubular degeneration were observed. From these results, CDDP’s hepatotoxicity was slight while its nephrotoxicity was more extensive in fibrotic rats.

KEY WORDS: Cisplatin (CDDP), Rats, Hepatic fibrosis, Acute toxicity, CYP2E1, GSH

INTRODUCTION

Cisplatin (CDDP) is a widely used antitumor agent and recently useful for treatment of advanced hepatocellular carcinoma (HCC) in combination with 5-fluorouracil (5-FU) (Tanioka et al., 2003). Its major toxicity is nephrotoxicity (Dentino et al., 1978; Davis et al., 1980; Hardaker et al., 1974; Macleod et al., 1988; Tirelli et al., 1985), but at high dose, it can produce undesirable side effects such as hepatotoxicity (Kim et al., 2004).

HCC is one of the most frequent malignancies worldwide. There has been a conspicuous trend towards an increase in the number of cases of HCC in the United States, Japan and the United Kingdom over the decades (Sangro et al., 2002). HCC is usually associated with liver cirrhosis or fibrosis, mainly chronic hepatitis or excessive alcohol intake. Notably, chronic hepatitis B and C infections are identified as the major risk factors for HCC (Komorizono et al., 2003). Hepatic fibrosis is a terminal feature of chronic hepatitis and enhances portal blood pressure.

It has been reported that hepatic fibrosis was induced in rats by repeated intraperitoneal injections of pig serum (Kitamura et al., 1984; Sun et al., 1990; Wada et al., 1996). Injection of pig serum into rats twice a week for 8 weeks induced stellate cell activation resulting in liver fibrosis without parenchymal cell injury including hepatocyte cell death and proliferation (Bhunchet and Wake, 1992; Mitasumura et al., 1997; Rubin et al., 1968; Sakaida et al., 1998a, 1998b).

In this study, we investigated the CDDP-induced acute toxicity in an experimental model of liver fibrosis induced with repeated intraperitoneal injections of swine serum.

MATERIALS AND METHODS

Animals and treatments

Sixty male Sprague-Dawley rats (Japan Slc Co., Ltd., Hamamatsu, Japan) were housed five per cage on a 12 hr light, 12 hr dark cycle prior to the experiment, and fed a cereal-based standard chow with free access to water. Pig serum (Gibco, USA) was intraperitoneally injected into 45 rats at 3.5 ml/kg twice a week for 8 weeks. Fifteen normal control rats were treated with saline at 3.5 ml/kg, twice a week for 8 weeks. After the final injection, fifteen rats each were injected intrave-
nously with CDDP at a dose of 4 or 7 mg/kg in saline or injected with saline as controls. The animal experiments were conducted according to the Guidelines for Animal Experiments, Research & Development Division, Toray Industries, Inc.

**Blood chemistry**

The blood chemical markers were determined in plasma samples from blood obtained from the abdominal aorta under anesthesia with pentobarbital sodium, 1, 3 and 10 days after CDDP treatment. An Automatic Analyzer 7070 (Hitachi Instruments Service Co., Ltd., Japan) and Autosera kits were used to determine the ALP (GSCC method), ASAT (IFCC method), ALAT (IFCC method), LAP (L-leucyl-pNA method), LDH (Wróblewski-LaDue’s method), total protein (burette method), albumin (BCG method), total bilirubin (azo-bilirubin method), glucose (hexokinase method), BUN (urease-GLDH method), creatinine (Jaffe’s method), triglyceride (enzyme method), total cholesterol (enzyme method), bile acid (3α-HSD-Δ4DH method), Na (ion-electrode method), K (ion-electrode method) and Cl (ion-electrode method) levels.

**Histology**

The rats were euthanized at 1, 3 or 10 days after CDDP treatment. Then the kidneys and liver were quickly removed and dissected. Two pieces of each organ were fixed in 10% buffered formalin. After fixation, the pieces were rinsed in tap water, dehydrated in a graded alcohol series, embedded in paraffin and cut at a thickness of 5-μm. Cross-sections were used for hematoxylin-eosin (HE) staining. Histopathological changes were observed under a light microscope.

**Statistical analysis**

The changes in plasma markers levels on each day were analyzed statistically with Student’s t-test (Kobayashi et al., 1996) or Aspin-Welch’s t-test (Kobayashi et al., 1996) compared to the normal control rats.

**RESULTS**

**Blood chemistry**

1. All results are shown in Fig. 1

Plasma ALP levels were significantly decreased 1, 3 or 10 days after CDDP-treatment in fibrotic rats. Plasma ASAT and LDH levels were significantly increased 1 or 3 days after CDDP-treatment in fibrotic rats. Plasma ALAT levels were not significantly changed in any groups. In the fibrotic rats treated with CDDP (7 mg/kg), plasma LAP levels were significantly increased 1 and 3 days after CDDP-treatment but were significantly decreased 10 days after treatment. Plasma glucose levels were significantly increased 1 and 3 days after CDDP (7 mg/kg)-treatment in fibrotic rats. Plasma total cholesterol levels were remarkably increased after CDDP-treatment in fibrotic rats. Low plasma triglyceride levels were found in control fibrotic rats, but the levels in fibrotic rats at 10 days after CDDP (7 mg/kg)-treatment were the same as those in the normal control rats. There was little effect on plasma total bilirubin levels in fibrotic rats after CDDP-treatment or saline-treatment. Plasma bile acid levels were increased more in fibrotic rats than normal control rats. In the fibrotic rats treated with CDDP, plasma total protein levels were significantly increased 1 and 3 days after CDDP-treatment, but were not significantly changed in any of the groups 10 days after treatment. In the fibrotic rats treated with CDDP (7 mg/kg), plasma albumin levels were significantly increased 1 and 3 days after CDDP-treatment, but were significantly decreased 10 days after treatment. Plasma BUN and creatinine levels were remarkably increased after CDDP-treatment in fibrotic rats. Plasma sodium levels were significantly decreased 1 and 3 days after CDDP (7 mg/kg)-treatment in fibrotic rats. Plasma potassium levels were significantly decreased 3 days after CDDP (7 mg/kg)-treatment in fibrotic rats. Plasma chloride levels were significantly decreased 1, 3 and 10 days after CDDP-treatment in fibrotic rats.

**Histology**

1. Liver

Pseudolobule and fibrotic septa were observed in all of the fibrotic rats. Pseudolobules were remarkably seen in all of animals and fibrotic septa tended to gradually thin out after the final serum treatment. In surviving animals, the necrosis, vacuolization of hepatocyte, pseudolobule, fibrotic septa and proliferation of bile ducts were more remarkable in the CDDP-treated fibrotic rats than control fibrotic animals. Necrosis was seen in the perilobular area in the control animals and also intralobular and perilobular areas in the CDDP-treated fibrotic rats. At 10 days after CDDP treatment in fibrotic rats, the necrosis was remarkable. Some vacuolization of hepatocytes was observed in the control fibrotic rats, but changes were more remarkable 3 days after CDDP-treatment in the CDDP-treated fibrotic rats. Pseudolobules did not differ between the control and CDDP-treated fibrotic rats. Changes to fibrotic
CDDP-induced toxicity in hepatic fibrotic model.

Fig. 1-1. Changes in blood chemistry after CDDP treatment in fibrotic rats. (■), saline control rat; (○), saline-treated fibrotic rat; (▲), CDDP (4 mg/kg)-treated fibrotic rat; (△), CDDP (7 mg/kg)-treated fibrotic rat. Each bar and line is presented as the mean± SD. Significantly different from saline controls, *: p<0.05, **: p<0.01.
Fig. 1-2. Changes in blood chemistry after CDDP treatment in fibrotic rats. □, saline control rat; ○, saline-treated fibrotic rat; △, CDDP (4 mg/kg)-treated fibrotic rat; ▴, CDDP (7 mg/kg)-treated fibrotic rat. Each bar and line is presented as the mean ± SD. Significantly different from saline controls, *: p<0.05, **: p<0.01.
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Fig. 1-3. Changes in blood chemistry after CDDP treatment in fibrotic rats. ( ), saline control rat; ( ), saline-treated fibrotic rat; ( ), CDDP (4 mg/kg)-treated fibrotic rat; ( ), CDDP (7 mg/kg)-treated fibrotic rat. Each bar and line is presented as the mean±SD. Significantly different from saline controls, *: p<0.05, **: p<0.01.
septa were more remarkable in the CDDP-treated fibrotic rats than the control fibrotic rats. The proliferation of bile ducts was frequently observed at portal areas in the control fibrotic rats and observed in the portal and intralobular areas in the CDDP-treated fibrotic rats. On the other hand, in all three dead CDDP (7 mg/kg)-treated fibrotic rats, marked pseudolobules and fibrotic septa were observed, and perilobular necrosis, vacuolization of hepatocytes, fibrosis and proliferation of bile ducts were also observed. (Fig. 2 and 3)

2. Kidney

In surviving animals, tubular necrosis, tubular dilatation, hyaline casts in tubules, and inflammatory cell infiltration were observed 1 day after CDDP (7 mg/kg) treatment in fibrotic rats. At 3 days after treatment, tubular necrosis, tubular dilatation, tubule hyaline cast and inflammatory cell infiltration were observed. At 10 days after treatment, tubular necrosis, tubular dilatation, basophilic tubules, hyaline casts in tubules and inflammatory cell infiltration were observed. No remarkable changes were observed in the saline control rats or control fibrotic rats. On the other hand, in all three dead CDDP (7 mg/kg)-treated fibrotic rats, tubular necrosis, tubular dilatation, basophilic tubules, hyaline casts in tubules and inflammatory cell infiltration were observed. (Fig. 4 and 5)

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**Fig. 2.** Light microscopy of the liver 3 days after CDDP treatment. No remarkable changes are seen in the saline control rat (a). Marked pseudolobules and fibrotic septa are seen in the saline-treated fibrotic control rat (b). Marked pseudolobules, moderate numbers of fibrotic septa and marked vacuolar degeneration are seen in the CDDP (7 mg/kg)-treated fibrotic rat. HE staining. ×25.

**Fig. 3.** Light microscopy of the liver 10 days after CDDP treatment. No remarkable changes are seen in the saline control rat (a). A slight proliferation of bile ducts (arrows) is seen in the fibrotic rat (b). A moderate proliferation of bile ducts (arrow) is seen in the CDDP (7 mg/kg)-treated fibrotic rat (c). HE staining. ×25.
DISCUSSION

We investigated the acute toxicity of CDDP in an experimental model of liver fibrosis established in rats with repeated intraperitoneal injections of swine serum. CDDP was administered at 4 or 7 mg/kg to the fibrotic rats. The animals were sacrificed 1, 3 or 10 days after administration and subjected to analysis of blood chemistry and histology.

Levels of hepatic markers, such as plasma ASAT, LDH, glucose, total cholesterol and bile acid levels, were significantly increased and plasma triglyceride levels significantly decreased in all fibrotic rats. These changes were more remarkable in the CDDP-treated fibrotic rats than in the control fibrotic rats. Histologically, pseudolobules and fibrotic septa were observed in all fibrotic rats. Necrosis, the vacuolization of hepatocytes, pseudolobules, fibrotic septa, and proliferation of bile ducts were more remarkable in the CDDP-treated fibrotic rats than control fibrotic rats. In alcoholic liver fibrogenetic rats, the induced CYP2E1 was enhanced by oxidative stress. Subsequently a striking depletion of GSH in the mitochondria was observed (Tsukamoto, 1993). CDDP-induced hepatotoxicity was enhanced by elevated expression of CYP2E1, and may involve increased production of ROS and oxida-

Fig. 4. Light microscopy of the kidney 3 days after CDDP treatment. No remarkable changes are seen in the saline control rat (a). Marked tubular necrosis, moderate tubular dilatation and some hyaline casts are seen in the CDDP (7 mg/kg)-treated fibrotic rat (b). HE staining. ×10.

Fig. 5. Light microscopy of the kidney 1 day after saline treatment. No remarkable changes are seen in the fibrotic rat (a). A slight amount of exudate in Bowman’s capsule and a slight increase in mesangial cells are seen in the CDDP (7 mg/kg)-treated fibrotic rat (b). HE staining. ×80.
tive stress (Lu and Cederbaum, 2006). From these findings, it is suggested that CDDP-induced hepatotoxicity occurred by single administration in fibrotic rats while CEP2E1 may be increased and GSH may be decreased in the liver.

Levels of renal markers such as plasma BUN and creatinine levels were significantly increased and plasma sodium, potassium and chloride levels significantly decreased in CDDP-treated fibrotic rats. Histologically, tubular necrosis, tubular dilatation, hyaline casts in tubules, inflammatory cell infiltration and basophilic tubules were observed in CDDP-treated fibrotic rats, though no remarkable changes were observed in control rats. In swine serum-injected fibrotic rats, it is reported that fine electron-dense deposits were diffusely distributed within the slightly broadened mesangial area, but there was no distinct thickening of the glomerular basement membrane (Kitamura et al., 1984). In the present study, we did not observe any histological changes in the kidneys of the control fibrotic rats. CDDP-induced nephrotoxicity may be accelerated in fibrotic rats.

In conclusion, hepatotoxicity was slight and nephrotoxicity was more progressed after a single CDDP treatment in an experimental model of liver fibrosis induced by repeated intraperitoneal injections of swine serum in rats.

REFERENCES


CDDP-induced toxicity in hepatic fibrotic model.


