Original Article

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Preclinical anticancer effects and toxicologic assessment of hepatic artery infusion of fine-powder cisplatin with lipiodol *in vivo*

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ABSTRACT: We conducted an *in vivo* study to evaluate the anticancer effect and toxicity of fine-powder cisplatin suspended in lipiodol (fCDDP/LPD suspension) after a single administration of three different doses to rats *via* the intrahepatic artery after transplantation of rat ascites hepatoma cells. The toxicity of the fCDDP/LPD suspension was also assessed in the same protocol in noncancer-bearing rats and the observed toxicologic changes were compared among groups administered saline (Sal), an aqueous solution of fCDDP (fCDDP/Sal solution), and LPD alone. In parallel with the toxicity test, plasma CDDP concentrations were compared between the fCDDP/LPD suspension and fCDDP/Sal solution. The mean weight of the tumors in the fCDDP/LPD suspension groups was significantly less than in the LPD-alone group. The pathologic changes in the liver observed in the fCDDP/LPD suspension group increased with dose, were more marked compared with those in the fCDDP/Sal solution and LPD-alone groups, and were reversible. No other toxicologic effects were observed. The concentration of CDDP in the plasma in the fCDDP/LPD suspension group was slightly lower than that in the fCDDP/Sal solution group. In conclusion, the results indicate that the fCDDP/LPD suspension has sufficient anticancer efficacy and tolerability for use in the clinical treatment of hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma, fine-powder cisplatin, lipiodol, transcatheter arterial chemoembolization, rats

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1. Introduction

At present, patients with unresectable or postoperatively recurrent hepatocellular carcinoma are generally treated with transcatheter arterial embolization (TAE) or transcatheter arterial infusion (TAI) of anticancer drugs. There have been numerous reports of excellent therapeutic results when hepatocellular carcinoma patients were administered a suspension of cisplatin (CDDP) in lipiodol (LPD) (*1*), an oil-soluble iodinated contrast medium that accumulates in liver cancer lesions in proportion to their vascularity (*2*,*3*). In the case of TAI, a high drug concentration at the tumor site is essential, requiring administration as close as possible to the tumor. In that context, a fine-powder formulation of CDDP (fCDDP; mean particle diameter: 20-30 μm) was developed to yield a solution with a high drug concentration. fCDDP was approved for the treatment of hepatocellular carcinoma in Japan in 2004 (*4*). Subsequently, fCDDP/LPD suspension has been used as a TAI (Lip-TAI) and transcatheter arterial chemoembolization (TACE) agent (*5*,*6*). However, no basic studies have been conducted to evaluate the efficacy and safety of Lip-TAI and TACE employing an fCDDP/LPD suspension, and the detailed mechanism of action of this combination therapy is not fully understood. This study was designed to clarify the mechanism as well as to evaluate the degree of fCDDP/LPD and CDDP bulk substance/LPD suspendability.

2. Materials and Methods

2.1. *Anticancer effects of fCDDP suspension*

2.1.1. *Experimental animals*

Fifty-seven male Donryu rats at 14 weeks of age (Charles River Japan Co., Ltd., Yokohama, Japan) were used for the study of anticancer efficacy. At 17 to 18 weeks of age, they underwent tumor cell transplantation with AH109A, a rat ascites hepatoma cell line obtained from the

Tohoku University Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Sendai, Japan. The cells were suspended in Matrigel at a concentration of 5×10^6 cells/mL. Transplantation of the AH109 cells was performed by infusing 20 µL of cell suspension into the parenchyma of the left lateral lobe of the liver of anesthetized (mixed anesthesia using ketamine 37.5 mg/kg and xylazine 5 mg/kg) rats.

Rats with confirmed tumor engraftment without ascites were selected on day 10 following transplantation and then administered the test samples. Handling of the rats and ethical considerations for all experiments were in accordance with the Regulations for Animal Experiments issued by the Committee on Animal Experiments of the Nippon Kayaku Co., Ltd. Pharmaceutical Research Laboratory.

2.1.2. *Preparation of test samples*

fCDDP (IA-call) was obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan). The fCDDP/LPD suspensions were prepared by mixing LPD (Lipiodol Ultra-Fluide, Laboratoire Guerbet, Aulnay-sous-Bois, France) with weighed amounts of fCDDP to yield CDDP concentrations of 5, 10, and 20 mg/mL and then suspensions were created in a vortex mixer.

2.1.3. *Treatment groups*

Three groups of rats (n = 8 in each group) received 20 µL of the fCDDP/LPD suspension with a fCDDP dose of 0.1, 0.2, and 0.4 mg, respectively. Two control groups received LPD alone or saline (Sal). The maximum fCDDP dosage in the fCDDP/LPD suspension groups was 20 mg/mL, corresponding to that used in clinical practice.

2.1.4. *Test sample administration*

Administration of test samples was performed under the same anesthesia used for tumor transplantation described above. A midline abdominal incision was performed to expose the injection site, the gastroduodenal artery. The test sample was drawn into a microsyringe attached to a polyethylene tube (PE-10, Becton Dickinson & Company, Franklin Lakes, NJ, USA). Then the polyethylene tube was inserted against the blood flow from the gastroduodenal artery, and the test sample was slowly administered so that it would flow into the hepatic artery with the blood.

2.1.5. *Observations, measurements, and assessments*

The tumors were enucleated carefully from the liver lobe using forceps on day 8 following test sample administration and weighed. The mean tumor weight was calculated for each treatment group, and the results are shown as mean ± SD. The tumor weight in each group was analyzed using a nonparametric method. The Dunnett test was used to analyze the anticancer effects in each fCDDP/LPD suspension group versus the LPD-alone group and in the Sal group versus the LPD-alone group. Statistical analyses were performed using the SAS system preclinical package, ver. 5.0.

2.2. *Toxicity study of fCDDP/LPD suspension*

2.2.1. *Experimental animals*

Thirty-two male Donryu rats at 10 weeks of age (Charles River Japan Co., Ltd., Yokohama Japan) were used for the study of fCDDP/LPD suspension toxicity. They were quarantined, acclimatized, and used in the experiments at 19 weeks of age, as in the anticancer experiments.

2.2.2. *Preparation of test samples*

fCDDP/LPD suspensions for use in the toxicity study were prepared at a concentration of 10 mg/mL, as in the anticancer experiments. In addition, fCDDP/Sal solution was prepared at a concentration of 1.43 mg/mL to reflect the dose used in clinical practice.

2.2.3. *Treatment groups*

The rats were divided into the Sal, fCDDP/Sal solution, LPD-alone, and fCDDP/LPD suspension groups. The fCDDP dosage for the fCDDP/LPD suspension group was 0.2 mg, because the results of the anticancer experiments showed efficacy at this dose. The same dosage was also used in the fCDDP/Sal solution group. The volume administered was 140 µL for the Sal and fCDDP/Sal solution, reflecting that used in clinical practice (1.43 mg/mL), and 20 µL (fCDDP concentration: 10 mg/mL) for the LPD-alone and fCDDP/LPD suspension groups. Each group consisted of 8 rats. Four rats were killed and autopsied on days 7 and 28 after the administration of the test samples.

2.2.4. *Test sample administration*

The test samples were administered to the rats in each group as in the anticancer experiments.

2.2.5. *Observations, measurements, and assessments*

The general condition including survival was confirmed in the rats in each group for 28 days after the administration of the test samples. Their body weight and food consumption were measured using an electrobalance scale (model PB8001, Mettler, Tokyo, Japan) at baseline and on days 2, 4, 6, 10, 14, 21, and 27 after test sample administration.

After fasting for approximately 16 h on days 7

and 28, the rats were anesthetized with diethyl ether, and blood was drawn from the abdominal aorta into a syringe. Ethylenediaminetetraacetic acid-2K was then added to the collected blood samples. A blood cell analyzer (XT-2000iV, Sysmex Corporation, Kobe, Japan) was used to analyze the following parameters for each specimen: red blood cell count; hemoglobin; hematocrit; white blood cell count, platelet count, reticulocyte count, and differential leukocyte count; corpuscular volume average; corpuscular hemoglobin; and mean corpuscular hemoglobin concentration. In addition, for blood biochemical assessment, heparin was added to blood samples in the same manner as for the hematology analyses. The specimens were then centrifuged (4°C, 1,600 × g, 10 min) to obtain the plasma, which was analyzed for the following using a Hitachi model 7180 autoanalyzer (Hitachi High-Technologies Corporation, Tokyo, Japan): aspartate aminotransferase; alanine aminotransferase; alkaline phosphatase; lactate dehydrogenase; creatine phosphokinase; total bilirubin; direct bilirubin; total cholesterol; triglycerides; phospholipids; total protein; albumin; albumin/globulin ratio; urea nitrogen; creatinine; calcium; glucose; sodium; potassium; and chloride.

After blood sampling for the hematology and blood biochemistry studies on days 7 and 28, the rats were euthanized by exsanguination from the abdominal aorta, and the injection site, heart, lungs, liver, spleen, kidneys, adrenal glands, thymus, testes, pituitary, brain, prostate, submaxillary glands, thyroid, pancreas, esophagus, stomach, duodenum, jejunum, ileum, colon, urinary bladder, epididymis, seminal vesicles, sternum, femur (including bone marrow), spinal cord, mesenteric lymph nodes, mandibular lymph nodes, thigh muscle, sciatic nerve, eyes, harderian glands, skin, mammary glands, aorta, trachea, and tongue were isolated and inspected macroscopically. Specimens of all organs were prepared to assess histopathologic changes by fixation in 10% phosphate-buffered formalin solution (for the eyes, a mixture of equal volumes of 3% glutaraldehyde and 10% phosphate-buffered formalin solution) and were stained with hematoxylin-eosin.

2.3. *Determination of CDDP concentrations in plasma*

2.3.1. *Experimental animals*

Eighteen of the 57 male Donryu rats used for the toxicologic assessment were quarantined, acclimatized, and then used for the determination of CDDP plasma concentration

2.3.2. *Preparation of test samples*

The fCDDP/LPD suspension and the fCDDP/Sal solution prepared for the toxicologic assessment were used to determine plasma CDDP concentrations.

2.3.3. *Treatment groups*

Nine rats each from the fCDDP/LPD suspension group and fCDDP/Sal solution group in the toxicologic assessment were used in this study, and the CDDP plasma concentration was measured 5 min, 6 h, and 24 h after test sample administration in 3 rats in each group.

2.3.4. *Test sample administration*

The test samples were administered to rats in the 4 groups as in the anticancer experiments.

2.3.5. *Measurement of CDDP concentration in plasma*

The plasma concentrations of total CDDP and protein-unbound (free) CDDP were analyzed. The same rats as used in the toxicokinetic (TK) studies in the fCDDP/Sal and fCDDP/LPD groups were anesthetized with diethyl ether 5 min, 6 h, and 24 h after test sample administration. Blood was collected from the abdominal aorta using a heparin-treated syringe. The blood was centrifuged (4°C, 1,800 × g, 10 min) to separate plasma, and the plasma was chilled on ice. Inductively coupled plasma mass spectrometry was used to measure the total platinum (total Pt) concentration. The protein-unbound platinum (free Pt) concentration in the plasma was analyzed with 1 ml of the plasma ultrafiltered through a Centrifree membrane filter (Millipore, Billerica, MA, USA) (4°C, 1,800 × g, 30 min). The results were converted to the CDDP concentration. The TK parameters (C_{5min}, $AUC_{0–24h}$) were calculated based on the CDDP concentration using WinNonlin software, ver. 5.2.1 (Pharsight, Mountain View, CA, USA).

2.4. *Comparison of suspendability of fCDDP and CDDP bulk substance in LPD*

For comparisons of suspendability, CDDP bulk substance was obtained from Nippon Kayaku Co., Ltd. (mean particle diameter: approximately 100 μm). One hundred milligrams of fCDDP and CDDP bulk substance in LPD was weighed and placed in vials, and then 5 mL of LPD was added to each. After vortex mixing, the 5-mL LPD suspensions were drawn into syringes. The syringes were placed in a horizontal position to observe the sedimentation visually over a 10-min period.

2.5. *Statistical analyses*

The body weight, food consumption, hematologic parameters, and blood biochemical parameters (mean ± SD) were calculated for the LPD-alone, fCDDP/Sal solution, and fCDDP/LPD suspension groups and compared with the Sal control group. The data were first tested for homogeneity of variance using the *F* test, after which a Student's *t*-test (two-sided) was employed in the

case of equal variance, while a Aspin-Welch *t*-test was applied in the absence of unequal variance. Statistical analyses were performed using the Toxi-Win system (SAS statistical package, Sumisho Computer Systems Corporation, Tokyo, Japan). A two-tailed *p* value of less than 0.05 and a *p* value of less than 0.01 were considered to represent statistically significant differences.

3. Results

3.1. *Anticancer effects of fCDDP suspension*

In the study of anticancer effects, the mean weight of tumors in the LPD group was 0.854 ± 0.875 g, whereas those in the 0.4-, 0.2-, and 0.1-mg fCDDP/LPD suspension groups were 0.198 ± 0.311 g, 0.190 ± 0.263 g, and 0.226 ± 0.134 g, respectively (Figure 1). All tumor weights in the fCDDP/LPD suspension groups were significantly lower than that in the LPD-alone group, indicating the efficacy of the fCDDP/LPD suspension dosages. There was no difference in tumor weight between the Sal and LPD-alone groups (0.963 ± 1.269 g).

Five deaths occurred during the study of one rat each time, on day 4 in the LPD group, on day 6 in both the Sal and fCDDP/LPD 0.4-mg groups, on day 7 in the fCDDP/LPD 0.4-mg group, and on day 8 in the fCDDP/LPD 0.1-mg group.

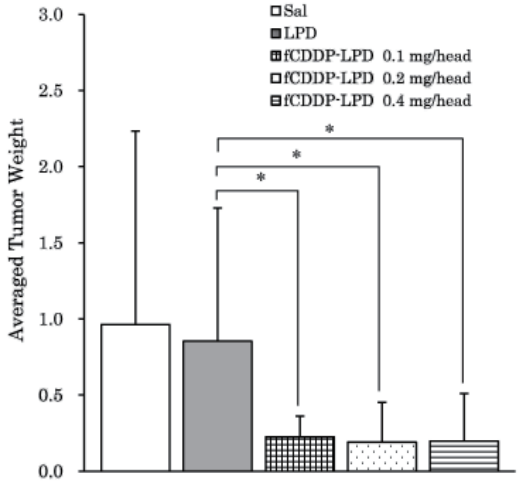

Figure 1. Antitumor activity of fCDDP suspended in LPD against orthotopically allografted rat ascites hepatoma AH109A. Mean tumor weights are shown by each bar. * $p <$ 0.05, fCDDP suspended in LPD *vs.* LPD.

3.2. *Toxicity study of fCDDP/LPD suspension*

In the study examining the toxicity of the fCDDP/LPD suspension, there were no treatment-related deaths or abnormalities among the experimental rats in the Sal, fCDDP/Sal solution, LPD-alone, or fCDDP/LPD suspension groups. Although body weight and food consumption decreased from the day after the test sample administration, both improved several days later. Compared with the Sal group, there were no statistically significant differences between the fCDDP/Sal solution, LPD-alone, and fCDDP/LPD suspension groups. The hematologic assessments performed on days 7 and 28 after test sample administration did not show any statistically significant changes in any experimental group. In addition, the blood biochemistry tests showed no significant changes in any value examined and no liver or kidney damage was observed (Figure 2).

The pathology studies found adhesion of cavity organs in all groups on days 7 and 28. In the histopathologic studies, single-cell necrosis of cholangiocytes and periportal hepatocytes was observed on day 7 in the fCDDP/Sal solution and fCDDP/LPD suspension groups, while cholangiocyte hypertrophy and vacuolation in the interlobular artery were observed in the fCDDP/LPD suspension and LPD-alone groups. Moreover, in

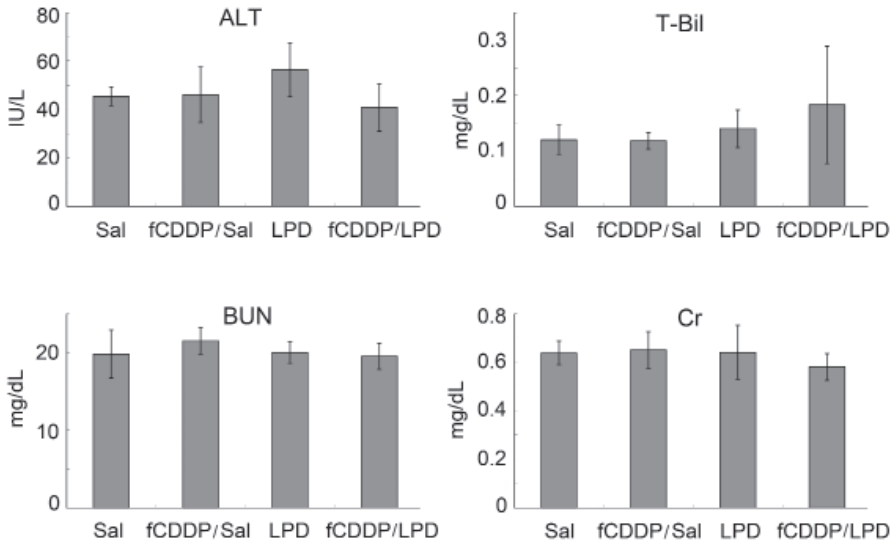

Figure 2. Serum chemistry in male rats after a single intrahepatic artery administration of each test sample.

the fCDDP/LPD group, microgranulomas around the interlobular arteries were also observed (Figure 3).

Granulomas of the gastroduodenal artery injection site and of the hepatic artery were also seen in all experimental groups. On day 28, interlobular artery vacuolation was observed in the fCDDP/LPD suspension and LPD-alone groups, and vacuolar degeneration of hepatocytes was seen in the fCDDP/LPD suspension and fCDDP/Sal solution groups. Microgranulomas were observed around the interlobular arteries except in the Sal group, while granulomas were seen in the gastroduodenal artery injection site and hepatic artery in all groups. In the kidney, regeneration of the tubular epithelium was observed on day 7 in more rats in the fCDDP/Sal solution, LPD-alone, and fCDDP/LPD suspension groups compared with the Sal group. However, no enhanced effects on the renal tubules in the fCDDP/LPD suspension group were noted. In addition, fewer rats in the fCDDP/Sal solution, LPD-alone, and fCDDP/LPD suspension groups showed abnormal changes on day 28 (Tables 1 and 2). Changes were also observed in the heart, lungs, spleen, pituitary, small intestine, and other organs of these three groups, although they were also seen to some extent in the Sal group. The changes were not considered to be caused by test sample administration.

3.3. *Determination of CDDP concentrations in plasma*

The results of the study measuring the CDDP plasma

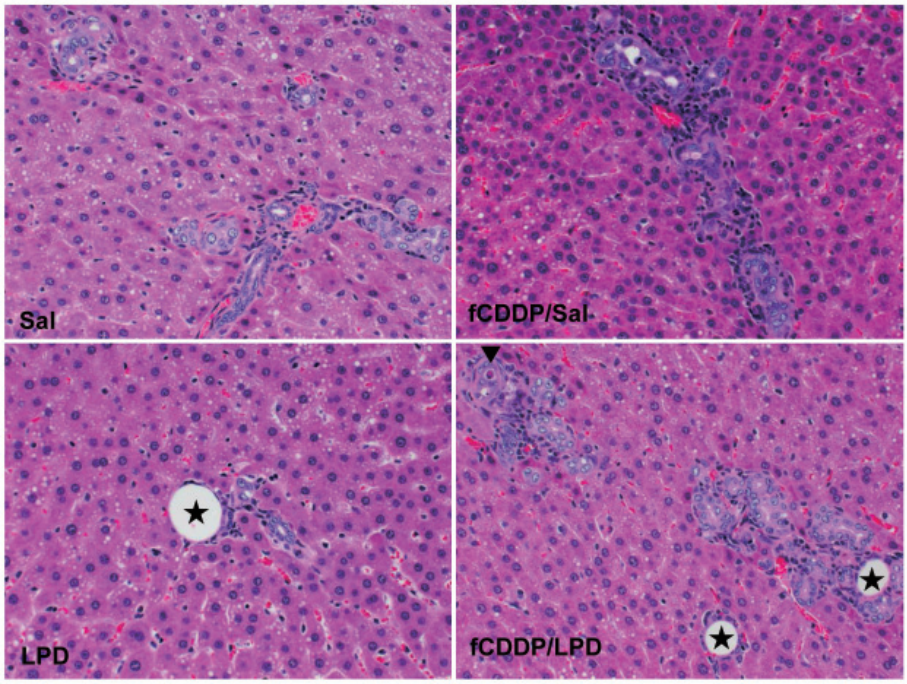

Figure 3. Single-cell necrosis of cholangiocytes (▼) and vacuolation in the interlobular artery (★) in the liver of rats treated with fCDDP/LPD suspension and LPD alone. Original magnification, ×20.

Table 1. Histopathologic findings on day 7

Object of observation	Grade	Number of rats with pathological changes			
		Sal	fCDDP/Sal	LPD	fCDDP/LPD
Liver					
Hypertrophy of cholangiocytes	1+	0	0	4	2
Vacuolation in interlobular artery	1+	0	0	3	4
Microgranuloma around interlobular artery	1+	0	0	0	3
Single-cell necrosis of hepatocytes, periportal	1+	0	0	0	1
Single-cell necrosis of cholangiocytes	1+	0	1	0	2
Microgranuloma in capsule	1+	0	2	2	2
Injection site (hepatic & gastroduodenal arteries)					
Granuloma	1+	2/3	2/3	3	1
Kidney					
Regeneration of tubular epithelium	1+	1	3	4	4
Hyaline casts	1+	0	3	3	3

Grade: 0, none; 1+, mild; 2+, moderate; 3+, severe. The fCDDP dosage for the fCDDP/Sal and fCDDP/LPD suspension group was 0.2 mg, respectively. Four rats were sacrificed and examined in each group.

Table 2. Histopathologic findings on day 28

Object of observation	Grade	Number of rats with pathological changes			
		Sal	fCDDP/Sal	LPD	fCDDP/LPD
Liver					
Microgranuloma around interlobular artery	1+	0	2	2	2
Degeneration of interlobular artery	1+	0	2	0	2
Vacuolation in interlobular artery	1+	0	0	4	4
Microgranuloma in capsule	1+	4	3	4	1
Injection site (hepatic & gastroduodenal arteries)					
Granuloma	2+	3/3	1/3	0	3
	1+	0/3	0/3	1	1
Kidney					
Regeneration of tubular epithelium	1+	0	2	1	3
Hyaline casts	1+	0	0	2	1

Grade: 0, none; 1+, mild; 2+, moderate; 3+, severe. The fCDDP dosage for the fCDDP/Sal and fCDDP/LPD suspension group was 0.2 mg, respectively. Four rats were sacrificed and examined in each group.

concentrations in the four experimental groups showed that the mean concentrations in the fCDDP/Sal solution group were 1,290 ng/mL at C_{5min}, which decreased to 114 ng/mL 6 h and to 101 ng/mL 24 h after administration. In the fCDDP/LPD suspension group, the corresponding values were 942 ng/mL, 99.9 ng/mL, and 67.8 ng/mL, respectively. In addition, the AUC_{0-24h} value was 6,200 ng^{-1} h/mL in the fCDDP/Sal solution group and 4,670 ng^{-1} h/mL in the fCDDP/LPD suspension group. Therefore these two groups had similar TK parameters.

On the other hand, the mean CDDP concentrations in the ultrafiltered plasma (free CDDP concentrations) were 729 ng/mL at C_{5min} and 2.22 ng/mL at 24 h, with an AUC_{0-24h} value of 2,300 ng^{-1} h/mL in the fCDDP/Sal solution group. In the fCDDP/LPD suspension group, the respective values were 649 ng/mL, 1.21 ng/mL, and 2,030 ng^{-1} h/mL, which were similar to those in the fCDDP/Sal group (Figure 4). However, all TK parameters in the fCDDP/LPD suspension group were lower than those in the fCDDP/Sal solution group at each time point measured (Table 3).

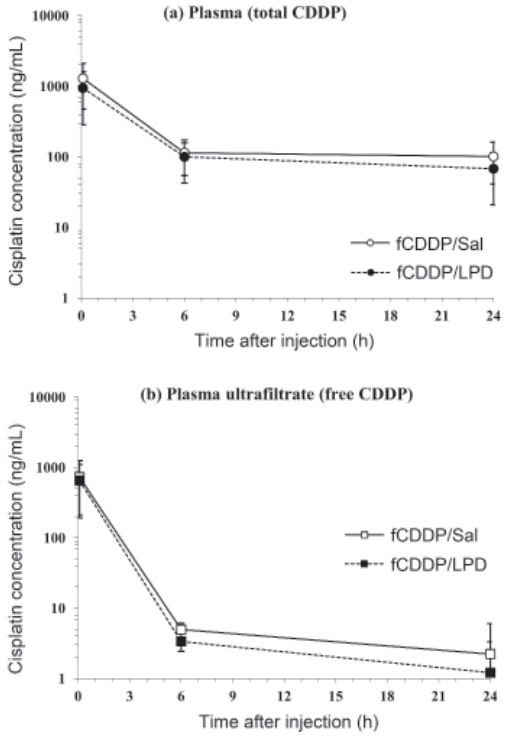

Figure 4. Plasma and ultrafiltered plasma concentration-time profiles of cisplatin in rats after a single intrahepatic artery injection of fCDDP/Sal or fCDDP/LPD (0.2 mg/rat).

3.4. *Comparison of suspendability of fCDDP and CDDP bulk substance in LPD*

When the sedimentation rate of the fCDDP and CDDP bulk substance in LPD in syringes was examined, it was found that the CDDP bulk substance rapidly began to sink to the bottom of the horizontal syringe, and nearly complete sedimentation occurred within 3 min. In contrast, the sedimentation rate of fCDDP was slower (Figure 5).

4. Discussion

Under the experimental conditions in this study, the fCDDP/LPD suspension showed anticancer effects against orthotopically transplanted AH109A hepatoma cells even at a dose of 0.1 mg of fCDDP, corresponding to the minimum fCDDP concentration administered in clinical practice. The mean tumor weight in the LPD-alone group was almost the same as that in the Sal group. This means that the tumor growth-inhibitory effects of the fCDDP/LPD suspension were likely due to the activity of fCDDP. Because an fCDDP/Sal solution group was not used in this study, we could not compare the tumor growth inhibition between the fCDDP/LPD suspension and fCDDP/Sal solution. Sonoda et al., however, performed this comparison in VX2 tumor-bearing rabbits and found that the fCDDP/LPD suspension group showed greater tumor growth inhibition than the fCDDP/Sal solution group (*7*). In addition, Morimoto *et al*. (*8*) administered fCDDP/LPD

Table 3. Toxicokinetic parameters of CDDP (0.2 mg/rat)

Test sample	Toxicokinetic parameter	Plasma (total CDDP)	Ultrafiltrated plasma (free CDDP)
fCDDP/Sal	C_{5min} (ng/mL)	1,290 ± 816	729 ± 520
	AUC_{0-24h} (ng•h/mL)	6,200	2,300
fCDDP/LPD	C_{5min} (ng/mL)	942 ± 659	649 ± 457
	AUC_{0-24h} (ng•h/mL)	4,670	2,030

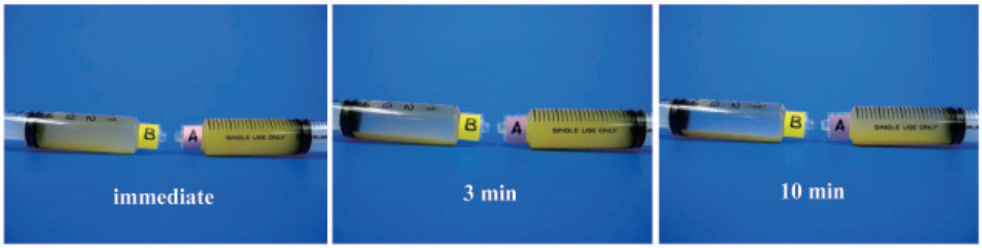

Figure 5. Comparison of time-courses of sedimentation of fCDDP and CDDP bulk substance following suspension in LPD. A: fCDDP/LPD suspension; **B:** CDDP bulk substance/LPD suspension.

suspension *via* the intrahepatic artery to VX2 tumor-bearing rabbits and compared the Pt concentrations in the tumors and normal liver tissue after TACE with fCDDP/LPD suspension and TACE with fCDDP/Sal solution. They found that the Pt concentration in normal tissues did not differ between the two treatments, whereas its concentration in tumors was 10-fold higher when fCDDP/Sal solution was used in combination with LPD (*8*). Based on that report and the results of the present study, it appears that the combined use of LPD with fCDDP in TAI and TACE for the treatment of liver carcinoma may have greater efficacy than fCDDP/Sal solution monotherapy.

The toxicologic effects of fCDDP 0.2 mg were investigated, a dosage that had shown marked effects in the anticancer activity experiments. In nontumor-bearing rats, histopathologic changes in the liver and interlobular artery vacuolation in the 0.2-mg fCDDP/LPD suspension group were caused by LPD emboli, and degeneration and single-cell necrosis were seen in the periportal hepatocytes, around the interlobular artery, and in cholangiocytes. However, the same changes were also observed in the fCDDP/Sal solution and LPD-alone groups. The changes that appeared in the fCDDP/LPD suspension group appeared to be simply extra effects. Findings that were associated with the pathologic changes observed in the liver were concluded to be mild reactions because there were no changes in other liver function markers and the pathologic changes reversed after day 28. In addition, granulomas were observed from the gastroduodenal artery (the injection site) to the hepatic artery. However, they were observed in all experimental groups, were not especially more marked in the drug-treated groups, and were considered to be caused by the administration technique.

The results indicate that the administration of fCDDP suspended in LPD does not induce a strong inflammatory reaction or other changes at the blood vessel injection site. In the combined administration of fCDDP with LPD in TAI and TACE, fCDDP is retained in the blood vessel for a long time because of the efficacy of LPD. Long-term exposure to fCDDP was expected to damage blood vessels. However, our histopathologic studies of blood vessel injection sites and the interlobular arteries of the liver did not reveal any evidence of such damage. Histopathologic examinations of the kidney showed that regeneration rates of the tubular epithelium were higher in the fCDDP/Sal, LPD-alone, and fCDDP/LPD groups than in the Sal group. However, those changes were mild in each drug-treatment group, and there were no abnormal changes in the levels of plasma urea nitrogen or creatinine. Therefore, we conclude that the administration of fCDDP suspended in LPD does not adversely affect kidney function.

When the CDDP concentrations in plasma and ultrafiltered plasma were compared, little difference was found between the fCDDP/Sal solution and fCDDP/LPD suspension groups in terms of the TK parameters. Therefore, we believe that the administration of fCDDP/Sal solution formulated as a suspension in LPD has almost no effect on the systemic exposure to CDDP. Morimoto et al. found that the Pt concentrations in both plasma and ultrafiltered plasma were lower in TACE performed using fCDDP combined with LPD than in TACE using an fCDDP aqueous solution without LPD (*8*). Compared with the results after the administration of fCDDP without LPD, the LPD suspension may alleviate the adverse reactions associated with CDDP, *i.e.*, kidney damage, nausea and vomiting, *etc*. Moriguchi *et al*. (*9*) also found that the total Pt concentration in plasma after intrahepatic artery administration of an LPD suspension of the clinically recommended dosage of fCDDP, 65 mg/m^2, to patients with unresectable hepatocellular carcinoma was lower

than when fCDDP was not suspended in LPD for 1 h following administration, and it increased thereafter. Therefore, the results from this study showing that the CDDP values in the fCDDP/LPD suspension group were lower than those in the fCDDP/Sal solution group at all measurement times is similar to the results from the previous two reports (*8*,*9*).

In summary, we found that a single intrahepatic artery administration of fCDDP/LPD suspension to rats with orthotopically transplanted hepatoma showed striking anticancer efficacy attributed to CDDP. In the toxicology study, the toxicity of the treatment with the suspension at a dosage that had shown anticancer efficacy was similar to that of fCDDP/Sal solution and LPD alone. The main toxicity was liver dysfunction and was reversible. The side effects caused by systemic exposure to CDDP were milder when it was combined in the LPD suspension.

Based on the present results, we conclude that the fCDDP/LPD suspension has sufficient anticancer efficacy and is well tolerated in the treatment of hepatocellular carcinoma. In addition, our results and those of previous studies (*8*,*9*) indicate that fCDDP has more potent efficacy in combination with LPD than when combined with Sal solution when treating hepatic cancer *via* TAI or TACE. Regarding the suspendability of fCDDP and CDDP bulk substance in LPD at a CDDP concentration of 20 mg/mL, the concentration used in clinical practice, the sedimentation of fCDDP was much slower than that of CDDP bulk substance. Future studies should be conducted to determine CDDP tumor concentrations in both fCDDP/LPD and fCDDP/Sal solution groups.

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