Hepatic Arterial Infusion Therapy with Cisplatin using Protein Binding Inhibition: Pharmacokinetics and Antineoplastic Effects of Cisplatin Combined with L-Cysteine in Rats

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Abstract

Covalent binding is involved in the protein binding of cisplatin. L-cysteine reduces the covalent binding of cisplatin. We investigated hepatic arterial infusion therapy with cisplatin using protein binding inhibition. In the present experiment, the pharmacokinetics and antineoplastic effects of cisplatin combined with L-cysteine in male Donryu rats were investigated. As a result, no significant difference was noted in the total and free concentrations of cisplatin combined with L-cysteine. In an *in vivo* experiment using rats with liver cancer, the hepatic arterial infusion of cisplatin combined with L-cysteine showed that it was the tendency that tumor growth rate was inhibited in comparison with administration only for cisplatin. In addition, concentrations of cisplatin increased significantly between tumor and non-tumor regions in liver tissue when combined with L-cysteine (p<0.01). Thus, L-cysteine can be combined with cisplatin for hepatic arterial infusion therapy.

Key words :cisplatin, protein binding inhibition, covalent binding, hepatic arterial infusion, L-cysteine

INTRODUCTION

The effects of drug therapy generally depend on the concentration of free drug that is not bound to human serum proteins and thus remains free to exert its therapeutic effect. The protein binding rate of cisplatin (*cis*-diamminedichloroplatinum [II]) in the blood is about 90%[1]. Cisplatin plays a major role in cancer chemotherapy. Cisplatin may not exert sufficient effects after administration because of a low free drug concentration. Cisplatin is locally administered around the tumor by hepatic arterial infusion as well as systemic administration. In transcatheter hepatic arterial chemoembolization (TACE), cisplatin should accumulate around the tumor, when combined with an embolus material, to disrupt the nutrient supply to the tumor. Inhibiting the protein binding of cisplatin may increase the free cisplatin concentration to facilitate cisplatin migration to tumor tissue. We have demonstrated that cisplatin binds mainly to human serum albumin (HSA) among human plasma proteins, and reported the involvement of covalent binding because of a time-dependent increase in the protein binding rate of cisplatin. The covalent

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binding site with HSA is a free SH group in Cys34[2]. We considered it beneficial to make this free SH group bind to a certain safe substance beforehand. Then, we demonstrated that L-cysteine with a SH group reduces the covalent binding of cisplatin[3]. Thus, we examined the application of hepatic arterial infusion therapy with cisplatin using protein binding inhibition. In the present study, we report the effects of the concomitant use of L-cysteine on the pharmacokinetics and protein binding of cisplatin in rats and the antineoplastic effects of cisplatin combined with L-cysteine in tumor-bearing rats.

MATERIALS AND METHODS

Animals

Male Donryu rats (Charles River Japan) were used. The rats were allowed free access to food and water for 1-week preliminary rearing.

Reagents

Cisplatin powder was supplied by Nippon Kayaku Co., Ltd. L-cysteine were purchased from Wako Pure Chemical Industries, Ltd. All other reagents used were special grade.

Pharmacokinetics Experiment

A test protocol on pharmacokinetics is shown in Figure 1. The rats were anesthetized with pentobarbital (50 mg/kg). A polyethylene tube PE50 (Becton Dickinson, 0.58-mm internal diameter, 0.965-mm external diameter) was inserted into the carotid artery and jugular vein. The rats were divided into three groups. The control group received the intravenous (i.v.) administration of saline (2 mL/kg), and, subsequently, cisplatin (2 mg/2 mL/kg) through a jugular cannula. The IV group received L-cysteine i.v. (100 mg/2 mL/kg) through a jugular cannula, and, subsequently, cisplatin i.v. (2 mg/2 mL/kg). The intravenous drip injection (DIV) group received L-cysteine d.i.v. (100 mg /2 mL/kg) through a jugular cannula for 1 hour before and after cisplatin administration. To prevent blood coagulation, the cannula was flushed with 0.1 mL of heparin (100 unit/mL). At 2, 10, 60, and 180 min after cisplatin administration, 750μL of blood was collected from the jugular cannula. The blood was left at room temperature for 30 min and centrifuged at 3,000 rpm for 15 min to fractionate serum.

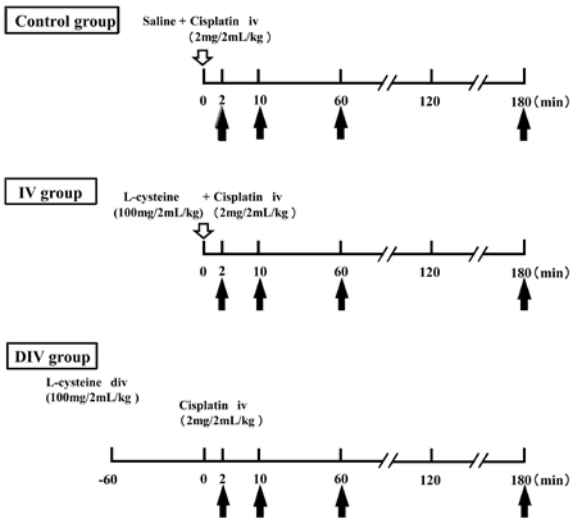

Figure. 1 Test Protocol on Pharmacokinetics.

The rats were divided into three groups. Control group: saline + cisplatin i.v., IV group: L-cysteine + cisplatin i.v., DIV group: L-cysteine d.i.v. + cisplatin i.v..

Measurement of Serum Cisplatin Concentration

The total serum cisplatin (bound and free cisplatin) concentration was determined as described below. To determine the serum total cisplatin concentration, the collected serum (8μL) was 5-fold diluted with blank serum (32μL), and the diluted serum was 5-fold diluted with 0.05% Triton-X/0.4N HCl (160μL). The 25-fold diluted serum thus obtained was used as a measurement sample. This sample was used for measurement with an atomic absorption photometer (Z-5010, Hitachi). Cisplatin reference solutions (0-0.2μg/mL) were prepared to create a calibration curve. The free cisplatin concentration in serum was determined as described below. The serum (250μL) was centrifuged at 3,200 rpm for 15 min with an ultrafilters (MINICENT-10, Tosoh). The obtained filtrate (20μL) was 5-fold diluted with purified water (80μL) to prepare a measurement sample.

This sample was used for measurement with an atomic absorption photometer. Cisplatin reference solutions (0-1.0 μg/mL) were prepared to create a calibration curve.

Preparation of Liver Cancer Rat Model

A rat ascites hepatoma strain, AH-109A, was kindly provided by Dr. Kishimoto Shuichi of the Faculty of Pharmaceutical Sciences, Kobe Gakuin University. The AH-109A cells were intraperitoneally subcultured in the ascites of a 4-week-old rat. A suspension of the subcultured AH-109A cells was subcutaneously transplanted into another 4-week-old rat. The solid tumor which grew up after subcutaneously transplantation was removed. The necrotic region was removed from the tumor. The tumor was cut into 1-mm pieces in a Hanks' balanced salt solution to be used for transplantation. A 9-week-old rat weighing about 400 g underwent laparotomy under Nembutal anesthesia. A graft was transplanted into the parenchyma of the left lobe of the liver using a commercial indwelling venous catheter with a 20G needle. Specifically, an intravascular emboli-promoting agent, Gelpart® (Nippon Kayaku), and a graft were used to fill the catheter tip in this order. The catheter was inserted into the parenchyma of the liver using a polyethylene tube PE10 (Becton Dickinson, 0.28-mm internal diameter, 0.61-mm external diameter) from the connection of the catheter syringe. Then, the catheter was removed. Figure 2 shows tumor transplantation and hepatic arterial infusion sites in a rat model of liver cancer.

Hepatic Arterial Infusion Therapy

On day 20 after transplantation, the rat underwent laparotomy under anesthesia. The major and minor axes of the tumor were measured. Then, the common hepatic, gastroduodenal, and proper hepatic arteries were identified with sutures. Subsequently, a polyethylene tube was inserted from the gastroduodenal artery to the bifurcation of the common hepatic and proper hepatic arteries and fixed with a suture. Then, an agent was slowly infused into the bloodstream from the common hepatic to proper hepatic arteries. After the completion of administration, the polyethylene tube was removed. The gastroduodenal artery was ligated, and the abdomen was sutured. Then, the rat was reared under normal conditions.

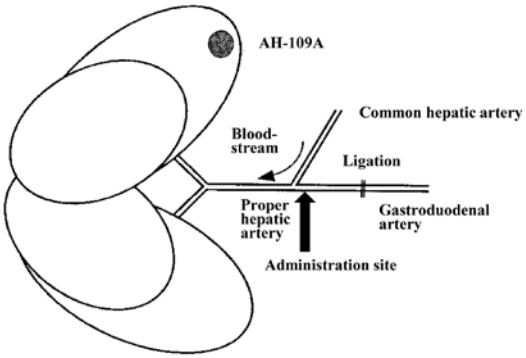

Figure. 2 Tumor Transplantation and Hepatic Arterial Infusion Sites in Rat Model of Liver Cancer.

A polyethylene tube was inserted from the gastroduodenal artery to the bifurcation of the common hepatic and proper hepatic arteries and fixed with a suture. Then, an agent was slowly infused into the bloodstream from the common hepatic to proper hepatic arteries.

Antineoplastic Effects

The rats were divided into three groups. The control group received a hepatic arterial infusion of saline (2 mL/kg). The cisplatin group received a hepatic arterial infusion of cisplatin (2 mg/2 mL/kg). The L-cysteine + cisplatin group received L-cysteine d.i.v. (100 mg /2 mL/kg) through a jugular cannula for 1 hour before and after cisplatin administration. Cisplatin was administered by hepatic arterial infusion. To prevent blood coagulation, the cannula was flushed with 0.1 mL of heparin. On day 7 after administration (i.e., on day 27 after transplantation), the rat was anatomized to remove the liver. The major and minor axes of the tumor were measured. Blood was collected from a lower leg artery.

Tumor growth rate (%) = (major axis x minor axis after administration / major axis x minor axis before administration) x 100

Measurement of Cisplatin Concentration in Liver Tissue

The removed liver was divided into tumor and non-tumor regions to determine the cisplatin concentration in liver tissue. The measurement was conducted by NAC Co., Ltd (Tokyo, Japan).

Statistical Analysis

All results were expressed as the mean ± S.E.. The unpaired Student *t*-test, Bonferroni test, and SNK test were employed for two-group, control, and multiple- group comparisons, respectively.

RESULTS

Effects of L-Cysteine on Pharmacokinetics of Cisplatin

No significant difference was noted in the total and free concentrations among the three groups (Figures 3(A) and 3(B)). No effect of L-cysteine was noted even in the free fraction (Figure 3(C)).

Effects of L-Cysteine on Antineoplastic Effects of Cisplatin

The cisplatin group and L-cysteine + cisplatin group showed significantly stronger inhibition of tumor growth rate compared with the control group ($p<0.01$) together. No significant difference was noted between the L-cysteine + cisplatin group and cisplatin group, although a tendency to inhibit of tumor growth rate was present (Figure 4). A comparison of the cisplatin concentrations in the tumor and non-tumor regions of liver tissue between the L-cysteine + cisplatin and cisplatin groups demonstrated no difference in the concentrations in the non-tumor region between the groups, but a higher concentration in the tumor region for the L-cysteine + cisplatin group (Table 1). The concentrations of cisplatin increased significantly between tumor and non-tumor regions in liver tissue when combined with L-cysteine ($p<0.01$). The cisplatin group showed significant weight loss compared with the control group ($p<0.05$). However, no significant difference was noted between the L-cysteine + cisplatin and control groups (Table 2). All the groups showed elevated AST values. However, L-cysteine caused no change in AST values. No change was observed for the other laboratory values.

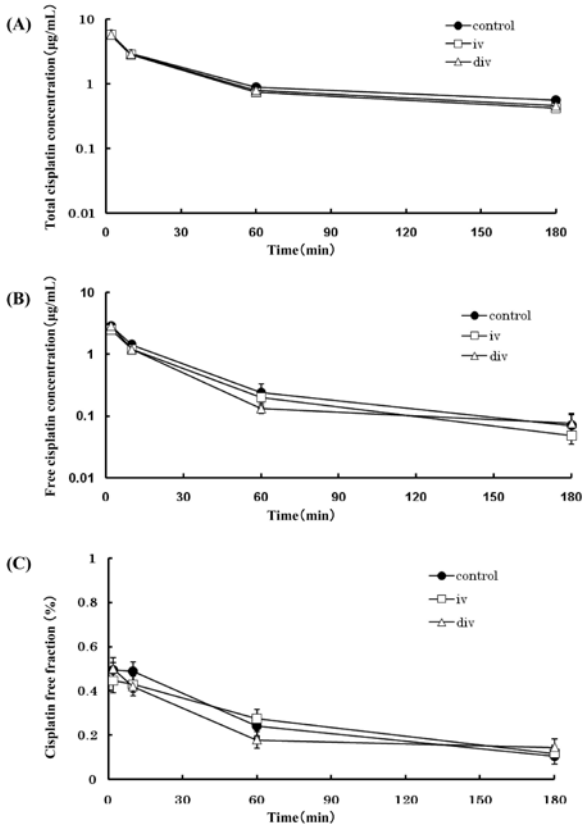

Figure. 3 Effects of L-Cysteine on Pharmacokinetics of Cisplatin.

(A) Effects of L-Cysteine on Pharmacokinetics of Total Cisplatin.

(B) Effects of L-Cysteine on Pharmacokinetics of Free Cisplatin.

(C) Effects of L-Cysteine on Pharmacokinetics of Free Fraction Cisplatin.

There is no significant difference. Each value represents the mean ± S.E. of seven or eight rats.

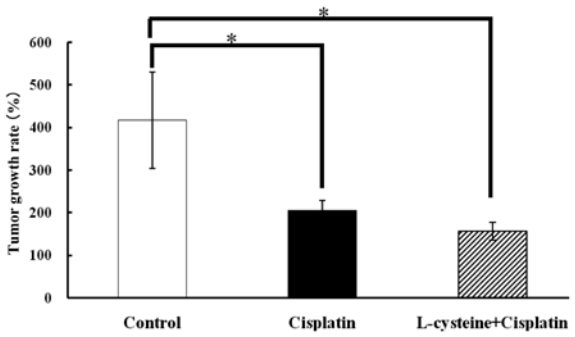

Figure. 4 Effects of L-Cysteine on Antineoplastic Effects of Cisplatin.

The cisplatin group and L-cysteine + cisplatin group showed significantly stronger inhibition of tumor growth rate compared with the control group ($p<0.01$) together. No significant difference was noted between the L-cysteine + cisplatin group and cisplatin group, although a tendency to inhibit of tumor growth rate was present. Each value represents the mean ± S.E. of eight or nine rats.

Table 1 Cisplatin level in liver tissue of cisplatin-hepatic arterial infusion 7 days later

Cisplatin concentration (μg/g)	Cisplatin	L-Cysteine + Cisplatin
Tumor region	0.12±0.09	0.23±0.08
Non-Tumor region	0.02±0.01	0.02±0.016 **

** $p<0.01$ (L-cysteine + cisplatin tumor region vs L-cysteine + cisplatin non-tumor region), Each value represents the mean ± S.E. of eight or nine rats.

Table 2 Tumor growth rate / weight gain rate / laboratory values of cisplatin-hepatic arterial infusion 7 days later

	Control	Cisplatin	L-cysteine + Cisplatin
Number of the rats (n)	6-8	5-8	9
Tumor growth rate (%)	417.5±113	206.6±23*	157.5±20.6*
Weight gain rate (%)	101.7±6	83.5±4.6*	94.2±0.9
Albumin(g/dL)	2.5±0.05	2.7±0.12	2.9±0.12
Total Protein(g/dL)	5.5±0.09	5.3±0.13	5.5±0.09
Total Billirubin(mg/dL)	0.2±0.03	0.2±0.04	0.2±0.02
AST(IU/L)	184.8±12.3	186.1±22.6	166.3±16.9
ALT(IU/L)	38.2±3.5	43.6±11.1	38.2±3.3
BUN(mg/dL)	23.4±2.2	28.8±4.1	21.9±1.2*
S-Cr(mg/dL)	0.3±0.04	0.3±0.04	0.2±0.02

* $p<0.05$ (control group vs cisplatin group or L-cysteine + cisplatin group), Each value represents the mean ± S.E. of five or nine rats.

DISCUSSION

In an *in vivo* experiment using rats with liver cancer, the hepatic arterial infusion of cisplatin combined with L-cysteine showed that it was the tendency that tumor growth rate was inhibited in comparison with administration only for cisplatin. The cisplatin concentrations were significantly different between tumor and non-tumor regions in liver tissue when cisplatin was combined with L-cysteine ($p<0.01$).

To examine the effects of L-cysteine on the pharmacokinetics of cisplatin under normal conditions, the pharmacokinetics after hepatic arterial infusion therapy should be examined on day 20 after transplantation. In the present study, another experiment was conducted because the effects of pharmacokinetic changes, caused by blood collection on day 20 after transplantation, on the antineoplastic effects on day 7 after hepatic arterial infusion (on day 27 after transplantation) were undeniable. Thus, in the present study, the dosage of L-cysteine (100 mg/2 mL/kg) was 200-fold higher than that required to achieve a routine blood level to examine pharmacokinetic changes. However, this dosage is safe in view of the results of a single-dose toxicity study (L-cysteine LD50: 6,350 mg/kg p.o.) [4]. The results demonstrated no significant difference in the total and free concentrations among the three groups. In addition, no effect was noted on the free fraction. The following reasons were considered: 1) the inhibitory effects of L-cysteine were not reflected on the blood concentration because of a low binding rate at the beginning of cisplatin administration (the protein binding rate immediately after the addition of cisplatin in human serum was about 18%[3]); 2) cisplatin was systemically administered through an intravenous route; and 3) the timing of the intravenous drip injection of L-cysteine was inappropriate in the DIV group. The dosage and administration timing should be examined in detail.

In the experiment on the antineoplastic effects of cisplatin when combined with L-cysteine, the protein binding inhibition of L-cysteine may have increased the free cisplatin concentration in the tumor and enhanced inhibitory effects on tumor growth. L-cysteine increases the blood 5-FU concentration and enhances antineoplastic effects[5, 6]. Iigo and Kawabata *et al.* reported that when 5-FU is orally administered with L-cysteine, it inhibits drug-metabolizing enzymes to delay 5-FU metabolism and blood concentration reduction, thereby enhancing the antineoplastic effects. L-cysteine is converted to disulfide cystine or glutathione, an important biological sulfhydryl compound,

in the living body. Glutathione is medicine for chronic liver diseases. Hepatic glutathione is depleted during liver injury. L-cysteine prevents the glutathione depletion to protect the liver[7]. The L-cysteine + cisplatin group showed a more marked inhibition of tumor growth rate than the cisplatin group. For this reason, it may be the interaction that added the unknown mechanism that is different from the protein binding inhibition of cisplatin, which we think about it. All the groups showed elevated AST values, which may have resulted from elevated values in liver cancer.

Thus, the concomitant use of L-cysteine may be applicable for TACE. For the application, the following dosing regimens may be possible. First, L-cysteine solution is used in hydration conducted before and after cisplatin administration to a patient with liver cancer. Routinely, 1,000-2,000 mL hydration is conducted to alleviate serious side effects (*e.g.*, renal damage). The administration of an amino acid agent containing L-cysteine should inhibit the protein binding of cisplatin to increase the free cisplatin concentration, thereby enhancing the antineoplastic effects. Second, the hepatic arterial infusion of high concentrations of L-cysteine through a catheter is employed before the local hepatic arterial infusion of cisplatin. Third, L-cysteine is orally administered before TACE to maintain a high blood L-cysteine concentration, thereby inhibiting the protein binding of cisplatin. Thus, these dosing regimens of anticancer agents using protein binding inhibition should be effective even at low doses. To establish these regimens, further basic research is needed.

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蛋白結合阻害を利用した cisplatin 肝動注療法に関する研究：ラットにおける L-cysteine 併用時における cisplatin の体内動態と抗腫瘍効果

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要　旨

Cisplatin の蛋白結合には共有結合が関与しており、L-cysteine は cisplatin の共有結合を低下させる。そこで、この蛋白結合阻害を利用して cisplatin の肝動注療法の応用性について検討を行った。実験では、Donryu 系雄性ラットにおける L-cysteine 併用時における cisplatin の体内動態と抗腫瘍効果の影響について検討した。その結果、L-cysteine 併用において cisplatin の total と free 濃度に有意な差はみられなかった。また肝癌ラットを使用した *in vivo* 実験系において、L-cysteine を併用した cisplatin の肝動注は cisplatin のみの投与に比べ、腫瘍増殖率が抑えられる傾向であることを示した。さらに肝組織中における腫瘍部と非腫瘍部における cisplatin 濃度において、L-cysteine 併用により腫瘍部と非腫瘍部に有意な差を認めることができた ($p<0.01$)。以上の結果より、L-cysteine の併用は cisplatin の肝動注療法へ応用できると考えられる。

キーワード：シスプラチン、蛋白結合阻害、共有結合、肝動注、L- システイン