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LABORATORY INVESTIGATION

Transarterial Chemoembolization Using Cisplatin Powder in a Rabbit Model of Liver Cancer

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Abstract The purpose of this study was to investigate the pharmacological advantages of transarterial chemoembolization (TACE) with cisplatin powder for hypervascular hepatic tumors in animal experiments. VX2 tumors were transplanted to the livers of nine rabbits. Cisplatin (1 mg/kg) was infused into the proper hepatic artery. In the cisplatin-HAI group, cisplatin solution was infused. In the cisplatin-GS-TACE group, after infusion of cisplatin solution, gelatin sponge particles were used for embolization. In the cisplatin-Lp-TACE group, after infusion of a cisplatin powder and lipiodol (10 mg/ml) suspension, gelatin sponge particles were used for embolization. Before and after administration, platinum concentrations in plasma were measured. Using liver specimens that were excised 60 min after infusion, platinum concentrations in tumorous and nontumorous liver tissues were measured. The mean platinum concentration in tumorous tissue was 0.88 μg/ml for the cisplatin-HAI group, 1.23 μg/ml for the cisplatin-GS-TACE group, and 12.65 μg/ml for the cisplatin-Lp-TACE group. The platinum concentration for the cisplatin-Lp-TACE group was significantly higher than that for the cisplatin-HAI group ($p = 0.004$) and the cisplatin-GS-TAE group ($p = 0.004$). The mean platinum concentration in nontumorous liver tissue was 0.98 μg/ml for the cisplatin-HAI group, 1.13 μg/ml for the cisplatin-GS-TACE group, and 1.09 μg/ml for the cisplatin-Lp-TACE group; no significant differences were seen. At both 5 and 10 min after infusion, the platinum concentrations for the cisplatin-Lp-TACE group were lower than those for the other two groups. The present results suggest that TACE using cisplatin powder/lipiodol suspension and gelatin sponge for hypervascular hepatic tumors has a number of pharmacological advantages.

Keywords Rabbit with VX2 tumor · Experimental study · Chemoembolization · Hepatocellular carcinoma · Cisplatin powder

Introduction

Transarterial chemoembolization (TACE) is widely performed in the treatment of advanced hepatocellular carcinoma, and randomized controlled trials have documented better vital prognosis for hepatocellular carcinoma [1, 2]. Many studies on TACE have been reported; a method using lipiodol, an oily contrast medium used as a drug delivery system (DDS), is now widely employed, and anticancer drugs such as doxorubicin, epirubicin, and other anthracyclines are often used [3–6]. However, hepatocellular carcinoma is not necessarily sensitive to these drugs [7–9]. Hence, the therapeutic results of TACE for hepatocellular carcinoma should improve with anticancer drugs being more efficient.

Cisplatin (*cis*-dichlorodiammineplatinum) is a platinum compound that has been used as an effective anticancer drug for various cancers, including head and neck cancer and bladder cancer [10], and studies have reported that hepatocellular carcinoma is more sensitive to cisplatin compared to anthracyclines [11, 12]. Cisplatin has been used on TACE for

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hepatocellular carcinoma in Europe and America since 1992. However, because cisplatin was only available as a solution, it was difficult to prepare a high-dose cisplatin emulsion using lipiodol. Therefore, cisplatin has hardly been used for TACE in Japan [13, 14]. In recent years, cisplatin powder (IA-CALL; Nippon Kayaku, Tokyo) has been developed, and its use in hepatic artery infusion for hepatocellular carcinoma was approved in July 2004 in Japan [15]. Using this cisplatin powder, it is easier to prepare lipiodol suspensions, and we suspect that there will be more clinical studies on TACE using cisplatin powder and lipiodol in the treatment of hepatocellular carcinoma. To the best of our knowledge, there have been no basic studies on TACE using cisplatin and lipiodol, and in the present study, we investigated the pharmacological usefulness of TACE using a cisplatin powder and lipiodol suspension (cisplatin-lipiodol-TACE) for hepatocellular carcinoma by performing cisplatin-lipiodol-TACE on rabbits in which VX2 tumors (hypervascular liver tumors) were transplanted into the liver.

Materials and Methods

Outline of Study Design

VX2 tumors were transplanted into the liver of nine Japanese white rabbits, and each rabbit was then subjected to one of the following three treatments: (1) three rabbits made up the cisplatin-HAI group, in which a cisplatin infusion was administered from the proper hepatic artery; (2) three rabbits were in the cisplatin-GS-TACE group, where, after administration of cisplatin infusion from the proper hepatic artery, embolization was performed using gelatin sponge particles; and (3) three rabbits comprised the cisplatin-Lp-TACE group, in which, after infusion of a cisplatin powder/lipiodol suspension into the proper hepatic artery, embolization was performed using gelatin sponge particles. Before and after treatment of the nine rabbits in the three groups, platinum concentrations in plasma, tumor tissue, and nontumorous liver tissue were measured and analyzed.

Animal Care and Rabbits

Experiments were performed in accordance with standard guidelines and adhered to local regulations, as specified by the animal committee of our institute.

Nine Japanese white rabbits with VX2 tumors transplanted into the livers were used. VX2 tumors were purchased from Japan SLC (Shizuoka, Japan). Tumors were implanted by embedding 1-mm tissue cubes below the left lobe capsule for 2 weeks. The body weight of rabbits receiving liver tumors ranged from 2.60 to 2.95 kg, with an average of 2.79 kg.

Angiographic Procedure

Rabbits were anesthetized by 5% isoflurane inhalation. Through surgical cutdown, a 4-Fr sheath (Terumo, Tokyo) was inserted from the common femoral artery, and under fluoroscopic guidance, a 4 Fr hockeystick catheter (CATHEX: Cathex, Kanagawa, Japan) was inserted into the celiac artery. By the coaxial method, a 2.0 -Fr microcatheter (Prograte α; Terumo) and a 0.016-in. guide wire (GT wire 0.016; Terumo) were used for selective catheterization of the proper hepatic artery. After confirmation of vessel insertion and tumor staining by contrast radiography, arterial infusion and TACE were performed (Fig. 1).

Preparation of CDDP and Gelatin Sponge

In the cisplatin-HAI group (cisplatin solution for hepatic arterial infusion) and cisplatin-GS-TACE group (chemoembolization with gelatin sponge), 100 mg of cisplatin powder (IA CALL; Nippon Kayaku, Tokyo) was dissolved in 70 ml of saline solution (1.43 mg/ml). In the cisplatin-Lp-TACE group (cisplatin-lipiodol suspension for lipiodol chemoembolization with gelatin sponge), 100 mg of cisplatin powder was mixed with 10 ml of lipiodol (Lipiodol Ultra-Fluid; Gelbe Japan-Terumo, Tokyo; 10 mg/ml), stirred manually just before infusion for about 1 min, and then subjected to ultrasonic mixing for 3 min. Gelatin sponges (Spongel; Astellas, Tokyo) were manually cut into $\sim$1-mm particles and mixed with a small amount of iopamidol (Iopamiron 370; Shering, Osaka, Japan).

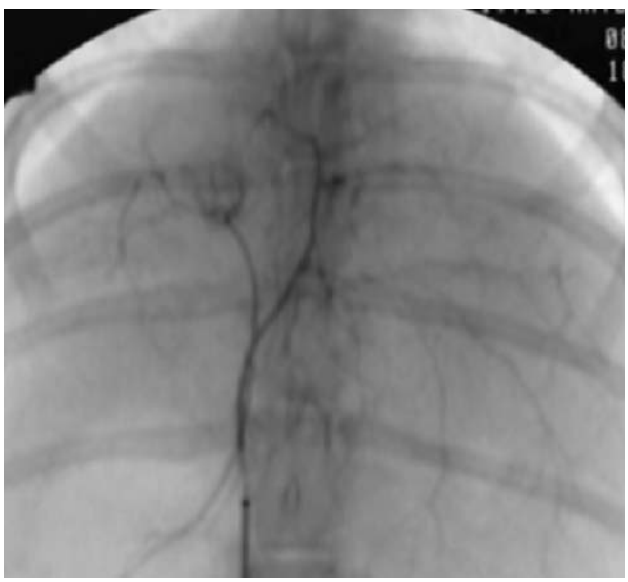

Fig. 1 Intense staining of intrahepatic tumor by angiography through the proper hepatic artery

Administration of Cisplatin

To three rabbits, a 0.7 ml/kg cisplatin solution was infused in one shot, followed by immediate injection of 1 ml of physiological saline (cisplatin-HAI group). To another set of three rabbits, the same amount of the cisplatin solution was infused in one shot, then gelatin sponge particles were immediately injected until hepatic artery flow became congested (cisplatin-GS-TACE group). To the last set of three rabbits, a 0.1 ml/kg cisplatin-lipiodol suspension was gradually infused, and then gelatin sponge particles were injected until hepatic artery flow became congested (cisplatin-Lp-TACE group). In all groups, the cisplatin dosage was set at 1 mg/kg body weight according to the usual clinical dosage of 1 mg/kg to the human. The injection speed of cisplatin saline and the timing of gelatin sponge were set equally in each group.

Blood and Tissue Sampling and Drug Concentration Assay

Blood (3 ml) was sampled from each rabbit at 5, 10, 30, and 60 min after administration of cisplatin. Each sample was centrifuged, the resulting plasma was filtered using Centriflo for determining filterable platinum concentration, and the filtrate was immediately stored frozen (−7°C). After collection of blood samples at 60 min after administration, rabbits were euthanized by deep anesthetization and the entire liver was excised. Liver tumors were carefully recovered. In addition, the left lobe was excised as a nontumorous liver tissue specimen. All specimens were immediately stored frozen (−7°C). Analysis was performed via atomic absorption spectroscopy, with each sample measured twice.

Statistical Analysis

Mean and SD of platinum concentrations in each tissue were calculated, and a post hoc test (Scheffe method) was used to statistically assess intergroup differences (Dr. SPSS II 11.0.1 J; SPSS Inc., Chicago, IL). The level of significance was set at $p < 0.05$. AUC analysis was conduced by the noncompartment method (Model: Plasma data, Bolus IV Administration; WinNonlin Profession Ver. 4.1; Pharsight Corp., Mountain View, CA).

Results

Tissue Platinum Concentration (Table 1)

The platinum concentration in tumorous tissue was 0.88 ± 0.26 μg/g ($p = 0.004$) for the cisplatin-HAI group, 1.23 ± 0.85 μg/g ($p = 0.004$) for the cisplatin-GS-TACE group, and 12.65 ± 3.45 μg/g for the cisplatin-Lp-TACE group was. These results showed that that platinum concentration for the cisplatin Lp-TACE group was significantly higher than that for the other two groups. No significant differences were seen in platinum concentrations in tumorous tissue between the cisplatin-GS-TACE and cisplatin-HAI groups.

Table 1 Platinum concentrations (μg/g) in tumorous and nontumorous liver tissues

Tissue	HAI	GS-TACE	Lp-TACE
Tumorous	0.88 ± 0.26	1.23 ± 0.85	12.65 ± 3.46
Nontumorous	0.98 ± 0.42	1.13 ± 0.42	1.09 ± 0.30

Platinum concentrations in nontumorous liver tissue for the cisplatin-HAI, cisplatin-GS-TACE, and cisplatin-Lp-TACE groups were 0.98 ± 0.42, 1.13 ± 0.42, and 1.09 ± 0.30 μg/g, respectively, and no significant intergroup differences were seen.

Plasma Platinum Concentration (Tables 2 and 3)

Table 2 reports the sequential changes in total platinum concentration in plasma. At 5 and 10 min after administration, the total plasma platinum concentration for the cisplatin-Lp-TACE group was significantly lower compared to those for the cisplatin-GS-TACE and cisplatin-HAI groups (5 min after, $p = 0.017$ and $p = 0.01$, respectively; and 10 min after, $p = 0.039$ and $p = 0.017$, respectively). At 5 and 10 min after administration, no significant differences were seen in total plasma platinum concentration between the cisplatin-GS-TACE and the cisplatin-HAI groups. At 30 and 60 min after administration, no significant differences were seen in total plasma platinum concentration among the three groups.

Table 3 reports the sequential changes in filterable platinum concentration in plasma. At 5 and 10 min after

Table 2 Plasma total platinum concentration (μg/ml)

	HAI	GS-TACE	Lp-TACE
5 min	2.45 ± 0.41	2.31 ± 0.32	1.16 ± 0.27
10 min	1.94 ± 0.17	1.79 ± 0.26	1.10 ± 0.28
30 min	1.00 ± 0.07	0.94 ± 0.20	0.82 ± 0.05
60 min	0.50 ± 0.06	0.49 ± 0.11	0.57 ± 0.11

Table 3 Plasma filterable platinum concentration (μg/ml)

	HAI	GS-TACE	Lp-TACE
5 min	2.08 ± 0.36	1.99 ± 0.32	1.02 ± 0.33
10 min	1.61 ± 0.21	1.58 ± 0.17	0.94 ± 0.24
30 min	0.81 ± 0.08	0.84 ± 0.11	0.59 ± 0.25
60 min	0.36 ± 0.07	0.37 ± 0.06	0.36 ± 0.16

Table 4 AUC at 0–60 min for total platinum and filterable platinum (μg min/ml)

Platinum	IA	GS-TACE	Lp-TACE
Total	77.0 ± 7.4	72.5 ± 12.45	51.7 ± 6.1
Filterable	62.9 ± 7.2	62.5 ± 8.5	39.8 ± 12.0

administration, the filterable platinum concentration for the cisplatin-Lp-TACE group was significantly lower than that for the cisplatin-GS-TACE and cisplatin-HAI groups (5 min after, $p = 0.034$ and $p = 0.023$, respectively; and 10 min after, $p = 0.03$ and $p = 0.024$, respectively). At 5 and 10 min after administration, no significant differences were seen in filterable platinum concentration between the cisplatin-GS-TACE and the cisplatin-HAI groups. At 30 and 60 min after administration, there were no significant differences in plasma filterable platinum concentration among the three groups.

Table 4 shows the AUC (area under the concentration-time curve) at 0–60 min for total and filterable platinum concentrations. The $AUC_{0–60\ min}$ (μg min/ml) for the total platinum concentration for the cisplatin-Lp-TACE group was significantly lower than that for the cisplatin-HAI group ($p = 0.038$). The $AUC_{0–60\ min}$ (μg min/ml) for the filterable platinum concentration was lower for the cisplatin-Lp-TACE group compared to the cisplatin-HAI group, but no significant difference was seen ($p = 0.064$). Between the cisplatin-HAI and the cisplatin-GS groups, there were no significant differences in the $AUC_{0–60\ min}$ of total and filterable platinum concentrations.

Discussion

TACE is a treatment that is performed for hepatocellular carcinoma, a hypervascular tumor, to achieve embolization and antitumor effects using an anticancer drug. In order to selectively deliver the anticancer drug to the tumor and make the drug remain inside the tumor for a long period of time, an emulsion made of lipiodol and an anticancer drug (DDS) is infused from the hepatic artery, and hepatic arterial flow is blocked using an embolizing material, such as gelatin sponge.

Anthracyclines, such as doxorubicin and epirubicin, are most frequently being used in TACE. Hepatocellular carcinoma is known as a tumor with a poor sensitivity to anticancer agents, and studies have documented the response rate of systemic chemotherapy and hepatic arterial infusion chemotherapy using anthracyclines at about 3% and 15%, respectively [7–9]. On the other hand, studies have documented relatively high response rates for systemic chemotherapy and hepatic arterial infusion chemotherapy using cisplatin at 15% and 45%, respectively [11, 12].

If cisplatin is used in TACE, even higher antitumor effects may be achieved. However, since cisplatin was supplied as a solution in the past, it was difficult to prepare a high-dose cisplatin emulsion using lipiodol. In fact, cisplatin in the form of a powder rather than a solution has been used since 1992 in Europe and America. However, in Japan, it has not been used for TACE except at a few institutions because cisplatin has only been available as a solution. At limited institutes of Japan, a dry powder of cisplatin was made from a solution to make a mixture with lipiodol, however, these techniques have not been practiced widely because of the complicated procedures [13, 14].

In July 2004, a cisplatin powder (IA CALL; Nippon Kayaku, Tokyo) was developed and approved for use in hepatic arterial infusion for hepatocellular carcinoma [15]. This made it easier to prepare a lipiodol/cisplatin suspension by mixing lipiodol and cisplatin powder. Therefore, TACE using the cisplatin powder should improve the therapeutic results for hepatocellular carcinoma, and while there will probably be more clinical studies on TACE using lipiodol and cisplatin powder (cisplatin-Lp-TACE), there have not been sufficient basic studies on TACE using cisplatin.

The VX2 liver tumor used in the present study is a hypervascular tumor, is mostly vascularized by the hepatic artery [16], and resembles human hepatocellular carcinoma in terms of hemodynamics. By assuming TACE for hepatocellular carcinoma, in the present study, the drug was infused by the coaxial method using a microcatheter under fluoroscopic guidance.

In the cisplatin-Lp-TACE group, the platinum concentration in tumorous tissue was higher than that in nontumorous liver tissue. The results were the same in past studies on doxorubicin or epirubicin [17, 18], and the reason for this was that nontumorous liver tissue was vascularized by the hepatic artery and portal vein, while tumorous tissue was only vascularized by the hepatic artery. Another reason is the hypervascular nature of the tumor in relation to normal liver tissue. Also, the platinum concentration in tumorous tissue was about 14 times higher for the cisplatin-Lp-TACE group than for the cisplatin-HAI group. When simply infused from the hepatic artery, the extraction rate for cisplatin is low, and the usefulness of arterial infusion is relatively low [19, 20]. However, by infusing a lipiodol suspension and performing embolization using gelatin sponge, the intratumoral concentration of the anticancer drug in the present method was just as high as with TACE using doxorubicin or epirubicin [17, 18]. The reason for this was the long-term retention of cisplatin in vessels and the increased vascular wall permeability.

In the cisplatin-GS-TACE group, there was no significant difference in platinum concentration between tumorous and nontumorous liver tissues, and there was no

marked difference in intratumoral anticancer drug concentration compared to that in the cisplatin-HAI group. Also, there was no significant difference in plasma platinum concentration between the cisplatin-GS-TACE and the cisplatin-HAI groups. In one past study, cisplatin solution and gelatin sponge were mixed and then administered into the uterine artery [21], but there has not been a pharmacological study on TACE where cisplatin was administered and then an embolizing agent, such as gelatin sponge, was used. In TACE for hepatocellular carcinoma, if a cisplatin solution is mixed with an embolizing agent, it is difficult to adjust the total cisplatin dose, and as a result, cisplatin is often administered before the embolizing agent. The results of the present study suggest that before blocking blood flow by gelatin sponge, most of the cisplatin was not retained in the liver but flowed outside the liver.

The plasma platinum concentrations for the cisplatin-Lp-TACE group at 5 and 10 min after administration were lower than those for the cisplatin-GS-TACE and cisplatin-HAI groups. This agreed with previous studies on plasma concentrations following TACE using doxorubicin/lipiodol emulsion and gelatin sponge injection [18]. Therefore, cisplatin-lipiodol-TACE using a cisplatin powder/lipiodol suspension should reduce the side effects associated with cisplatin, such as nephrotoxicity, nausea, and vomiting.

In conclusion, TACE using a cisplatin powder/lipiodol suspension (cisplatin-lipiodol-TACE) achieved very high platinum concentrations in hepatic tumors, and should lead to marked antitumor effects. In addition, because platinum concentrations in peripheral blood were low, side effects would be minimized. Furthermore, in TACE where a cisplatin solution was arterially infused and gelatin sponge used for embolization, there were no marked differences in plasma and tissue platinum concentrations compared to those with ordinary hepatic arterial infusion, and as a result, the pharmacological usefulness of cisplatin-GS-TACE was low. In terms of limitations, the present study did not investigate the effects of cisplatin TACE on the liver parenchyma. In the future, clinical studies need to be conducted to confirm both therapeutic efficacy and safety.

References

1. Lo CN, Ngan H, Tso WK et al (2002) Randomized controlled trial of transarterial lipiiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 35:1164–1171
2. Llovet JM, Real MI, Montana X et al (2002) Arterial embolizasion or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellur carcinoma: a randomized controlled trial. Lancet 359:1734–1739
3. Ohishi H, Yoshimura H, Uchida H et al (1989) Transcatheter arterial embolization using iodozed oil (lipiodol) mixed with an anticancer drug for the treatment of hepatocellular carcinoma. Cancer Chemother Pharmacol 23:S33–S36
4. Uchida H, Ohishi H, Matsuo N et al (1990) Transcatheter hepatic segmental arterial embolization using lipiodol mixed with an anticancer drug and gelfoam particles for hepatocellur carcinoma. CardioVasc Interv Radiol 13:140–145
5. Nakamura I, Hashimoto T, Oi H et al (1989) Transcatheter oily chemoembolization of hepatocelluar carcinoma. Radiology 170: 783–786
6. Takayasu K, Shima Y, Muramatsu et al (1987) Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. Radiology 162:345–351
7. Lai CL, Wu PC, Chan CG et al (1988) Doxorubicin versus no antitumor thrapy in inoperable hepatocellular carcinoma. A prospective randomized trial. Cancer 62:479–483
8. Bokemeyer C, Kynast B, Harstrick A et al (1995) No synergistic activity of epirubicin and interferon-alpha 2b in the treatment of hepatocellular carcinoma. Cancer Chemother Pharmacol 35:334–338
9. Nagasue N, Yukawa H, Okamura J et al (1986) Intraarterial administration of epirubicin in the treatment of non-resectable hepatocellular carcinoma. Epirubicin Study Group for Hepatocellular. Jpn J Cancer Chemother 13:2786–2792
10. Einhorn LH, Williams SD (1979) The role of cis-platinum in solid-tumor therapy. N Engl J Med 300:289–291
11. Okada S, Okazaki N, Nose H et al (1993) A phase 2 study of cisplatin in patients with hepatocellular carcinoma. Oncology 50:22–26
12. Abe R, Akiyoshi T, Koba F et al (1988) 'Two-route chemotherapy' using intra-arterial cisplatin and intravenous sodium thiosulfate, its neutralizing agent, for hepatic malignancies. Eur J Cancer Clin Oncol 24:1671–1674
13. Maeda S, Shibata J, Fujiyama S et al (2003) Long-term follow-up of hepatic artrial chemoembolization with cisplatin suspended in lodized oil for hepatocellular carcinoma. Hepato-Gastroenterology 50:809–813
14. Ueno K, Miyazono N, Inoue H et al (2000) Transcatheter arterial chemoembolization therapy using lodized oil for patients with unresectable hepatocellular carcinoma. Cancer 88:1574–1581
15. Inose H (2004) Through a fusion of intellectual assets from within and beyond the company, and through accelerated product development, we are aiming for growth in the health care field, and particularly growth in cancer-related treatment areas. Annual Report 10-13. Available at: http://www.nipponkayaku.co.jp/english/ir/report/pdf/2004/04e10-13.pdf
16. Burton MA, Kelleher DK, Codde JP et al (1991) Changes in hepatic blood flow during regional hyperthermia. Int J Hyperthermia 7:271–277
17. Sakaguchi H, Yoshimura H, Nishimura Y et al (1990) Behavior of Anti-cancer drug of transcatheter hepatic segmental arterial chemoembolization using lipodol mixed with an anti-cancer drug followed by gelatine sponge assessed by Tc-99m perthechnetate. Jpn J Cancer Chemother 17:1725–1730
18. Raoul JL, Heresbach D, Bretagne JF et al (1992) Chemoembolization of hepatocellular carcinomas. A study of the biodistribution and pharmacokinetics of doxorubicin. Cancer 70:585–590
19. Garnick MB, Ensminger WD, Israel M (1979) A clinical-pharmacological evalusion of hepatic arterial infusion of adriamycin. Cancer Res 39:4105–4110
20. Campbell TN, Howell SB, Pfeifle CE et al (1983) Clinical pharmacokinetics of intraarterial cisplatin in humans. J Clin Oncol 12:755–762
21. Harima K, Harima Y, Hasegawa T et al (1995) Transcatheter arterial emboliazion as a model of cisplatin-retention enhancement on the VX2 tumor uterus transplants. CardioVasc Interv Radiol 18:30–34