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Therapeutic effect of a CDDP-epirubicin-Lipiodol emulsion on advanced hepatocellular carcinoma

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Abstract. Prior injection of an anticancer agent and Lipiodol mixture is a key point for the treatment of hepatocellular carcinoma (HCC). We therefore prepared a new, improved emulsion of Lipiodol containing a high dose of *cis*-diamminedichloroplatinum (CDDP) and epirubicin by replacing the ionic contrast medium (Urografin 67) with a nonionic contrast medium (Iopamidol; Iopamiron 300) and adding phosphatidyl choline. This CDDP-epirubicin-Lipiodol emulsion (CELE) was examined pharmacologically and chemically with the following results. The size of these particles is less than 10 μm (diameter) for up to 24 h; the release of 28%–34% of the CDDP and 80%–90% of the epirubicin was estimated in the dissolution test, and 85% of the CDDP and 35% of the epirubicin was retained in the organs in the moment calculation. CELE was injected into 58 HCC patients via a celiac angiographic catheter. In 36 of these patients, the CELE injection was followed by transcatheter arterial embolization (TAE) therapy. Following the administration of CELE as one-shot injection therapy for stage IV HCC, the 1-year survival rate was 59% and the 2-year survival rate was 27%. Moreover, in patients (stage II, 12; stage III, 8; stage IV, 16) who received CELE and subsequently underwent TAE therapy, the 1-year survival rate was 90% and the 2-year survival rate was 67%. The nonionic contrast medium with Lipiodol forms finer emulsified particles, and these particles are more capable of penetrating into the tumor. In addition, the greater pharmacological stability of these particles provides a slow-release effect and prolonged stability of their shape. Finally, theoretically, the use of two major anticancer agents such as CDDP and epirubicin showed a greater clinical effect in the treatment of HCC than either our earlier suspension or a single anticancer agent.

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Introduction

It is well known that in the treatment of hepatocellular carcinoma (HCC) by transcatheter arterial embolization (TAE) therapy, prior injection of an anticancer agent and Lipiodol mixture might be necessary. However, this mixture of emulsified or suspended particles is not stable either pharmacologically or theoretically. In a previous symposium, we introduced a new suspension consisting of two kinds of anticancer agent and Lipiodol for the treatment of HCC [1]. We tried to prepare an improved emulsion that would be more stable pharmacologically, would show more efficient accumulation in the tumor and slower release, and would achieve penetration of finer particles into the tumor capillaries and longer clinical survival in spite of advanced HCC. This report focuses on the clinical results we obtained in the treatment of HCC with a *cis*-diamminedichloroplatinum(CDDP)-epirubicin-Lipiodol emulsion (CELE) [2, 5].

Patients and methods

Preparation of CELE

CELE was prepared as follows. In all, 240 mg of phosphatidyl choline (Asahi Kasei Co., Tokyo) was mixed with 6 ml of Lipiodol (Kodama Co., Tokyo) in an agate mortar. The mixture was collected in a vial and heated to obtain a transparent liquid. The transparent liquid was again mixed with 80 mg of CDDP in an agate mortar (CDDP-Lipiodol suspension, CLS). In parallel, 60 mg of epirubicin hydrochloride (Kyowa Hakko Kogyo Co., Ltd., Tokyo) was dissolved in 3 ml of Iopamidol (Iopamiron 300; Nihon Schering Co., Tokyo), which is a representative nonionic contrast medium (epirubicin-Lipiodol emulsion, ELE). For our earlier CDDP-epirubicin-Lipiodol suspension (CELS) [1], 60 mg of epirubicin hydrochloride was dissolved in 1.8 ml of distilled water and then 4.2 ml of Urografin 76, which is a representative ionic contrast medium, was added. In this study, we changed this process and the materials. Then, immediately, the two mixtures (CLS and ELE) were combined and emulsified in a cylinder, resulting in well-dispersed CELE.

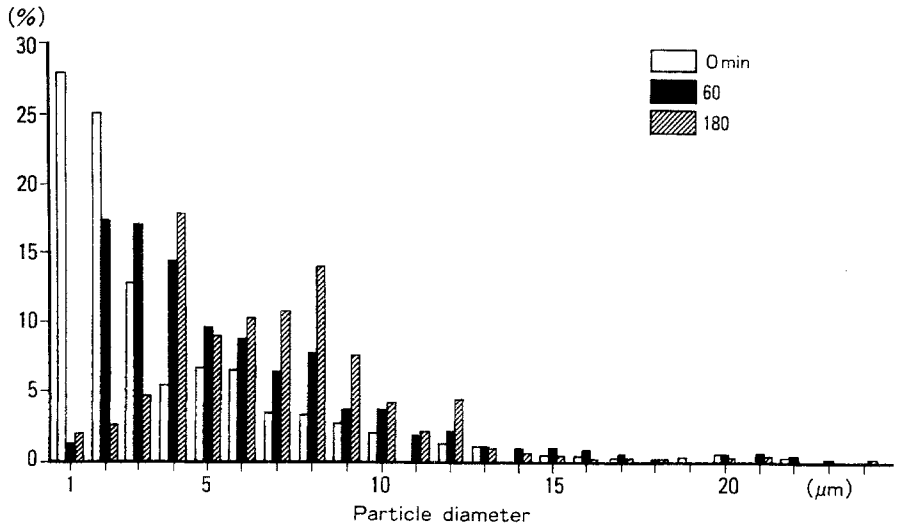

Fig. 1. Size distribution of CELE particles immediately, 60 min, and 180 min after preparation

Size distribution of prepared CELE particles

The size distribution of CELE was analyzed with an Auto Image Analyzer RAS-GN3000 (Amersham Co.). CELE was dissolved in Iopamidol to give a 40-fold dilution and then analyzed immediately after its preparation and at 30, 60, and 180 min after its preparation and dissolution.

Freeze-fracture scanning electron microscopy

CELE was subjected to observation of the distribution of particles in freeze-fracture scanning electron microscopy.

Sustained-release study in vitro

A dissolution test was performed in accordance with the Japanese Pharmacopeia. That is, 1 ml of CELE was placed in a saline solution (1000 ml) and stirred at 25 rpm at 37°C. The sample solution was then analyzed for CDDP and epirubicin using a high-performance liquid chromatography system equipped with an ultraviolet detector.

Table 1. Stage classification of hepatocellular carcinoma

Stage I		(T1)	Solitary tumor; less than 2 cm, vascular invasion (–)
Stage II	(A)	(T2)	Solitary tumor; less than 2 cm, vascular invasion (+)
	(B)	(T2)	Multiple tumor; less than 2 cm in one lobe
	(C)	(T2)	Solitary tumor; more than 2 cm, vascular invasion (–)
Stage III	(A)	(T3)	Solitary tumor; more than 2 cm, vascular invasion (+)
	(B)	(T3)	Multiple tumor; more than 2 cm in one lobe
	(C)	T1–T3	With lymph node metastasis (N1)
Stage IV-A	(A)	(T4)	Multiple tumors in both lobes
	(B)	(T4)	Vascular invasion of portal or hepatic vein
IV-B		T1–T4	With extrahepatic metastasis (M1)

According to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer, with TNM classification [3]

Serum levels of CDDP and epirubicin

The serum levels of both CDDP and epirubicin were analyzed in five patients until 24 h postinjection. Moment calculation was done using the results of the serum concentration of both CDDP and epirubicin with the area under the curve (AUC) as determined by Yamaoka's method.

Patients

In all, 22 patients with unresectable HCC (stage IV) received CELE via a celiac angiographic catheter, and 36 patients with HCC (stage II, 12; stage III, 8; and stage IV, 16) subsequently underwent TAE therapy with Gelfoam particles. In a comparative clinicopharmacology study, we also injected CLS via a celiac angiographic catheter into 22 HCC patients, 16 of whom subsequently underwent TAE therapy. None of these patients satisfied the indications for either surgical resection or percutaneous ethanol injection therapy. The decision of whether to perform TAE therapy depended on the liver function, the tumor anatomy, and the grade of portal vein invasion in each case.

Stage classification of HCC

HCC cases were divided into four groups on the basis of the number of lesions and their size, localization, and vascular invasiveness (TNM classification) according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [3] (Table 1).

Survival rate

Survival was estimated by Kaplan-Meier's method.

Results

Size distribution of CELE

The mean diameter of the CELE particles was 1.4 μm immediately after their preparation and then 3.3 μm at 30 min, 4.1 μm at 60 min, and 6.8 μm at 180 min after their preparation (Fig. 1).

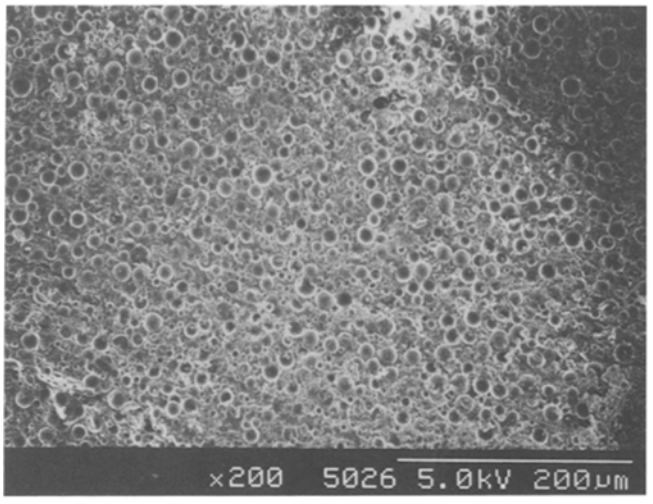

Fig. 2. Scanning electron microscopy by freeze-fracture of prepared CELE particles in the panoramic view

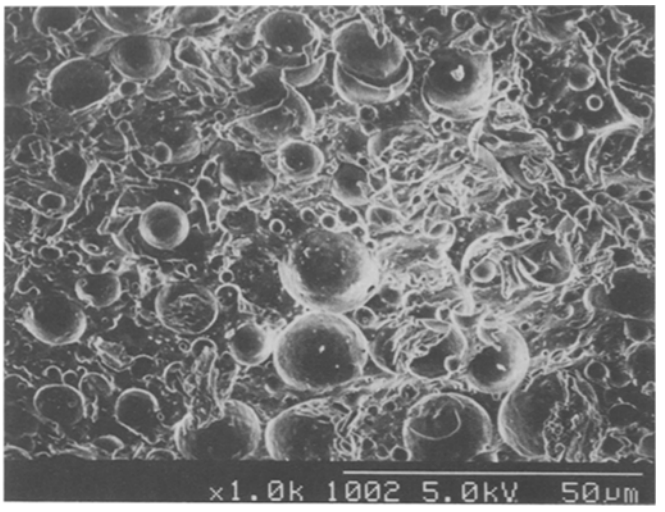

Fig. 3. High magnification of scanning electron microscopy by freeze-fracture of prepared CELE particles

Freeze-fracture scanning electron microscopy

Freeze-fracture scanning electron microscopy revealed round-shaped particles, which measured less than 10 µm in diameter (Figs. 2, 3).

Dissolution test on anticancer agents

The results of dissolution of CDDP indicated that the percentage of CDDP released from CELE was 28% after 1 h, 31% after 3 h, and 34% after 24 h. However, the results of dissolution of epirubicin showed that the percentage of epirubicin releasd from CELE was 80% after 1 h, 90% after 3 h, and 85% after 24 h (Fig. 4). In contrast, the percentage of CDDP released from CELS was approximately 50% over a 24-h observation period. Furthermore, the percentage of CDDP released from CLS was less than 20% over the same period. There was no difference in the release of epirubicin from CLS, CELS, and CELE.

Serum levels of anticancer agents

Figure 5 shows the time courses of the serum levels of CDDP and epirubicin. The moment calculation estimating the AUC showed that approximately 85% of the CDDP and 35% of the epirubicin was retained in the organs. Our earlier preparation showed that approximately 54% of the CDDP and 35% of the epirubicin was retained in the organs [1].

Survival

Following the administration of CELE and CLS as one-shot therapy, the 1-year survival rates were 59% in the CELE group and 25% in the CLS group. Moreover, the 2-year survival rates were 27% in the CELE group and 0 in the CLS group. In patients who received CELE or CLS and subsequently underwent TAE therapy, the 1-year survival rates were 90% (CELE group) and 70% (CLS group), and the 2-year survival rates were 67% (CELE group) and 10%

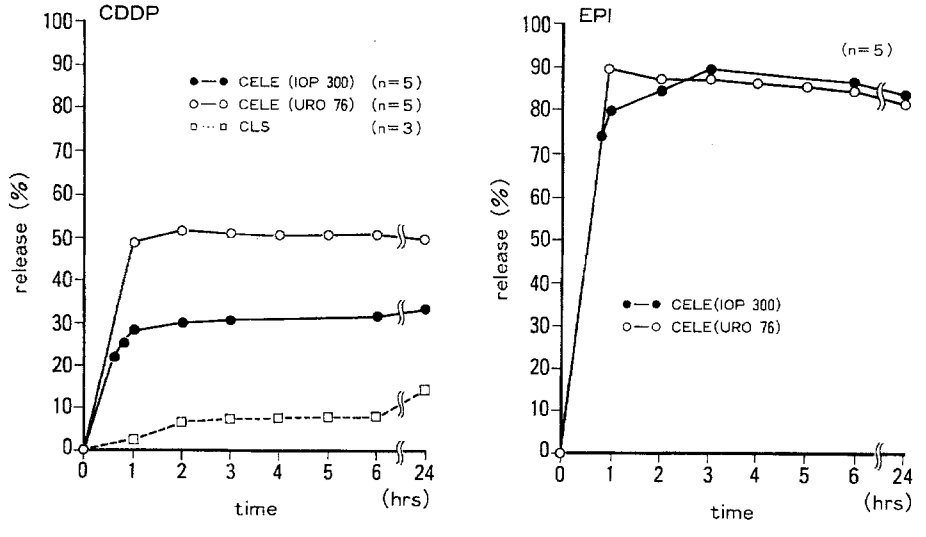

Fig. 4. Dissolution profiles of anticancer drugs at 37° C. *CELE*, CDDP-epirubicin-Lipiodol emulsion; *CLS*, CDDP-Lipiodol suspension; *IOP 300*, Iopamiron 300; *URO 76*, Urografin 76; *CDDP*, *cis*-diamminedichloroplatinum; *EPI*, epirubicin

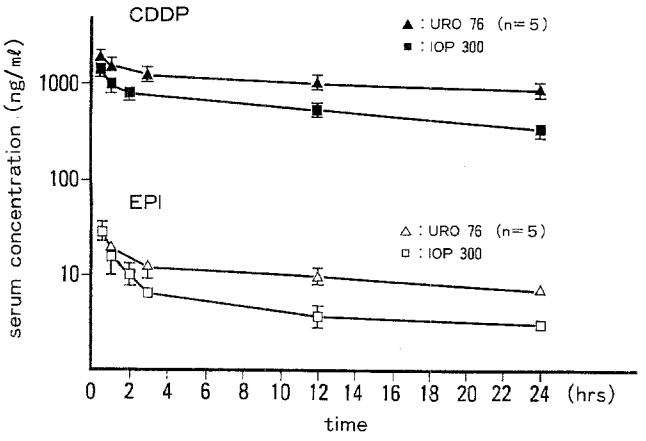

Fig. 5. Changes in serum levels of CDDP and epirubicin after administration of CELE. *URO 76*, Urografin 76; *IOP 300*, Iopamiron 300

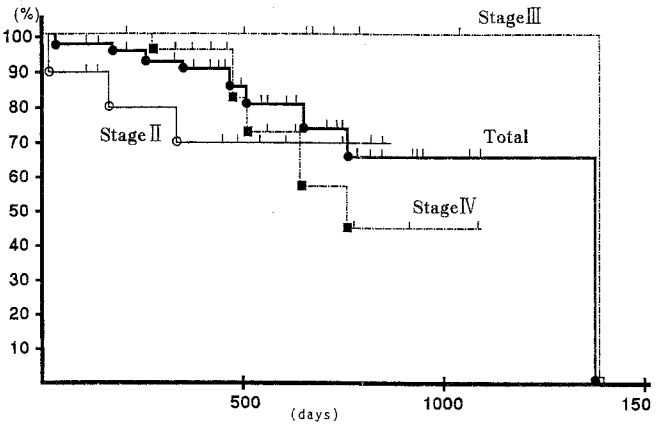

Fig. 6. Survival curves for HCC cases treated with CELE injection and subsequent TAE therapy as generated by the Kaplan-Meier method

Table 2. Summary of survival rates of HCC cases treated with CELE injection and subsequent TAE therapy

	CELE + TAE				CELE One-shot (n = 22)
	Stage			Total	
	II (n = 12)	III (n = 8)	IV (n = 36)	(n = 36)	
1 year	71%	100%	93%	90%	59%
2 year	71%	100%	43%	67%	27%
3 year	–	100%	–	67%	–

Table 3. Clinical results of TAE therapy for HCC (nonoperative cases)

	Conventional TAE[a] (n = 60)	CELE + TAE[a] (n = 36)	National survey (n = 4960)
1 year	48.3%	90%	58.3%
2 year	26.7%	67%	34.0%
3 year	3.3%	67%	20.7%
5 year	1.6%	–	8.3%

[a] Data from the Third Department of Internal Medicine, Niigata University School of Medicine (through 1992)

(CLS group). The 3-year survival rate was also 67% in the CELE group in this study. In patients who received CELE and subsequently underwent TAE therapy for stage II HCC, both the 1-year and the 2-year survival rates were 71%. The survival of stage III HCC cases was 100% at the end of the 3-year observation period. In CELE group patients with stage IV HCC, the 1-year survival rate was 93% and the 2-year survival rate was 43% (Fig. 6, Table 2).

Discussion

We compared our present clinical results with both the survival data from a national survey conducted by the Liver Cancer Study Group of Japan [3] and the data obtained with our former conventional TAE therapy through 1989. The survival rate obtained after CELE injection and subsequent TAE was much higher than those reported in the other sets of data (Table 3). This high survival rate suggests that use of the improved CELE and subsequent TAE therapy for advanced HCC gains its advantage by achieving a sharp accumulation in the tumor tissues, from its slow-release function, and from its being endowed with prolonged stability of the shape of the particles. By replacing the ionic contrast medium with a nonionic contrast medium in the preparation of CELE with phosphatidyl choline, we obtained an emulsion having greater pharmacological stability as shown by the results of the dissolution tests and the serum levels of both CDDP and epirubicin. In addition, the size distribution of CELE was less than 10 μm after its preparation. These fine particles are capable of penetrating into the tumor capillaries and tumor sinusoids.

Hepatic resection [4] and TAE therapy are not indicated as treatment for HCC in stage IV patients who have suffered from vascular invasion of both the hepatic vein and the portal vein. To date, we have tried to inject a high dose of an anticancer agent via a celiac artery and intravenously, but we could not get satisfactory clinical results. However, our results showed prolonged survival for CELE injection via a celiac artery, either repeatedly or with subsequent TAE therapy. This CELE injection therapy might become one of the key treatments for advanced HCC.

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