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Transcatheter hepatic arterial chemoembolization using epirubicin-lipiodol: experimental and pharmacological evaluation*

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Summary. The experimental and pharmacological characteristics of various formulations of an anticancer agent (epirubicin, EPI) and lipiodol were evaluated in vitro and in vivo. Three forms of EPI-lipiodol, i.e., an oil-in-water type of emulsion (O/W type), a water-in-oil type of emulsion (W/O type), and a suspension (S type), were prepared and investigated for their stability. An O/W-type emulsion using a stock solution of Iopamidol as the solvent for EPI was the most stable form in the stationary state in vitro. In 16 patients with malignant liver tumors (14 hepatocellular carcinomas and 2 liver metastases), the three forms of EPI-lipiodol were injected into the proper hepatic artery. The plasma EPI level was monitored periodically and analyzed pharmacokinetically. No significant difference in the pharmacokinetics of EPI was detected among the O/W, W/O, and S types.

Introduction

The efficacy of transcatheter hepatic arterial chemoembolization (TACE) in the therapy of malignant liver tumors has been highly evaluated, and TACE is now performed in various medical institutions. Along with the development of novel materials for chemoembolization of the hepatic artery and of new anticancer agents, clinical trials have been conducted on various antineoplastics mixed or suspended in lipiodol (a lipid contrast medium). Epirubicin (EPI), an anthracycline antineoplastic agent, is a new derivative of Adriamycin (ADR). The antitumor activity of EPI is equivalent to that of ADR, but its toxicity is lower.

In the present study, we prepared various EPI-lipiodol mixtures and investigated their stability in vitro. We also applied these EPI-lipiodol mixtures in TACE for 16 patients with malignant liver tumors and investigated the pharmacokinetics of EPI.

Patients and methods

Stability of various EPI-lipiodol mixtures. Three forms of EPI-lipiodol, i.e., an oil-in-water type of emulsion (O/W type), a water-in-oil type of emulsion (W/O type), and a suspension (S type) were prepared (Fig. 1, Table 1). Their state after preparation was observed under a light microscope, and the separation of the oil and water phases was monitored during their maintenance at room temperature in the absence of agitation (stationary state).

For preparation of the O/W and W/O types, three contrast media, i.e., Iopamidol 300 (I), Iohexol 300 (O), and 60% Urografin (U), were used as solvents for EPI. Both the O/W and the W/O types were divided into two subgroups: the O/W type was divided into forms A and B and the W/O type, into forms C and D. In forms A and C, a stock solution of contrast medium was used to dissolve EPI. In forms B and D, each solvent was diluted with distilled water to the equivalent specific gravity of lipiodol. We set the ratio of distilled water at 1 : 5 in consideration of the solubility of EPI. The solvent and lipiodol were mixed by a pumping method using two disposal syringes and a three-way stopcock. Monitoring and recording of the separation were done at 30 min and at 1, 2, 4, 8, 12, 24, 48, 72, and 96 h after EPI-lipiodol preparation.

Plasma concentration of EPI. The test subjects were 16 patients with a malignant tumor of the liver, consisting of 14 cases of hepatocellular carcinoma (HCC) and 2 cases of liver metastasis of rectal cancer. They included 15 men and 1 woman whose ages ranged from 54 to 84 years (mean, 65.8 years). Either the O/W type (4 cases), the W/O type (5 cases), or a suspension (7 cases) of EPI-lipiodol was injected into the proper hepatic artery of a patient, and the plasma EPI level was monitored periodically. The target dose of EPI was 40 mg/m^2.

For O/W and W/O types, a stock solution of Iopamidol 300 was used to dissolve EPI. Lipiodol (4–8 ml) was used, and each form of the drug was prepared by the pumping method. In principle, small pieces of gelatin sponge were used for embolization following the drug injection.

After the administration of EPI-lipiodol, peripheral blood was collected immediately before and after the injection and at 5, 15, 30, and 60 min as well as 2, 4, 24, and 48 h later. Plasma was immediately separated from the samples by centrifugation and was frozen until analysis. The levels of EPI and its metabolite epirubicinol were monitored by

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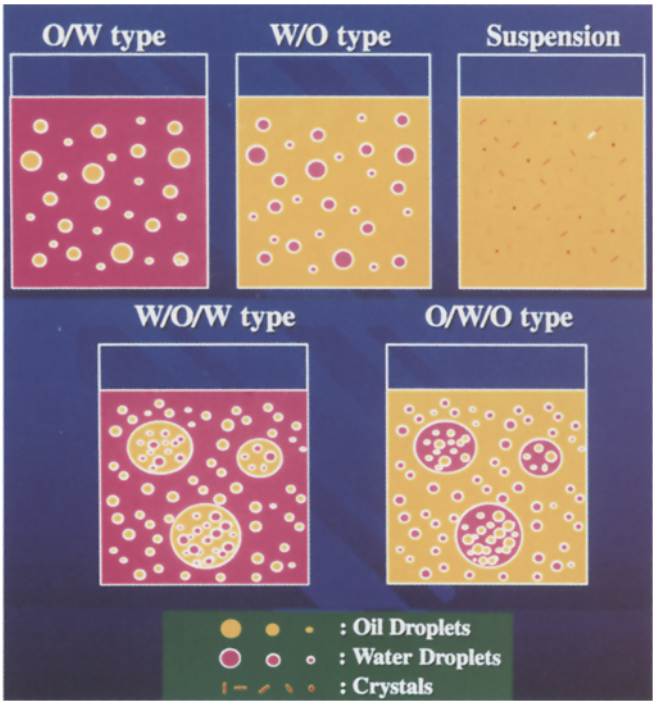

Fig. 1. Various EPI-lipiodol mixtures: O/W type of emulsion, oil droplets floating in water solution; W/O type of emulsion, water droplets floating in lipiodol; Suspension, drug crystals floating in lipiodol; W/O/W type of emulsion, drug appears as smaller water droplets within oil droplets in water solution; and O/W/O type emulsion, observed when a nonionic contrast medium is used as the solvent for EPI

Table 1. Methods used in the in vitro study

1. Oil-in-water type of emulsion (O/W): oil droplets of lipiodol float in the solution (solution: lipiodol, 1 : 1)
   Form A:
   EPI (10 mg) + stock solution of contrast medium (1 ml)
   Lipiodol (1 ml)
   Form B: (isogravity system):
   EPI (10 mg) + stock solution of contrast medium (0.8 ml) + distilled water (0.2 ml)
   Lipiodol (1 ml)
2. Water-in-oil type of emulsion (W/O): water droplets float in lipiodol (solution: lipiodol, 1 : 3)
   Form C:
   EPI (10 mg) + stock solution of contrast medium (1 ml)
   Lipiodol (3 ml)
   Form D (isogravity system):
   EPI (10 mg) + stock solution of contrast medium (0.8 ml) + distilled water (0.2 ml)
   Lipiodol (3 ml)
3. Suspension type (S): EPI crystals float in lipiodol
   Form S:
   EPI (10 mg)
   Lipiodol (3 ml)

high-performance liquid chromatography in cooperation with Farmitalia Carlo Erba and Kyowa Hakko Kogyo Co., Ltd.

For analysis of the pharmacokinetics, a three-compartment open model (Fig. 2) described by the following equation was applied, and the experimental parameters, i. e., A, B, D, p, q and r, were obtained by the repeated least-squares method:

$C = Ae^{-Pt} + Be^{-qt} + De^{-rt}$,

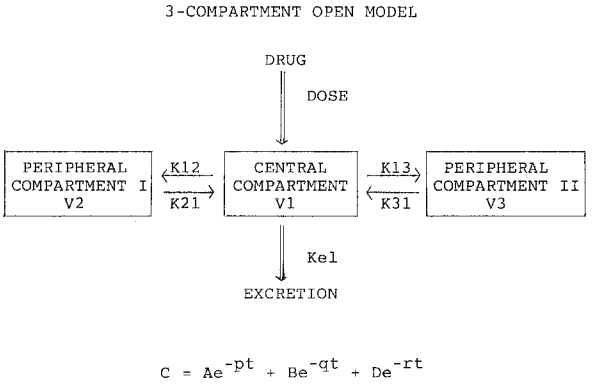

Fig. 2. Schema of the 3-compartment open model applied in the present study

where C represents the EPI level (μg/ml) in the plasma and t indicates the time elapsed after administration. On the basis of these experimental parameters, the following pharmacokinetic parameters were calculated: $t_{1/2\ (\alpha,\ \beta,\ \gamma)}$, the half-time of the plasma EPI level during the α, β, and γ phases; K_{el}, the velocity constant of drug elimination; V_1, V_2, and V_3, the apparent distribution volume in the central compartment and peripheral compartments I and II; V_d, the apparent distribution volume in the steady state ($= V_1+V_2+V_3$); Cl, plasma clearance ($= V_1 \times K_{el}$); C_{max}/D, the maximal plasma concentration/dose; and AUC/D, the area under the curve of the plasma concentration/dose. A two-compartment model was applied to patients whose data did not fit the three-compartment model.

Results

Stability of EPI-lipiodol preparations

Observations by light microscopy. For the drug forms in which Iopamidol or Iohexol was used as the solvent for EPI, no difference arising from the solvent or specific gravity was observed microscopically.

In the O/W-type emulsion, many lipiodol oil droplets ranging from 3 to 1,000 μm in diameter were floating in the red water solution. Inside oil droplets with a diameter of 10 μm or more, the EPI solution was observed as much smaller water droplets and represented a form of a water-in-oil-in-water (W/O/W) emulsion (Figs. 1, 3 A). In the W/O type, red water droplets with a diameter ranging from 3 to 500 μm floated in the lipiodol, and much smaller oil droplets of lipiodol were floating inside water droplets measuring 10 μm or more in diameter. This represented a form of oil-in-water-in-oil (O/W/O) emulsion (Figs. 1, 3 B). At 30 min after EPI-lipiodol preparation, almost no change was detected in the O/W type, whereas the W/O type showed coalescence of the water droplets, and the water and oil phases started to separate.

In the preparation using 60% Urografin as the solvent, crystals of EPI were deposited in both the O/W and the W/O types, and this preparation thus did not take the form of an emulsion. The diameters of floating droplets ranged from 10 to 2,000 μm. Neither W/O/W nor O/W/O types were observed (Fig. 3 C). In the suspension, crystals of EPI floated in the lipiodol, and their coalescence was detected at 30 min (Fig. 3 D).

Separation of the preparations in the stationary state. The stability of EPI-lipiodol is summarized in Table 2. A comparison of the O/W, W/O, and suspension forms revealed

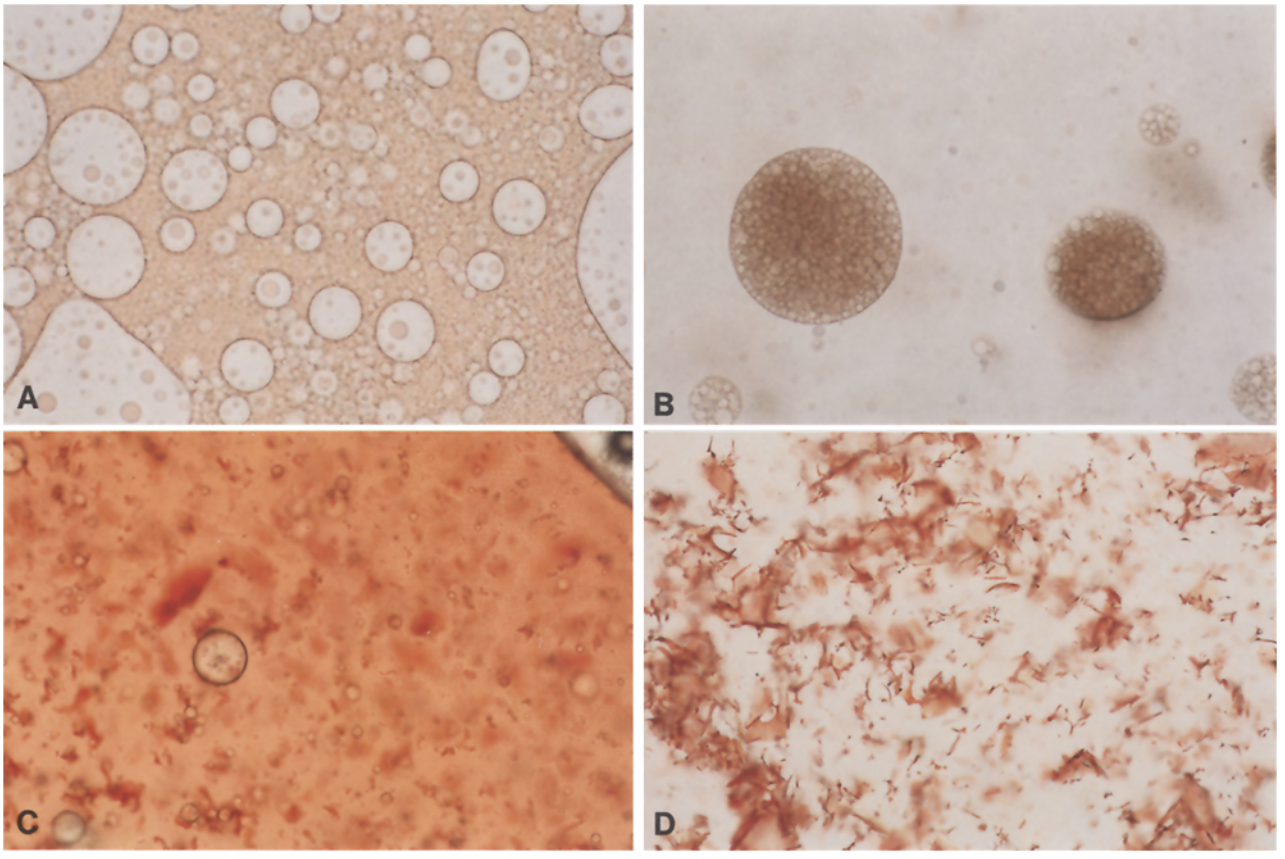

Fig. 3. A O/W type of emulsion using Iohexol. ×80. Many lipiodol oil droplets ranging from 3 to 1,000 μm in diameter are floating in the red water solution. Inside the oil droplets with a diameter of 10 μm or more, the EPI solution appears as much smaller water droplets and represents a form of W/O/W-type emulsion. **B** W/O type of emulsion using Iopamidol. ×80. Red water droplets ranging in diameter from 3 to 500 μm are floating in the lipiodol, and much smaller oil droplets of lipiodol are floating inside water droplets measuring 10 μm or more in diameter. This represents a form of O/W/O-type emulsion. **C** O/W type of emulsion using Urografin. ×40. In the preparation containing 60% Urografin as the solvent, many crystals of EPI are deposited, and the preparation does not take the form of an emulsion. The diameter of floating droplets ranges from 10 to 2,000 μm. W/O/W and O/W/O types of emulsion are not observed. **D** Suspension. ×80. In the form of a suspension, crystals of EPI are floating in the lipiodol

Table 2. Stability of EPI-lipiodol preparations

			0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h
	I	A										
		B							+	+	+	+
O/W	O	A								+	+	+
type		B									+	+
	U	A										
		B										
	I	C				+	+	+	+[a]	++[a]	++[a]	++[a]
		D	+	+	+	++	++	++[a]	+++[a]	+++[a]	+++[a]	+++[a]
W/O	O	C			+	+	+	+	++[a]	++[a]	++[a]	+++[a]
type		D	+	+	+	+	++	++[a]	++[a]	+++[a]	+++[a]	+++[a]
	U	C				+	+	+	+	++	++[a]	+++[a]
		D					+	+	+	+	++	++[a]
Suspension			+[a]	+[a]	++[a]	++[a]	++[a]	+++[a]	+++[a]	+++[a]	+++[a]	+++[a]

+, Slight separation; ++, moderate separation; +++, complete separation; I, Iopamidol; O, Iohexol; U, 60% Urografin; A, form A; B, form B; C, form C; D, form D

[a] Macroscopic crystal deposits (+)

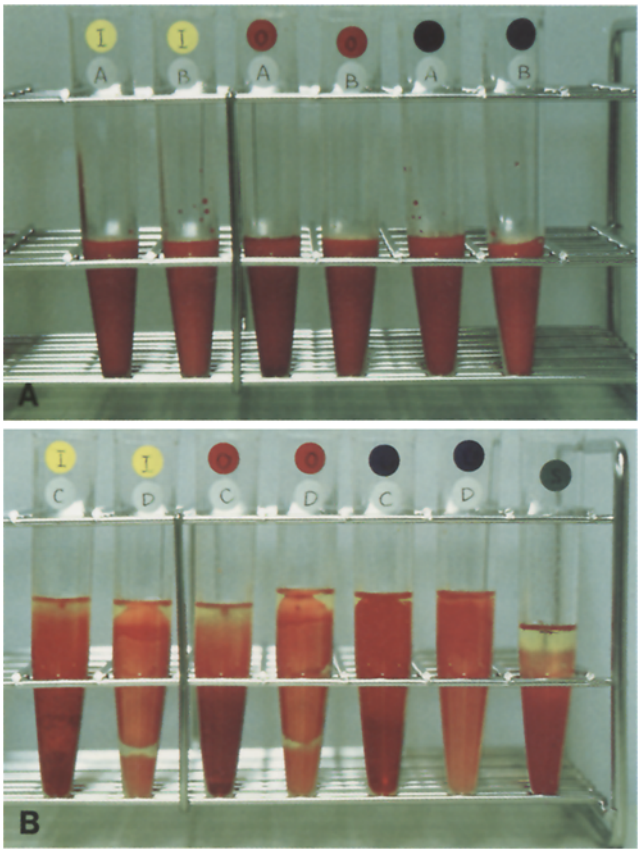

Fig. 4. A Separation of the preparations of forms A and B at 48 h. Slight separation is visible in form B (using Iopamidol) and form A (using Iohexol). **B** Separation of the preparations of forms C, D, and S at 48 h. Various degrees of separation are visible in all preparations. *I (yellow)*, Iopamidol; *O (red)*, Iohexol; *U (blue)*, Urografin; *A*, form A (O/W); *B*, form B (O/W); *C*, form C (W/O); *D*, form D (W/O); *S*, suspension

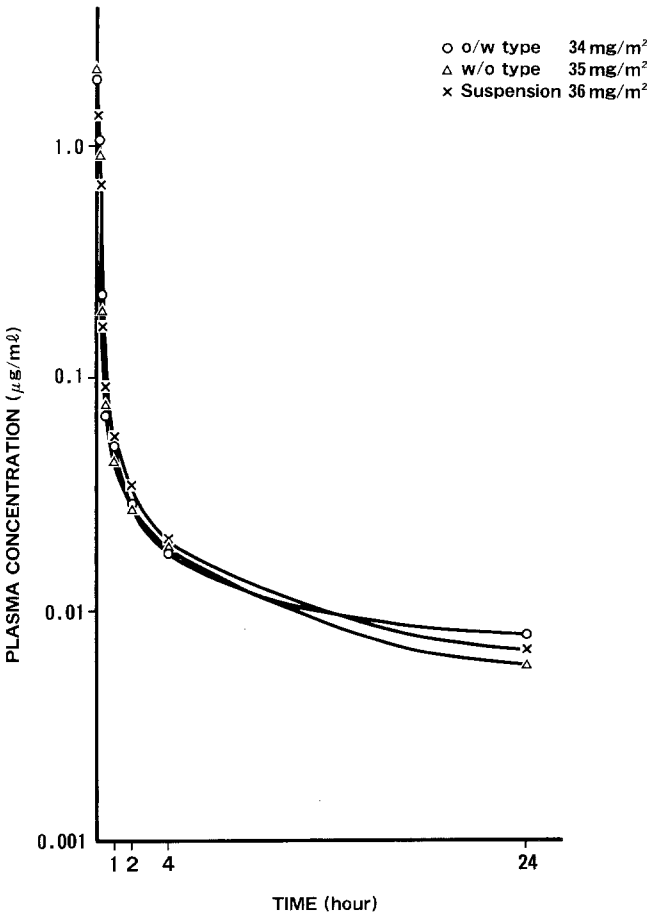

Fig. 5. Time-concentration curves constructed for the 3 patients who were included in the 3-compartment model. No significant difference was detected among the O/W type, the W/O type, and the suspension

that the oil and water phases separated earliest in the suspension, followed by the W/O type and the O/W type (Fig. 4).

In the observation of the stability of the preparations in different contrast media and under different gravities, the O/W type emulsion using 60% Urografin and the stock solution of Iopamidol were more stable in the stationary state. However, light microscopic observations revealed that many crystals were deposited in the Urografin system and that this preparation did not take the form of an emulsion. This might have been attributable to the poor solubility of EPI in 60% Urografin. Therefore, we concluded that the stock solution of Iopamidol was appropriate for our clinical study.

Plasma EPI level in clinical cases

The plasma EPI level reached a peak immediately after the administration of the EPI-lipiodol mixture in 12 of the 16 patients and at 5 min after the administration in 4 patients. The slope of the concentration curve constructed for nine patients fit the three-compartment model, whereas that of the curve generated for seven patients fit the two-compartment model. Three patients in whom chemoembolization using gelatin sponge pieces could not be performed following drug administration were included in the two-compartment analysis. The plasma level in each patient was analyzed using both models, and the values are shown in Table 3.

The plasma concentration curves generated for representative cases of each model are shown in Fig. 5. The curve for the three-compartment model showed a sharp decrease during the α phase, a slightly slower decrease during the β phase as compared with the α phase, and a prolonged decrease during the γ phase. In the two-compartment model, the curve showed α and β phases. In both models, the pattern of decrease in the plasma level was similar for the O/W type, the W/O type, and the suspension, and no statistically significant difference was detected by analyzing each parameter.

A metabolite of EPI, epirubicinol, possesses antitumor activity that is slightly weaker than that of the unchanged drug, and the production of epirubicinol differs greatly between individuals. In this study, the plasma level of epirubicinol differed from case to case, but epirubicinol generally appeared early after EPI administration and did not exceed the plasma EPI level except in one patient.

Table 3. Pharmacokinetic parameters

	3-Compartment model				2-Compartment model			
	O/W type ($n = 2$)	W/O type ($n = 4$)	Suspension ($n = 3$)	Total ($n = 9$)	O/W type ($n = 2$)	W/O type ($n = 1$)	Suspension ($n = 4$)	Total ($n = 7$)
$t_{1/2\alpha}$ (h)	0.072 ±0.002	0.109 ±0.037	0.083 ±0.041	0.092 ±0.035	0.100 ±0.008	0.080	0.104 ±0.018	0.099 ±0.016
$t_{1/2\beta}$ (h)	1.00 ±0.46	1.13 ±0.59	1.34 ±1.48	1.17 ±0.85	2.67 ±0.47	4.73	2.51 ±0.26	2.87 ±0.87
$t_{1/2\gamma}$ (h)	40.2 ±22.8	24.0 ±16.8	18.1 ±10.9	25.6 ±16.6	–	–	–	–
V_1 (l/kg)	1.17 ±0.99	1.17 ±0.50	1.17 ±0.82	1.17 ±0.62	1.67 ±0.12	1.38	3.51 ±3.15	2.67 ±2.46
V_2 (l/kg)	4.25 ±4.80	3.21 ±2.84	5.53 ±7.62	4.21 ±4.65	9.98 ±0.17	17.8	17.4 ±15.5	15.3 ±11.6
V_3 (l/kg)	30.5 ±7.2	28.4 ±8.1	25.6 ±1.1	27.9 ±5.9	–	–	–	–
V_d (l/kg)	35.9 ±1.4	32.8 ±6.6	32.3 ±8.9	33.3 ±6.2	11.7 ±0.1	19.2	20.8 ±18.7	18.0 ±13.9
Cl (l/h kg)	0.75 ±0.45	1.30 ±0.71	1.39 ±0.38	1.21 ±0.56	5.22 ±0.91	4.28	8.88 ±5.98	7.18 ±4.76
$C_{max}/D \times 10^2$ $\left(\frac{\mu g/ml}{mg/m^2}\right)$	3.82 ±3.27	2.98 ±1.91	3.11 ±1.58	3.21 ±1.86	1.67 ±0.01	2.22	1.36 ±0.97	1.57 ±0.76
$AUC/D \times 10^2$ $\left(\frac{\mu g\ h/ml}{mg/m^2}\right)$	4.67 ±2.71	2.79 ±1.64	2.11 ±0.70	2.98 ±1.75	0.54 ±0.05	0.72	0.41 ±0.18	0.49 ±0.18

Data represent mean values ± SD. D, Dose

Discussion

Stability of EPI-lipiodol preparations

Iopamidol and Iohexol, which are nonionic low-osmotic-pressure contrast media, act as surfactants [5, 16]. The diameter of the floating particles was smaller in the mixtures prepared using these contrast media than in those prepared using 60% Urografin (both O/W and W/O types). The minimal diameter of the particles was 3 μm in the O/W-type emulsion using a nonionic low-osmotic-pressure contrast medium as the solvent for EPI. It is considered that particles of lipiodol with a diameter of 5 μm or less can enter the hepatic vein and cause microembolization in the lung and other organs; therefore, such small particles are dangerous [6, 7, 9, 12–14]. However, in our clinical study only the usual side effects of TACE (i.e., fever, pain) were observed, and no dangerous sign attributable to small-diameter particles was detected.

Nakamura et al. [10] have reported that the stability of an emulsion consisting of an aqueous solution and lipiodol is enhanced when the ratio of lipiodol to water is increased; thus, the most stable form would be the W/O type. However, in the present study, separation of the oil and water phases was observed in some W/O-type samples beginning at 30 min and in all samples after 8 h. In contrast, none of the O/W types showed separation until 24 h. This difference could be attributable to the difference in the antineoplastic agent used, i.e., we used EPI whereas Nakamura et al. used ADR.

Plasma EPI level in clinical cases

The plasma level of anthracycline agents such as ADR and EPI following i.v. administration is generally known to decrease, showing α, β, and γ phases [1–4, 8, 11, 15]. The steep slope of the curve during the α phase is understood to represent rapid distribution of the drug from the blood to the tissues. The subsequent β and γ phases are considered to represent the elimination phase, resulting from excretion of the drug in the bile or urine or its elimination via hepatic metabolization.

In this study, nine patients showed a three-phase decrease in plasma EPI levels and seven patients showed a two-phase decrease. In the two-compartment model, the drug was not detectable at 24 or 48 h. This means that the plasma concentration of the drug decreased more sharply in these cases than in those analyzed using the three-compartment model. It is interesting that all three patients who were not injected with gelatin sponge pieces were included in the two-compartment-model group. This suggests that the drug was released more slowly and more continuously when gelatin sponge pieces were used.

In the analysis of the pharmacokinetic parameters, the mean maximal plasma level (C_{max}/D) was

$3.21 \pm 1.86 \times 10^{-2}$ μg ml^{-1}/mg m^{-2} in patients whose data fit the three-compartment model and $1.57 \pm 0.76 \times 10^{-2}$ μg ml^{-1} mg^{-1} m^2 in those whose data fit the two-compartment model. Our results were one-third to one-eighth lower than those reported by other authors, and this difference is interpreted as reflecting the slow release of EPI due to the properties of lipiodol. In terms of other pharmacokinetic parameters, our results were almost the same as those reported by other authors.

In the comparison of the three drug forms, no significant difference in the curve for the plasma level or other pharmacokinetic parameters was observed in clinical cases, whereas the O/W type was the most stable form in vitro. In vivo, agitation occurs due to the blood flow and to other factors, the result being that no significant difference are observed among the three drug forms.

References

1. Camaggi CM, Strocchi E, Tamassia V, Martoni A, Giovannini M, Iafelice G, Canova N, Marraro D, Martini A, Pannuti F (1982) Pharmacokinetic studies of 4′-epi-doxorubicin in cancer patients with normal and impaired renal function and with hepatic metastases. Cancer Treat Rep 66: 1819
2. Chan KK, Chlebowski RT, Tong M, Chen H-SG, Gross JF, Bateman JR (1980) Clinical pharmacokinetics of Adriamycin in hepatoma patients with cirrhosis. Cancer Res 40: 1263
3. Eksborg S, Cedermark BJ, Strandler HS (1985) Intrahepatic and intravenous administration of Adriamycin – a comparative pharmacokinetic study in patients with malignant liver tumors. Med Oncol Tumor Pharmacother 2: 47
4. Garnick MB, Ensminger WD, Israel M (1979) A clinical-pharmacological evaluation of hepatic arterial infusion of Adriamycin. Cancer Res 39: 4105
5. Hori S, Murakami T, Nishikawa M, Fujita N, Hashimoto T, Kozuka T, Narumi Y (1988) New contrast material for CT of the liver and spleen: iodinated oil emulsified by a non-ionic contrast material. Nippon Acta Radiol 48: 1503
6. Konno T (1987) Targeting chemotherapy for hepatocellular carcinoma by arterial administration of anticancer agents dissolved in lipiodol. Jpn J Cancer Chemother 14: 373
7. Konno T (1990) Targeting cancer chemotherapeutic agents by use of lipiodol contrast medium. Cancer 66: 1897
8. Koreeda C (1990) Studies on the effect of lipiodol during transcatheter arterial embolization for liver tumor. Acta Hepatol Jpn 31: 636
9. Maeda H, Konno T, Iwai K, Maki S, Tashiro S (1984) Tumor selective drug delivery with lipid contrast medium (SMANCS/lipiodol): sustained antitumor effect, enhanced diagnostic value and quantification of dosage regimen. Jpn J Cancer Chemother 11: 814
10. Nakamura H, Hashimoto T, Taguchi T, Oi H, Inoue K, Seki K, Mizumoto S, Tsukaguchi I, Araki Y, Sawada S (1987) Oily chemoembolization of hepatoma. Jpn J Cancer Chemother 14: 381
11. Ohno T, Shimoyama T, Kimura K, Yamada H (1986) Pharmacokinetics of epirubicin in cancer patients. Jpn J Cancer Chemother 13: 1881
12. Sasaki Y, Imaoka S, Nakamori N, Ishikawa O, Ohigashi H, Koyama H, Taniguchi K, Iwanaga T, Fujita M, Kawamoto S, Kasugai H, Kojima J, Tanaka S, Ishiguro S (1985) Effect of intra-arterial injection of lipiodol-Adriamycin suspension combined with arterial embolization on hepatocellular carcinoma. J Jpn Soc Cancer Ther 20: 1357
13. Sasaki Y, Imaoka S, Kasugai H, Fujita M, Kawamoto S, Ishiguro S, Kojima J, Ishikawa O, Ohigashi H, Furukawa H, Koyama H, Iwanaga T (1987) A new approach to chemoembolization therapy for hepatoma using ethiodized oil, cisplatin, and gelatin sponge. Cancer 60: 1194
14. Sonoyama T, Hironaka T, Inaba S, Tsukamoto K, Nonaka M, Makino H, Kohnosu H, Oka T (1986) Effect of arterial injection of lipiodol-Adriamycin emulsion on hepatocellular carcinoma. J Jpn Soc Cancer Ther 21: 773
15. Strocchi E, Camaggi CM, Rossi AP, Angelelli B, Comparsi R, Franchini A, Del Prete P, Cola B, Pannuti F (1985) Epirubicin pharmacokinetics after intrahepatic arterial and intraperitoneal administration. Drugs Exp Clin Res 11: 295
16. Yamashita Y, Nakagawa N, Miyawaki M, Bussaka H, Takahashi M, Fujimura N, Fukushima S, Nakano M (1985) Experimental study of hepatic arterial embolization therapy by various formulations of the anticancer agent lipiodol. Nippon Acta Radiol 45: 1313