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Adriamycin-Lipiodol suspension for i.a. chemotherapy of hepatocellular carcinoma

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Summary. Physicochemical properties of two types of adriamycin preparation, suspensions and emulsions prepared for i.a. chemotherapy of hepatocellular carcinoma, were investigated. A suspension was prepared by dispersing adriamycin directly into the lipid contrast medium, Lipiodol, whereas an emulsion was obtained by emulsifying an aqueous solution of adriamycin into Lipiodol. The dispersibility of the drug in each preparation was examined microscopically. The chemical stability of and drug release from the preparation were determined by high-performance liquid chromatography and spectrophotometry, respectively. The suspension was then given to ten patients with primary hepatocellular carcinoma. The suspension maintained good dispersibility without coagulation of drug particles, whereas coalescence of aqueous droplets and the resultant phase separation occurred 4 h after preparation of the emulsion. Both preparations maintained the initial drug content for at least 1 week at room temperature. The release of adriamycin was more prolonged in the suspension than in the emulsion. After i.a. administration of the suspension, a selective accumulation of Lipiodol in the tumor and decrease in serum α-fetoprotein (AFP) levels were found in most patients. A significant amount of adriamycin was still detected in hepatic specimens resected from two patients 1 and 2 months after treatment. These findings suggest that the adriamycin-Lipiodol suspension may be a useful preparation for targeting chemotherapy to hepatocellular carcinoma.

Introduction

The lipid lymphographic agent, Lipiodol Ultra-Fluid (Lipiodol), has been found to remain selectively in liver tumors long after its infusion via the portal vein or hepatic artery [3, 18, 19]. On the basis of this finding, Lipiodol has been used as a carrier of anticancer agents. Some authors have reported that the application of Lipiodol containing the lipophilic anticancer drug, copolymer of stylene maleic acid conjugated to neocarzinostatin (SMANCS), to hepatocellular carcinoma reduces the tumor size and serum α-fetoprotein (AFP) levels [6–8]. Kanematsu et al. [5] have prepared adriamycin (doxorubicin hydrochloride)-Lipiodol emulsions for the chemotherapy of hepatocellular carcinoma, as this anticancer drug is highly water-soluble. The emulsion was prepared by using an aqueous contrast medium, Urografin, as an intermediate to dissolve adriamycin. However, this emulsion was not sufficiently physically stable; shortly after its preparation, separation into oily and aqueous phases was observed. Therefore, the emulsion should be given to patients immediately after its preparation.

In the present study, we prepared the adriamycin suspension and emulsion using Lipiodol, compared the in vitro physicochemical stability and drug-release characteristics of the suspension with those of the emulsion, and carried out clinical administration of this suspension in ten patients with hepatocellular carcinoma.

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Materials and methods

Materials. Lipiodol, an ethyl ester of the fatty acid of poppyseed oil containing 38% iodine by weight, was purchased from Kodama Co., Ltd. (Tokyo, Japan). Urografin, an aqueous contrast medium containing 52.1% meglumine diatrizoate, 7.9% sodium diatrizoate, and 29.2% iodine by weight, was purchased from Nihon Schering K. K. (Osaka, Japan). Adriamycin was supplied by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan).

Preparation of suspension and emulsion. The suspension was prepared by pulverizing adriamycin and directly mixing it with Lipiodol using a mortar and pestle. The drug content of the suspension was 20, 30, or 40 mg in 6 ml of Lipiodol. The emulsion was prepared in two steps: adriamycin (30 mg) was first dissolved in 1.5 ml Urografin; this aqueous solution was then introduced into 6 ml Lipiodol and emulsified with an ultrasonicator (Sonifier, Branson Co., USA) for 5 min.

Examination of physical stability. Changes in the dispersibility of the drug in the suspension and emulsion with time were monitored by optical microscopy of each sample at room temperature (25° ± 2° C). Each sample was also examined macroscopically for the precipitation of adriamycin particles in the suspension and phase separation in the emulsion.

Measurement of adriamycin contents. A 1-ml sample of the freshly prepared suspension or emulsion was transferred into a centrifuge tube and stored at room temperature for 7 days. The adriamycin content was determined by high-per-

formance liquid chromatography (HPLC) after extraction by the following procedure. The suspension or emulsion was shaken with 20 ml phosphate-buffered solution (pH 3.0) and 10 ml chloroform. After centrifugation at 2500 rpm for 5 min, 5 ml supernatant solution was mixed with 4 ml 2-naphthalenesulfonic acid (as an internal standard) solution and 1 ml distilled water. A liquid chromatograph (Shimadzu LC-5A) equipped with a spectrophotometric detector (Shimadzu SPD-2A) and a data processor (Shimadzu C-R2A) was used for determinations of chemical stability. The HPLC conditions, which were modified from those in the United States Pharmacopeia (USP) XXI [17], were as follows: Nucleosil-5C_{18} column (4.6 mm inside diameter × 150 mm); mobile phase, 28% (v/v) acetonitrile and 72% (v/v) distilled water adjusted with phosphoric acid to pH 2; flow rate, 1.3 ml/min; UV detector at 254 nm.

Drug-release test. Drug release from the suspension or emulsion was investigated for 1 h by the rotating basket method of the dissolution test described in the Japanese Pharmacopeia (JP) X. The suspension or emulsion was gently introduced into the basket (36-mesh), which was agitated at 200 rpm in 500 ml isotonic phosphate-buffered solution (pH 7.4) at 37° C. A 4-ml sample of the buffer solution was withdrawn at appropriate intervals and an equal volume of the fresh buffer solution was added to maintain the initial volume. The concentrations of adriamycin released in the buffer solution (4 ml) were determined spectrophotometrically at 480 nm. The amount of drug released was expressed as the percentage of total absorbance corresponding to the total drug in each sample. For evaluation of the release characteristics, the mean dissolution time (MDT) was estimated by moment analysis [15]; there was no degradation of adriamycin during this test.

Clinical application. The present clinical study was carried out in Hirata Municipal Hospital after being approved by the local ethics committee. Ten patients (nine men and one women, 43–80 years old; average age, 61) with primary hepatocellular carcinoma gave their informed consent to undergo chemotherapy with 4–10 ml suspension (5 mg adriamycin/ml Lipiodol). The present dose levels were based on a previous report by Kanematsu et al. [5]. The suspension was percutaneously infused into the hepatic artery under X-ray monitoring according to Seldinger's method [14]. After administration of the suspension, deposit of Lipiodol was examined by plain X-ray and computerized axial tomography (CAT). The AFP level in serum before and after administration was measured by radioimmunoassay. Hepatic resection was done 1 and 2 months after administration in two patients with resectable liver cancer. The tissue concentration of adriamycin in the resected specimen was determined by an HPLC method developed by Masuike et al. [10] after extraction from the homogenized tissue.

Results

Physicochemical properties of the suspension and emulsion

The physical stability of the suspension and emulsion was examined at room temperature. Photomicrographs of the suspension and emulsion are shown in Fig. 1. Adriamycin particles in the suspension and aqueous droplets containing the drug in the emulsion were dispersed uniformly in the oily phase immediately after preparation (Fig. 1 a). At 1 h after preparation, the suspension maintained good dispersibility, whereas the partial coalescence of aqueous droplets was observed in the emulsion (Fig. 1 b). No coagulation of the particles was observed 4 h after preparation of the suspension, whereas most of the aqueous droplets had developed into larger droplets in the emulsion by this time (Fig. 1 c). The above findings were supported by the macroscopic observation that no precipitation of adriamycin particles occurred in the suspension 24 h after preparation, whereas a distinct phase separation took place in the emulsion shortly after its preparation.

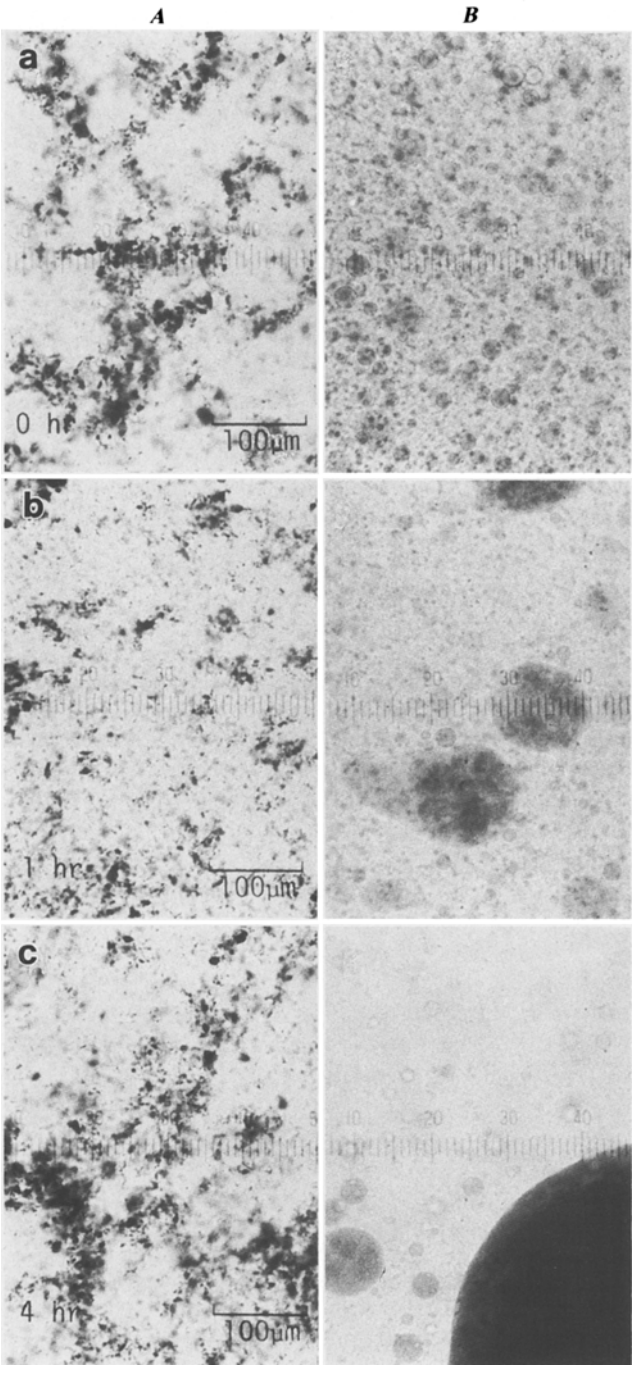

Fig. 1. Photomicrographs of the suspension (*A*) and the emulsion (*B*) immediately (**a**), 1 h (**b**), and 4 h (**c**) after their preparation

Table 1. Residual percentage of adriamycin in the suspension and emulsion

Preparation	Period (day)				
	1	2	3	5	7
Suspension	100.1 ±1.8	99.1 ±2.5	99.9 ±2.5	100.4 ±1.4	99.5 ±2.6
Emulsion	101.7 ±2.7	102.1 ±2.1	100.6 ±2.1	100.4 ±1.6	99.8 ±1.8

Each value represents the mean ± SD of eight experiments

Table 2. Mean dissolution time (MDT) of the suspension and emulsion

Preparation	MDT (min)[a]	C.V. (%)
Suspension		
20 mg/6 ml	17.59±1.09	6.2
30 mg/6 ml	17.33±0.97	5.6
40 mg/6 ml	16.82±0.76	4.5
Emulsion	10.29±1.31[b]	12.7

[a] Each value represents the mean ±SD of six experiments
[b] Significant difference from the suspension according to Student's *t*-test ($P<0.001$)

The adriamycin content of the suspension and emulsion during storage is shown in Table 1 as a percentage of the initial content. There was no decrease in drug content in either the suspension or the emulsion, which retained more than 99% of the initial content after 7 days at room temperature.

Figure 2 shows the release profiles of adriamycin in the suspension and emulsion. The release of adriamycin from the suspension was rather sustained, such that about 50% of the drug was released in 20 min. In contrast, the emulsion released the drug so rapidly that it achieved 90% release in 20 min. Figure 3 shows the release profiles of adriamycin in the suspension when the drug content was varied from 20 to 40 mg. Almost the same releasability with time was obtained in three suspensions with different contents. Table 2 shows the mean dissolution time (MDT) of the suspension and emulsion. There was no significant difference in MDT (about 17 min) between the various suspensions. The MDT of the emulsion was about 10 min, which was significantly shorter than that of the suspension.

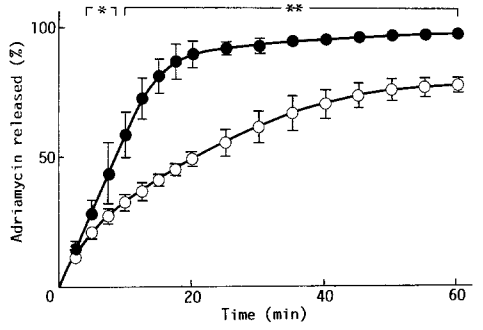

Fig. 2. Release patterns of adriamycin from the suspension (○) and the emulsion (●). Each point and vertical bar represents the mean ± SD of six experiments. There were significant differences between the suspension and the emulsion according to Student's *t*-test; $*P<0.05$, $**P<0.001$

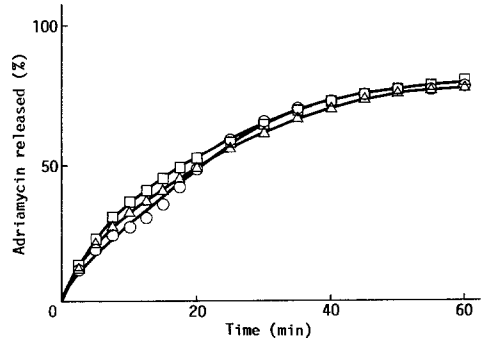

Fig. 3. Effect of adriamycin content, 20 mg/6 ml (○), 30 mg/6 ml (△), and 40 mg/6 ml (□), on its release from the suspension. Each point represents the mean of six experiments. The coefficient of variation at any data point ranged from 1.9% to 13.7%

Clinical application of the suspension

Figure 4 shows the plain radiograph, obtained immediately after i.a. infusion of the suspension (5 mg/ml) in one patient, demonstrating the presence of Lipiodol in the liver tumor. Figure 5 shows a CAT scan before and after i.a. administration in another patient. Before administration of the suspension, the tumor was recognizable as a low-density area localized in the right hepatic lobe (Fig. 5a). After 24 h, it could be seen as an area with markedly high density due to the selective accumulation of Lipiodol (Fig. 5b). This high-density area was still retained in the tumor 2 weeks after i.a. administration, and it decreased in size (Fig. 5c).

Figure 6 shows AFP levels in serum before and after treatment with the suspension. Serum AFP levels were reduced in 8 of 10 patients 7–10 days after treatment; how-

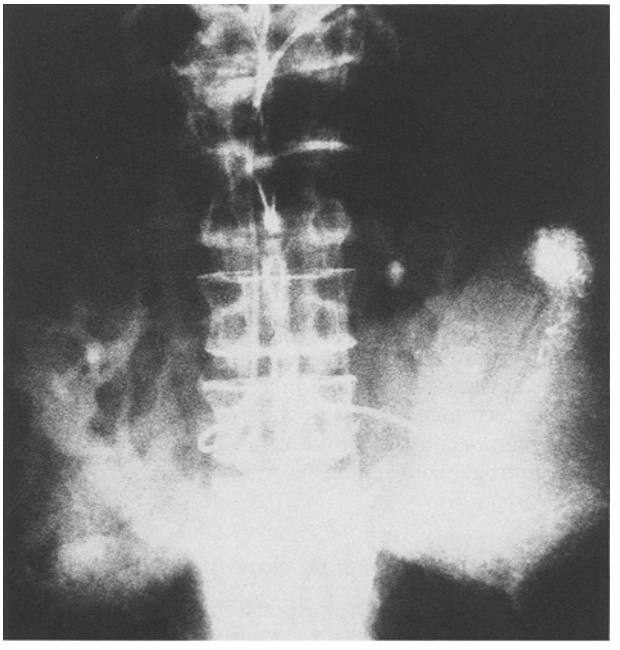

Fig. 4. Plain X-ray immediately after i.a. administration of adriamycin suspension in a patient

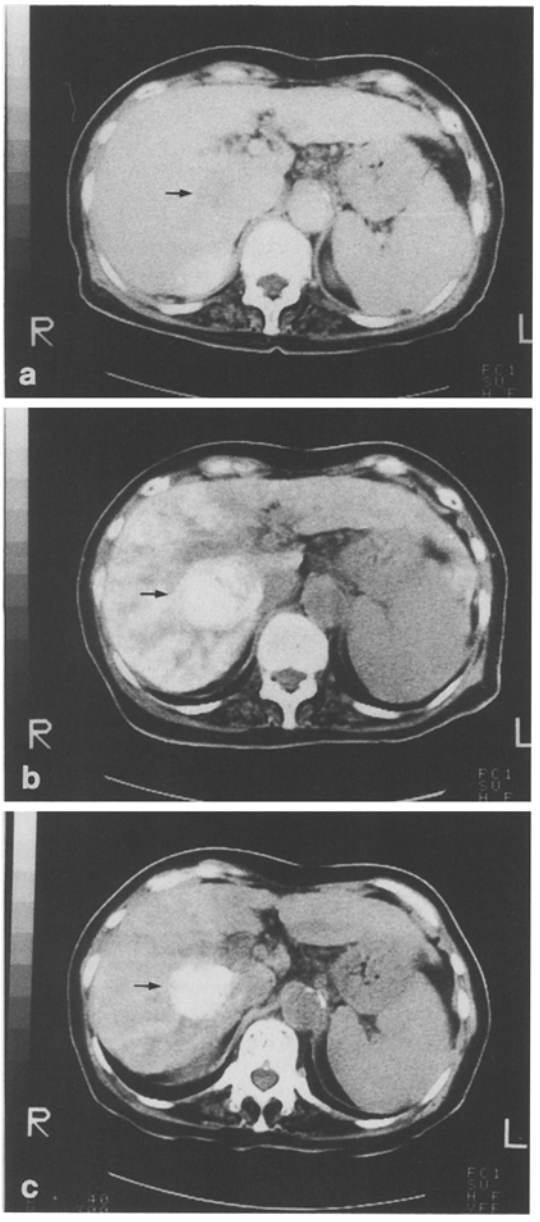

Fig. 5. CAT scan before (**a**), 24 h (**b**), and 2 weeks (**c**) after i.a. administration in a patient. The arrow shows the site of the tumor

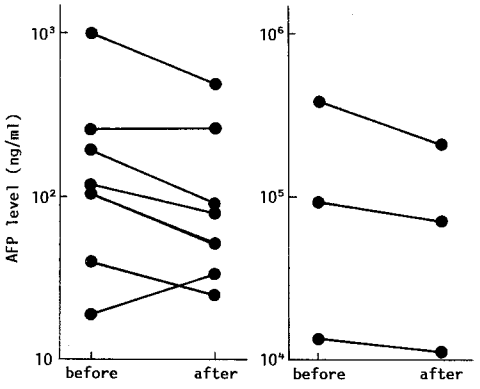

Fig. 6. AFP levels in serum before and after administration of the suspension in ten patients

Table 3. Adriamycin level in resected specimens from two patients with resectable liver cancer

Case	ADM level (μg/g tissue)		Period after administration
	Tumor	Parenchyma	
1	5.7	ND	1 month
2	0.3	ND	2 months

ADM, adriamycin; ND, not detected

ever, in 2 other patients there was neither a change nor a decrease in serum AFP levels.

Two patients with resectable liver cancer underwent hepatic resection 1 and 2 months after i.a. administration of the suspension. Histological examination showed that the resected main tumor and daughter nodule were completely necrosed. Table 3 shows adriamycin levels in the resected hepatic tissue. The drug level was 5.7 μg/g tissue in the tumor that was resected 1 month after administration, and 0.3 μg/g in the tumor resected 2 months after administration. In both cases, no trace of the drug was detected in the liver parenchyma.

No serious complications were encountered during and after administration of the suspension except transient fever, mild nausea, and slight elevations of glutamic oxalacetic transaminase (GOT) or glutamic pyruvic transaminase (GPT).

Discussion

In cancer chemotherapy, major efforts have been made to improve the forms for the selective, long-term delivery of anticancer agents to tumors [1, 2, 4, 11, 12]. Lipiodolized anticancer agents have been used for the treatment of hepatocellular carcinoma [5–8], as the selective accumulation of this contrast medium in the liver tumor after i.a. administration has been reported [3, 18, 19]. Adriamycin has commonly been prescribed for the treatment of hepatic cancer, and a water-in-oil (W/O) emulsion of this drug with Lipiodol has recently been prepared using Urografin [5]. However, this W/O-type adriamycin emulsion has been reported to undergo phase separation easily and release the drug too rapidly [16]. The physicochemical stability and drug release of W/O-type emulsions are very important factors in controlling the clinical efficacy of such preparations.

In this study, we aimed to achieve a stable adriamycin preparation with sustained drug release. The dispersibility of the suspension was sufficient, yielding neither coagulation nor precipitation of the drug particles for at least 24 h. However, the coalescence of aqueous droplets and the resultant phase separation were observed in the emulsion 4 h after its preparation. This phase separation may be due to the difference in specific gravity between Urografin (1.328–1.332) and Lipiodol (1.275–1.290). The release of adriamycin was found to be sustained in the suspension, and the adriamycin content did not affect its releasability. These results suggest that the present adriamycin suspension in Lipiodol is not only stable but also sustains drug release such that the dose can be modified according to clinical demand.

Konno et al. [8] have confirmed by CAT that the deposit of Lipiodol in liver tumors persists for more than

3 months. The accumulation of Lipiodol in liver tumors was visible on plain X-rays in all patients after i.a. administration of the present adriamycin-Lipiodol suspension. The CAT examination confirmed the selective accumulation of Lipiodol in the tumor with none in the liver parenchyma. Furthermore, after a long-term retention of Lipiodol, a marked decrease in the area of deposit (i.e., tumor size) was observed. This evidence by CAT was consistent with the clinical efficacy of the present suspension.

It is well recognized that serum AFP levels may provide an important and useful indicator of the activity of anticancer agents against hepatocellular carcinoma [11, 13]. The remarkable decrease in serum AFP levels in eight of ten patients after treatment suggests a sufficient antitumor effect for this suspension on hepatocellular carcinoma.

Lee et al. [9] have reported that adriamycin levels in tumor tissue from two patients with hepatoma at 2 h after bolus i.v. injection of 30 mg/m^2 were 3.51 and 4.62 μg/g, which was almost comparable to the level in normal liver tissue. On the other hand, Kanematsu et al. [5] have reported that adriamycin levels in tumor tissue 16 days after i.a. administration of Lipiodol emulsion containing 30 mg of the drug was 11.0 μg/g, which was much higher than the level in the liver parenchyma. In the present clinical study, certain levels of adriamycin were determined in resected tumors even after 1 and 2 months, whereas no trace of the drug was detected in the liver parenchyma. These findings confirm that Lipiodol plays a role as an adequate carrier of adriamycin, which may thereby be delivered and selectively accumulated in the tumor tissue for long periods.

On clinical use of the suspension, all side effects were transitory and mild, and no severe complications were experienced. The localization of adriamycin in the tumor, as discussed above, is required not only for enhancing its efficacy but also for minimizing its side effects. In conclusion, the present adriamycin-Lipiodol suspension maintained good long-term drug dispersibility and showed sustained releasability of the drug. The selective accumulation of Lipiodol and adriamycin in the tumor and a marked decrease in serum AFP levels were observed after i.a. administration of the suspension in most patients. Therefore, the adriamycin-Lipiodol suspension is a useful preparation for targeting chemotherapy to hepatocellular carcinoma.

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