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# Assessment of chemoembolization therapy for primary liver cancer using a stabilized Adriamycin-lipiodol suspension*

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**Summary.** We formulated a new lipiodol-Adriamycin suspension (ADM/lipiodol, 50 mg/10 ml) that remained stable for 48 h (half-life, 25 ± 3 days). In five cases of hepatocellular carcinoma (HCC) resected after intra-arterial infusion of this agent, the ADM concentration in the tumor was quite high and the tumor necrosis rate was more than 80% on histological examination. Over a 5-year period, 180 patients with unresectable HCC underwent transcatheter arterial embolization therapy (TAE) in the presence or absence of this agent. The regimens consisted of suspension injection alone (A, $n$ = 54), suspension injection + TAE using gelatin sponge (B, $n$ = 29), TAE followed by suspension injection (C, $n$ = 34), and TAE alone (D, $n$ = 63). The estimated 1-year survival values determined for patients treated with these regimens were 70%, 73%, 43%, and 39% respectively, and the corresponding 3-year survival values were 27%, 31%, 15%, and 10%. The survival achieved using suspension injection was thus superior to that obtained using conventional TAE, and combined therapy with suspension injection followed by TAE seemed to enhance survival, although there were some biases in tumor size and in the stage of tumor progression. For patients with tumors measuring 5 cm or more in diameter, the survival obtained using regimen A was lower than that achieved using regimen D, but the combination of TAE and suspension injection improved the 1-year survival value obtained using regimen D from 34% to 52%. For patients with tumors measuring less than 5 cm in diameter, the survival achieved using regimen A was markedly better than that obtained using regimen D, although no difference was found between the survival value achieved using regimen A and that obtained using regimens B and C. On the basis of these results, our newly formulated ADM-lipiodol suspension was surmised to be effective by itself against relatively small HCC tumors, whereas it enhanced the efficacy of conventional TAE in large lesions.

## Introduction

During the last several years, there have been many reports on the usefulness of iodized oil (lipiodol) as a chemoembolization material for the treatment of liver tumors, especially hepatocellular carcinoma (HCC) [3, 7]. Lipiodol can be selectively retained even in HCC with a small mass, whereas it has also been used in targeting therapy with anticancer drugs such as mitomycin C [4], doxorubicin (Adriamycin, ADM) [8], and cisplatin [5]. The use of combined anticancer drug targeting and arterial embolization therapy (TAE) therefore seems to be the best way to prolong the survival of patients with HCC associated with extremely advanced liver cirrhosis. The efficacy of this chemoembolization therapy is controversial due to differences in the methods applied to prepare the carcinostatic-lipiodol suspension (emulsion). Nakamura et al. [3] have reported the stability and effectiveness of a water-in-oil emulsion, but an even more stable agent would be expected to provide even better therapeutic effects.

We designed a new ADM-lipiodol suspension in 1985 and have used this agent for chemoembolization therapy in 117 patients with unresectable HCC over the past 5 years. In this report we present the data we obtained on the stability and efficacy of this newly designed suspension as based on the histological changes, the ADM concentration in resected specimens, and the survival of patients with unresected HCC.

## Patients and methods

*Preparation of the suspension.* The suspension was prepared at 1–2 h before arterial infusion in the Department of Biochemistry. Commercially available doxorubicin hydrochloride (Adriamycin, Farmitalia,

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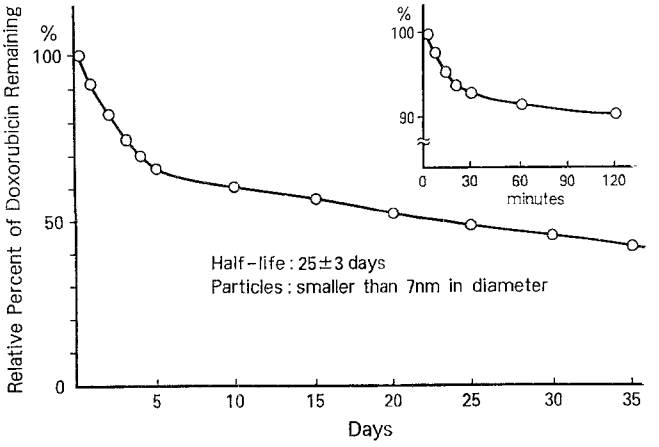


**Fig. 1.** Degradation curve of suspended ADM. The calculated half-life was 25 ± 3 days

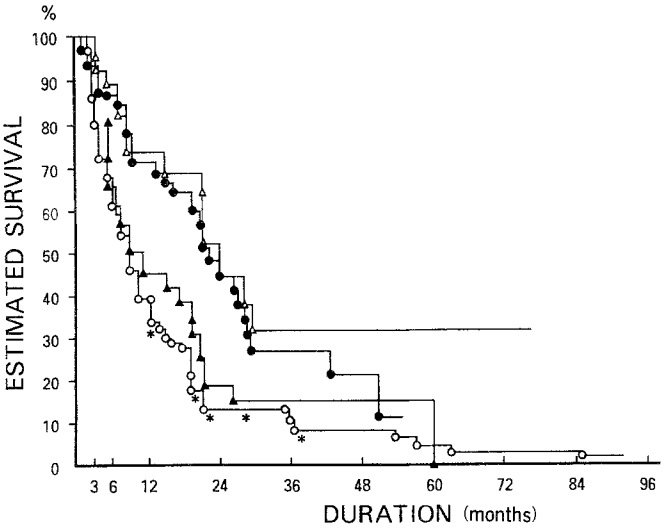


**Fig. 2.** Cumulative survival of patients in each group. ●, Group A (suspension infusion alone); △, group B (suspension infusion followed by TAE); ▲, group C (TAE with subsequent suspension infusion); ○, group D (TAE alone). * Significant difference between ○ and ● ($P < 0.05$)

Italy) in a dose of 50 mg was suspended in 10 ml iodized oil (Lipiodol, Andre Gelbe Laboratory, France) by means of ultrasonic treatment at 37° C. In some instances, continuous release of ADM from the suspension was estimated by spectrometry.

### *Chemistry and histology studies on resected specimens*

In six patients undergoing hepatectomy following intra-arterial injection of the suspension, the ADM concentrations in both the cancer tissue and the hepatic parenchyma were determined simultaneously by high-performance liquid chromatography (HPLC). The interval between suspension injection and hepatectomy ranged from 14 to 35 days. Resected specimens were also employed for evaluation of the pathological changes and estimation of the relative percentage of necrotic tissue in the whole lesion.

*Chemoembolization regimens.* During the period from November 1979 through August 1990, a total of 180 patients with unresectable HCC underwent chemoembolization in the presence or absence of this agent. The method used for selective catheterization was the same as that reported previously [4, 8]. The chemoembolization regimens consisted of suspension injection alone (group A, 54 cases), suspension injection followed by TAE (group B, 29 cases), TAE and subsequent suspension injection (group C, 34 cases), and TAE alone (group D, 63 cases). The mean age of the patients was 59.3 ± 9.3, 60.7 ± 9.0, 59.0 ± 8.9 and 59.1 ± 8.5 years, respectively. Survival values were calculated by the Kaplan-Meier method [1], and the significance of differences was evaluated by the generalized Wilcoxon test.

## Results

### *Stability and activity of the ADM-lipiodol suspension*

Microscopy studies revealed that the mean diameter of ADM-suspended oil particles was about 7 nm. When the suspension was left to stand at room temperature, the suspended dose of ADM gradually decreased to 65% of the initial dose within the first 5 days but declined slowly to 47% during the subsequent 30 days (Fig. 1). The calculated half-life was 25 ± 3 days.

**Table 1.** Tissue concentrations of ADM and pathological changes in resected specimens

| Specimen number | Tumor | | Infused dose | ADM concentration | | % Necrosis |
|---|---|---|---|---|---|---|
| | Location, size | Histology | | Tumor | Parenchyma | |
| 1 | $S_2$, 3.5 × 3.5 cm | Ed III, pseudoglandular | 25 mg | 6.7 μg/g | 0 | 93% |
| 2 | $S_2$, 3.5 × 3.2 cm | Ed III, trabecular | 20 mg | 6.7 μg/g | 0 | 97% |
| 3 | $S_8$, 3.5 × 2.5 cm | Ed III, trabecular | 20 mg | 1.2 μg/g | 0 | 88% |
| 4 | $S_5$ 2.7 × 2.5 cm | Clear cell, compact | 15 mg | 0 | 0 | 7% |
| 5 | $S_3$, 3.9 × 2.8 cm | Ed III–IV, trabecular | 20 mg | 1.9 μg/g | 0.6 μg/g | 100%[a] (5%)[b] |
| 6 | $S_3$, 3.7 × 3.7 cm | Uncertain | 50 mg | 0.5 μg/g | 0.1 μg/g | 100% |

[a] Intracapsular lesion
[b] Extracapsular lesion

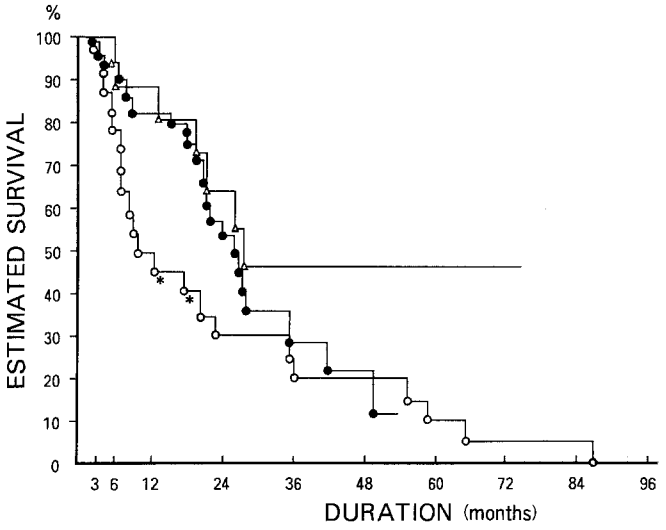


**Fig. 3.** Cumulative survival of patients with tumors measuring 5 cm or less in diameter. ●, Group A; △, groups B+C; ○, group D. * Significant difference between ○ and ● ($P$ <0.05)

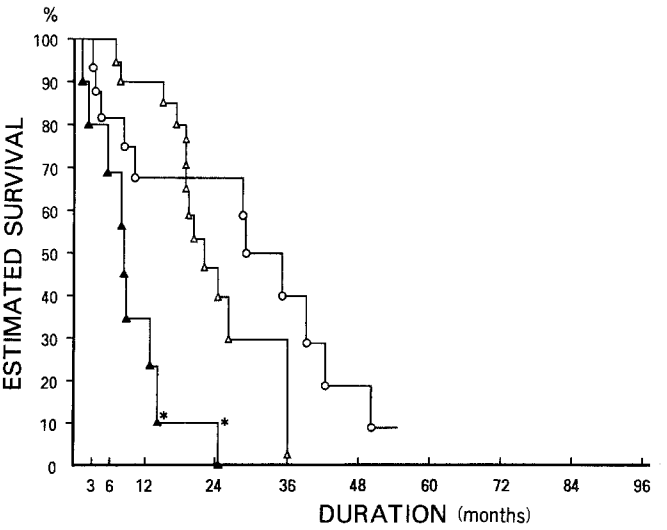


**Fig. 5.** Graded tumor size vs survival of patients treated with regimen A. ○, ≦2 cm in diameter; △, 2.1–5 cm; ▲, >5 cm. * Significant difference between △ and ▲ ($P$ <0.05)

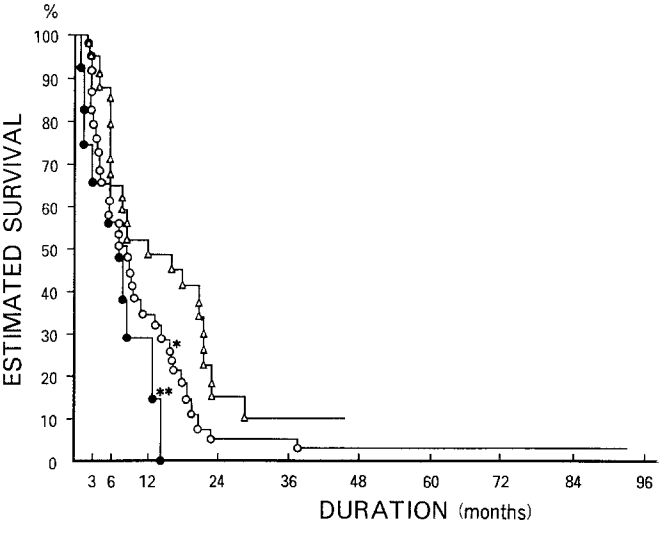


**Fig. 4.** Cumulative survival of patients with tumors measuring more than 5.1 cm in diameter. ●, Group A; △, groups B+C; ○, group D. * Significant difference between ○ and △ ($P$ <0.05); ** Significant difference between ○ and ● ($P$ <0.05)

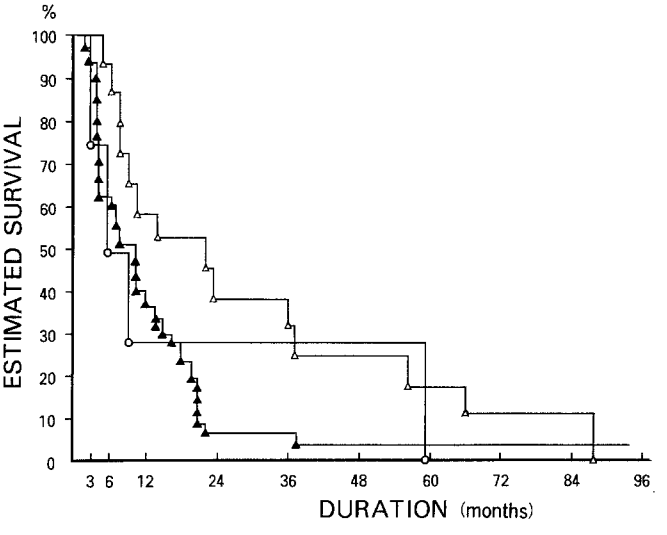


**Fig. 6.** Graded tumor size vs survival of patients in group D. ○, ≦2 cm in diameter; △, 2.1–5 cm; ▲, >5 cm

The ADM concentration in five resected specimens showed a range of 0.5–6.7 μg/g wet tumor tissue as opposed to 0–0.6 μg/g in the hepatic parenchyma, as shown in Table 1. Histologically, the percentage of tumor necrosis was estimated at 88%–100%, excluding case 4. This case showed poor vascularity on angiography and faint accumulation of lipiodol on CT following suspension infusion, which seemed to be a characteristic finding in clear-cell-type HCC.

*Survival of patients with nonresected HCC*

The estimated survival values for each group are presented in Fig. 2. The 1-year survival values determined for groups A, B, C, and D were 70%, 73%, 43%, and 39%, respectively, and the respective 2-year values were 45%, 45%, 17%, and 13%. The survival of patients undergoing suspension injection therapy was significantly better than that of patients undergoing only conventional TAE ($P$ <0.01). In terms of 3-year survival, both group A and group B differed significantly from group D, but no difference was found between group A (27%) and group B (31%) or between group C (15%) and group D (10%).

There was, however, a bias in these results, since some differences in tumor size and stage were observed among the four groups. To minimize the bias, we divided the tumor size (maximal diameters as measured on the angiogram) into two categories: 5 cm or smaller, and 5.1 cm or larger. For tumors measuring 5 cm or less in diameter, the

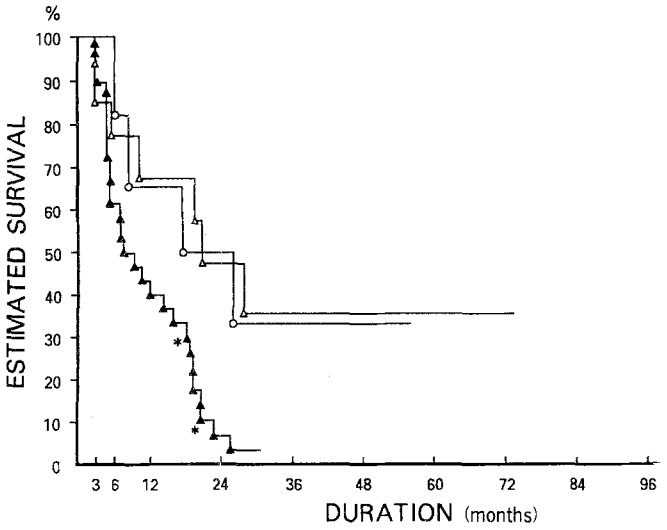


**Fig. 7.** Graded tumor size vs survival of patients in groups B and C. ○, ≦2 cm in diameter; △, 2.1–5 cm; ▲, >5 cm. * Significant difference between △ and ▲ ($P$ <0.05)

estimated 1-, 2-, and 3-year survival values for patients undergoing suspension injection therapy (group A) were 82%, 54%, and 28%, respectively, whereas the respective values estimated for group D were 45%, 30%, and 20% (Fig. 3). The cumulative survival of groups B and C was similar to that of group A, which was significantly better than that of group D ($P$ <0.01). For tumors measuring more than 5.1 cm in maximal diameter, on the other hand, conventional TAE achieved a 1-year survival value higher than that attained using suspension injection alone. Nevertheless, the combination of TAE and suspension infusion further enhanced the 1-year survival value to 52% (Fig. 4).

Next, the survival values obtained using each regimen were evaluated according to the tumor diameter, which was divided into three ranges: ≦2 cm, 2.1–5.0 cm, and >5 cm. In group A (suspension injection alone), the 1-, 2-, and 3-year survival value determined for the size of 2.1–5 cm were 90%, 47%, and 30%, respectively, as compared with 34%, 10%, and 0 for the size of >5 cm (Fig. 5). With respect to the tumor size of ≦2 cm, the 1-year survival value was lower than that determined for the size of 2.1–5 cm, whereas the 2-year survival was superior. In group D (TAE alone), no significant difference was found among the three size ranges, but the survival values obtained for a tumor size of 2.1–5 cm were higher than those determined for the size of >5 cm (Fig. 6). Figure 7 shows the survival curve generated for patients treated by combined therapy with the suspension and TAE (group B plus group C). The 2-year survival value obtained for a tumor size of 2.1–5 cm was significantly higher than that determined for the size of >5 cm ($P$ <0.05), whereas no difference was found between the size of ≦2 cm and the size of 2.1–5 cm.

## Discussion

More than 10 years have passed since the introduction of TAE as treatment for HCC, and many investigators [6, 10] have reported its advantages and limitations. Based on our experience, the limitations of gelatin sponge TAE relate to the following conditions: portal tumor thrombi, daughter nodules, invasive growth of the cancer, small masses, and collateral formation of feeding arteries. In 1983, Konno et al. [2] discovered that an oily contrast medium, lipiodol, was selectively accumulated within HCC tumors. Thereafter, many attempts to conjugate anticancer drugs with lipiodol have been made with the goal of using this agent as a carrier of anticancer drugs. Preparations of lipiodol-SMANCS [2], lipiodol-MMC [4], and lipiodol-ADM [3] emulsions have previously been studied, but their stability and anticancer effects were unsatisfactory as described by Taniguchi et al. [9].

In 1985 we prepared a new lipiodol-ADM suspension containing ADM at a dose of 3–10 mg/ml. The method of preparation used was as follows. Lipiodol in an adequate volume was added to vials of commercially available doxorubicin, and the mixtures were left at room temperature for 1 h. The vials were then exposed to ultrasonic waves for 5 min to suspend the ADM in the lipiodol particles. The suspension was homogeneous in appearance and remained free of phase separation for at least 48 h. The calculated half-life was 25 ± 3 days, as shown in Fig. 1.

These results indicated that this newly formulated suspension was more stable than were others reported previously and presumably had slow-release and long-acting properties. In fact, the tissue concentration of ADM in resected tumor specimens was quite high even at 35 days after intra-arterial infusion of this suspension. The efficacy of this agent as evaluated in histology studies was also satisfactory, although some viable tissue remained in the peripheral regions of the main tumor and daughter nodules in some cases. Takayasu et al. [8] reported that a lipiodol and doxorubicin emulsion achieved only a 13% "complete-necrosis" rate and a 46% "necrosis percentage" in 15 resected cases. As compared with these data, our results are superior in terms of both the 80% necrosis rate and the complete-necrosis rate.

On the basis of these fundamental data, we used this agent in the treatment of unresectable HCC. The regimens were given to four groups of patients as described in Materials and methods. First, the survival of group A (suspension injection alone) was better than that of group D (conventional TAE); the 1-, 2-, and 3-year survival values determined for group A vs group D were 70% vs 39%, 45% vs 13%, and 27% vs 10%, respectively. Above all, the suspension injection therapy seemed to be more effective in relatively small tumors measuring less than 5 cm in diameter but not as effective in tumors smaller than 2 cm (small liver cancer). In our series, most small liver cancers were associated with severe liver cirrhosis, and the outcome seemed to be poor even when the HCC had been successfully treated by chemoembolization therapy. These results suggest that percutaneous ethanol injection therapy is the treatment of choice for small liver cancer associated with extremely advanced cirrhosis.

The combination of suspension injection with conventional TAE improved the survival to some extent. The regimen of suspension injection soon after TAE enhanced survival as compared with TAE alone, especially in tumors

measuring more than 5 cm in diameter. The suspension is considered to act on the viable tumor tissue remaining after TAE, on daughter nodules, and possibly on tumor thrombi, which seem to resist repeated gelatin-sponge TAE. In fact, in some cases we observed the disappearance or regression of the portal thrombus after suspension injection. On the other hand, it has been reported that the simultaneous use of ADM-lipiodol suspension and gelatin sponge particles achieve longer survival and a better tumor-necrosis rate [3, 8]. In our series, the regimen consisting of the suspension injection followed by TAE also produced better survival in patients with medium-sized HCC. In tumors measuring at least 3–5 cm in diameter, the additional use of gelatin sponge as an embolizing material might be needed to inhibit early disease recurrence.

Finally, we address the problem of the therapeutic approach for the management of unresectable HCCs. In principle, administration of the suspension alone seems to be of limited value in the treatment of tumors measuring less than 2 cm in diameter, and the use of gelatin sponge in combination with the suspension is required to obtain more effective embolization in larger tumors. In patients with a portal tumor thrombus, suspension injection therapy must be given prior to TAE using gelatin sponge, and repeated chemoembolization will be necessary.

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