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Effect of Arterial Administration of High-molecular-weight Anticancer Agent SMANCS with Lipid Lymphographic Agent on Hepatoma: a Preliminary Report*

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Abstract—*A clinical evaluation of arterial infusion of high-molecular-weight antitumor agent SMANCS dissolved in lipid lymphographic agent (ethiodol®) in* 44 *patients with mostly unresectable hepatoma is described. The treatment regimen demonstrated significant merits both therapeutically and diagnostically. Marked antitumor effects were shown in the decreased serum alpha-fetoprotein levels* (86% *of cases) and tumor size* (95% *of cases), and in survival period and histological findings. Furthermore, there was increased diagnostic sensitivity using CT scan, plain X-rays or ultrasound. The procedure of selective arterial administration of* 3–4 mg *of SMANCS in* 3–4 ml *of ethiodol per dose was simple to perform and was required only once every* 3–4 weeks. *Both ethiodol and the drug accumulated more selectively in tumor than in any other tissues and their activity remained for more than* 3 weeks. *Only minimal side-effects were associated with SMANCS and ethiodol during this study.*

INTRODUCTION

THE LIPID lymphographic agent ethiodol has been found to selectively remain in hepatocellular carcinoma when injected into the hepatic end of the ligated hepatic artery [1]. This persistent and selective deposition of ethiodol also occurred without ligation of the hepatic artery, as described below. Based on this finding, a new therapeutic approach to the treatment of hepatoma was attempted using a high-molecular-weight anticancer agent, SMANCS [2–4], dispersed and solubilized in ethiodol. SMANCS is a chemical conjugate of a synthetic copolymer of styrene maleic acid and the proteinaceous antitumor agent neocarzinostatin [5]. SMANCS has a molecular weight of 15,000–30,000 daltons and dissolves in some organic solvents such as pyridine and acetone, and particularly in ethiodol. The homogeneous suspension of SMANCS in ethiodol, designated as SMANCS-ethiodol, was used for the present arterial administration. Forty-four patients with advanced hepatocellular carcinomas were injected via the hepatic or celiac artery by Seldinger's method [6]. The outstanding anticancer effects and significant improvement in subsequent diagnostic X-ray evaluation obtained using this approach are described.

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MATERIALS AND METHODS

Patients

Forty-four patients with advanced hepatoma, established either histologically or clinically, were subjected to the present study and are listed in Table 1. They were admitted to the Kumamoto University Hospital Department of Surgery, or our associated hospitals in Kumamoto City.

All patients or their families consented to the treatment protocol. Twenty-nine patients had unresectable, highly advanced hepatoma, and 15

Table 1. *Category of patients under present protocol*

(A) Unresectable hepatoma, 26 patients:

Case No.	Age, Sex	Location, size	Type of hepatoma*	Liver cirrhosis	Reasons for unrectability
1	70, ♀		M	+	widespread
2	48, ♀		D	+	widespread
3	75, ♂		D	+	widespread
4	62, ♂		N	+	widespread lung metastasis
5	77, ♂		M	+	widespread
6	63, ♂		D	+	widespread
7	46, ♂		D	+	widespread
8	39, ♂		M	-	widespread
9	39, ♀		D	+	widespread
10	58, ♂		M	+	ICG R_{max} value
11	54, ♂		M	+	ICG R_{max} value
12	51, ♂		N	+	ICG R_{max} value
13	66, ♂		M	-	bone metastasis

Case No.	Age, Sex	Location, size	Type of hepatoma*	Liver cirrhosis	Reasons for unresectability
14	60, ♂		D	+	widespread
15	52, ♂		M	+	invasion to chest bone mestasis
16	73, ♂		M	+	ICG R_{max} value
17	63, ♂		N	+	ICG R_{max} value
18	69, ♂		N	+	widespread
19	53, ♂		N	+	widespread
20	67, ♂		D	+	widespread
21	57, ♂		D	+	widespread
22	59, ♂		M	+	widespread
23	75, ♂		M	+	heart failure
24	47, ♀		M	-	lung metastasis
25	69, ♀		M	+	widespread
26	63, ♂		N	+	widespread

(B) Recurrence after hepatic resection, 5 patients (2 resectable, 3 unresectable)
(C) Resectable hepatoma, 13 patients—preoperative administration

*M, massive; D, diffuse; N, nodular.

patients had resectable hepatoma. Thirty-six of the 44 patients had liver cirrhosis.

Angiography, computerized tomography (CT), ultrasonography and abdominal plain X-ray examinations were performed routinely to evaluate the locations and sizes of the tumors. Serum alpha-fetoprotein levels were also measured when applicable and possible.

To evaluate the location of ethiodol, serial slices of 10 mm in thickness of the resected liver specimen were radiographed with a Softex using Fuji fine grain film for Softex, and the bioactivity of SMANCS was assayed as described below.

Drug preparation and administration

SMANCS was prepared at the Department of Microbiology, Kumamoto University Medical School. Briefly, the amino group of protein antitumor agent neocarzinostatin [5] was conjugated via a carbamide bond with the anhydride component of the synthetic copolymer of styrene maleic acid. The present preparation has a mean molecular weight between 15,000 and 30,000 daltons* according to elemental analysis and polyacrylamide gel (7.5%) electrophoresis in the presence of 0.2% sodium dodecylsulfate at pH 8.2. The drug, hereafter referred to as SMANCS-ethiodol, was prepared by suspending 1 mg of SMANCS in 1 ml of ethiodol. The 44 patients were given a total of 88 injections of the drug between February 1981 and June 1982. The 15 patients with resectable hepatomas received only one injection, ranging from 11 to 60 days before surgical resection. Selective catheterization was generally performed through the femoral artery under fluoroscopy using Seldinger's method. Arterial infusions of an average of 3–4 ml of SMANCS-ethiodol per dose were given through the celiac (18 times), hepatic (49 times) or other peripheral arteries (21 times). The maximum dose per patient per injection was 12 ml, 6 ml each for the right and left hepatic lobes.

Chemicals and bioassay

Ethiodol is an iodinated fatty acid ester of poppy-seed oil containing 38% iodine by weight and is a product of André-Gelbe Laboratories, France, obtained through Kodama Co. Ltd., Tokyo. The biological activity of SMANCS in the resected tumor specimens was determined by the technique previously described [7] and discussed briefly below. The specimen was not homogenized since inactivation of the sample during homogenization has been found to proceed faster even in the presence of protease inhibitor [7]. As an alternative, we punched out a specimen in the resected liver specimen with a cork borer ($\phi = 8$ mm) and the removed specimen was sliced to a thickness of about 5 mm. This punched slice was placed on the preinoculated agar plate and incubated at 4°C for 5–6 hr, then at 37°C for 15 hr. The diameter of the inhibition zone after calibration with standard SMANCS yielded the biological activity in the specimen.

Laboratory examinations

Most laboratory examinations such as serum alpha-fetoprotein levels, liver function tests and hematologic tests were carried out in the University Hospital using routine laboratory techniques.

RESULTS

Accumulation of ethiodol and SMANCS in the tumor

Selective accumulation of ethiodol was clearly demonstrated. As shown in Fig. 1 A and B, after a 3- to 4-ml injection of SMANCS-ethiodol, the hepatoma was indicated by high-density areas on the CT scan. When the resected liver specimen was examined by soft X-ray, the tumor regions, including a previously undiagnosed 4 mm satellite nodule, were revealed by the accumulation of ethiodol (Fig. 2). Plain abdominal films also revealed the hepatomas, including satellite nodules, due to the accumulated ethiodol (Fig. 3).

Using a Sudan III stain on microscopic sections of the resected specimens, the ethiodol was found to accumulate in the neovasculature of the tumor. It was not clear if the ethiodol was deposited in the extracapillary space before tumor necrosis occurred.

Biological activity of SMANCS was measured in both tumor and non-tumor areas of the resected specimens. The results showed that this biological activity persisted even after 22 days in tumor and closely adjacent non-tumor-containing tissue (Table 2).

Antitumor effect

The effect of this chemotherapy can be evaluated by CT and angiography, as shown in Fig. 4 A–C. The serum alpha-fetoprotein level and tumor size (Figs 5 and 6) showed decreases in 86 and 95% of cases respectively. Among them 9 of 21 cases showed 40–99% size reduction within 1–5 months. Other parameters utilized for tumor size determination such as ultrasound and plain X-ray also confirmed each of these results. The

*As a nature of synthetic polymer (in this case copolymer of styrene maleic acid), its molecular weight distributes within a limited range. Thus the polymer conjugates with this protein exhibit some deviation (median, approx. 20,000) while that of the protein part is exact (10,700). A further refinement in purification is under way to improve this point.

Table 2. Biological activity of SMANCS in resected liver

Cases	Dose of SMANCS	Administered artery	Period from administration to hepatectomy	Biological activity (μg SMANCS/g tissue) Hepatoma	Non-tumorous portion surrounding hepatoma	Non-tumorous portion distant from hepatoma (over 10 cm)
62 yr male	4 mg	common hepatic	15 days	0.94	1.06	0
54 yr male	4 mg	common hepatic	22 days	5.4	1.06	0

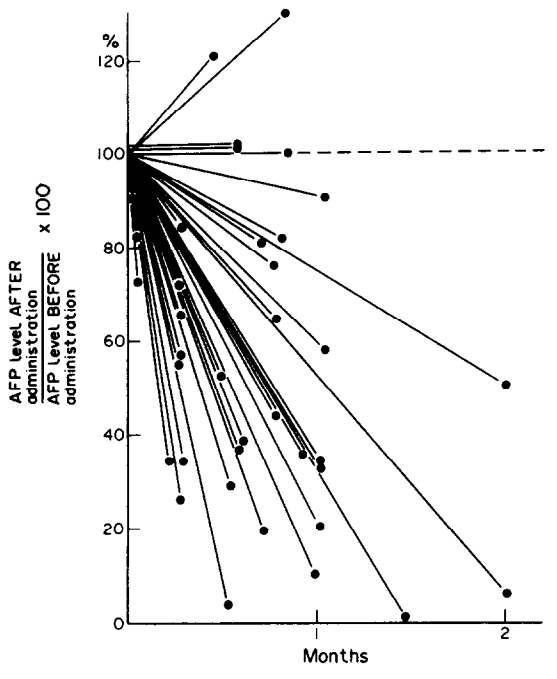

Fig. 5. Changes in α-fetoprotein after administration of SMANCS-ethiodol.

histological examination of the resected specimens of 15 cases revealed that extensive necrosis of the tumor had occurred. In these cases single administration of a dose of 2–9 mg of SMANCS-ethiodol was performed 1–8 weeks before hepatic resection. The degree of necrosis of the tumor tissue nearly paralleled the magnitude of the SMANCS-ethiodol dose delivered. In the patients administered a dose of SMANCS more than 0.25 mg/cm^2 of maximal cut surface area complete necrosis of the tumor was found and, importantly, non-cancerous liver tissue remained unaffected (Fig. 7).

Side-effects

After 88 selective arterial infusions with SMANCS-ethiodol, no adverse effects due to embolization in critical organs such as lung, heart or brain have been noted. Untrapped ethiodol disappeared from the fluoroscopic view very rapidly, though we speculate some may circulate bound to serum albumin and lipoprotein while others, although undetectable, may be lodged in the kidney, lung or bile.

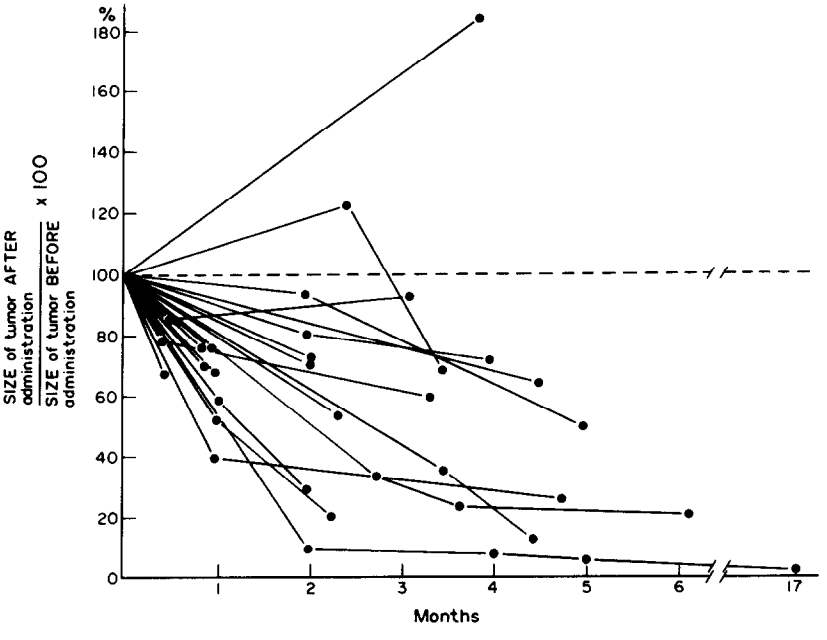

Fig. 6. Changes in size of hepatoma by administration of SMANCS-ethiodol.

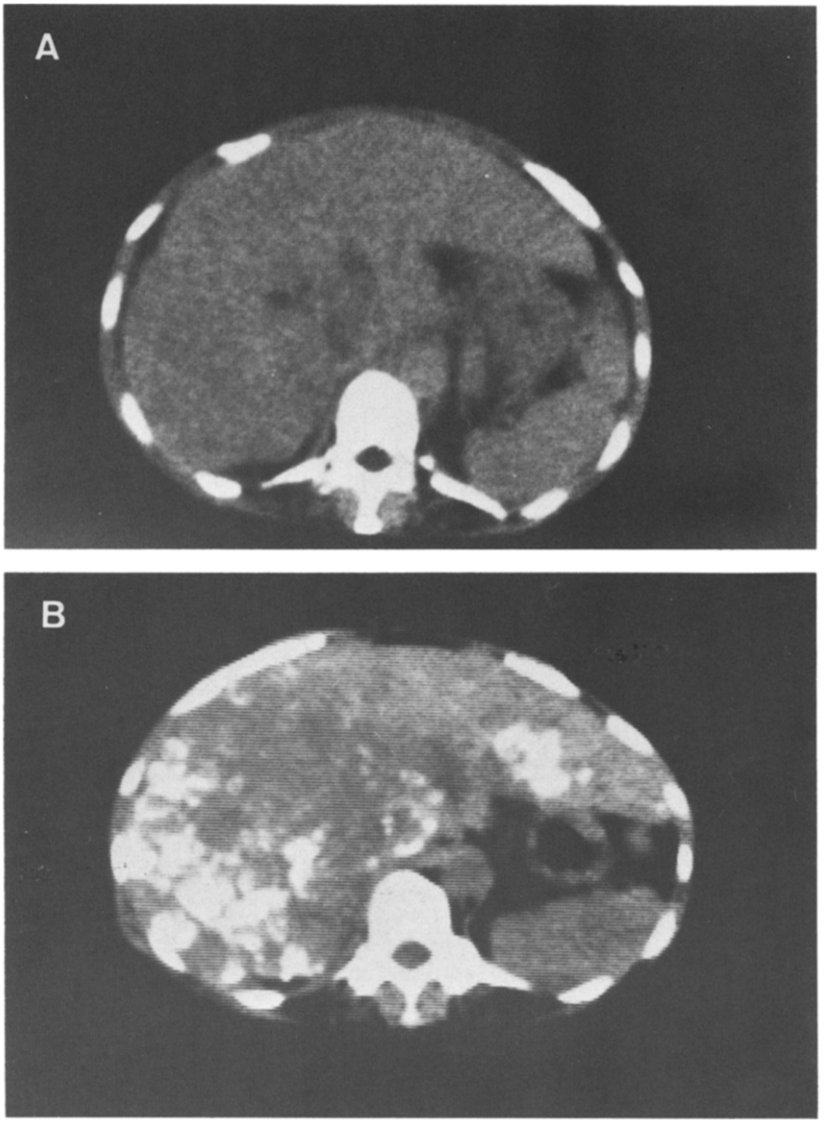

Fig. 1. Computerized tomogram (CT) of a 48-yr-old with unresectable hepatoma (case 2). (A) Before administration of SMANCS-ethiodol; (B) 1 week after administration of SMANCS-ethiodol. The tumorous area became clearly visible as a white high density area.

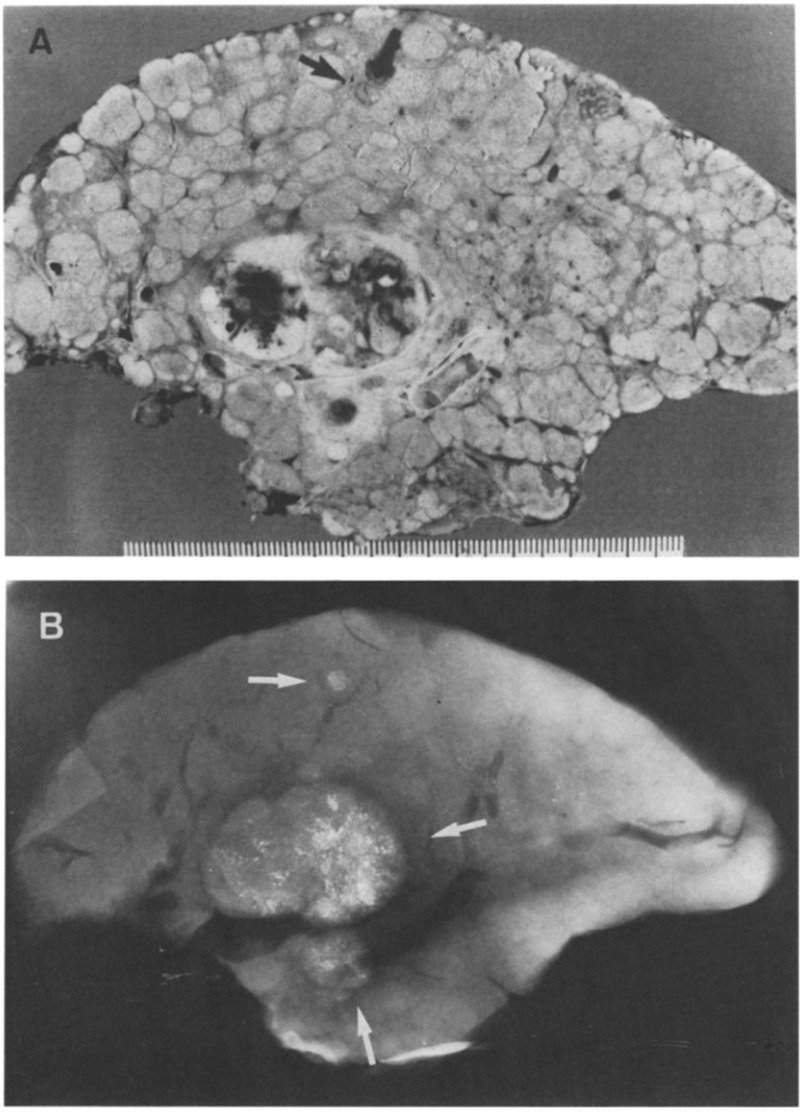

Fig. 2. 54-yr-old male with resectable hepatoma (a case in C group). Administration of SMANCS-ethiodol had been performed 22 days before hepatic resection. (*A*) A cut surface of resected hepatoma. A satellite nodule with a diameter of 4 mm is observed (arrow); (*B*) soft X-ray film reveals clearly the hepatoma as a high density area due to the selective remaining of ethiodol.

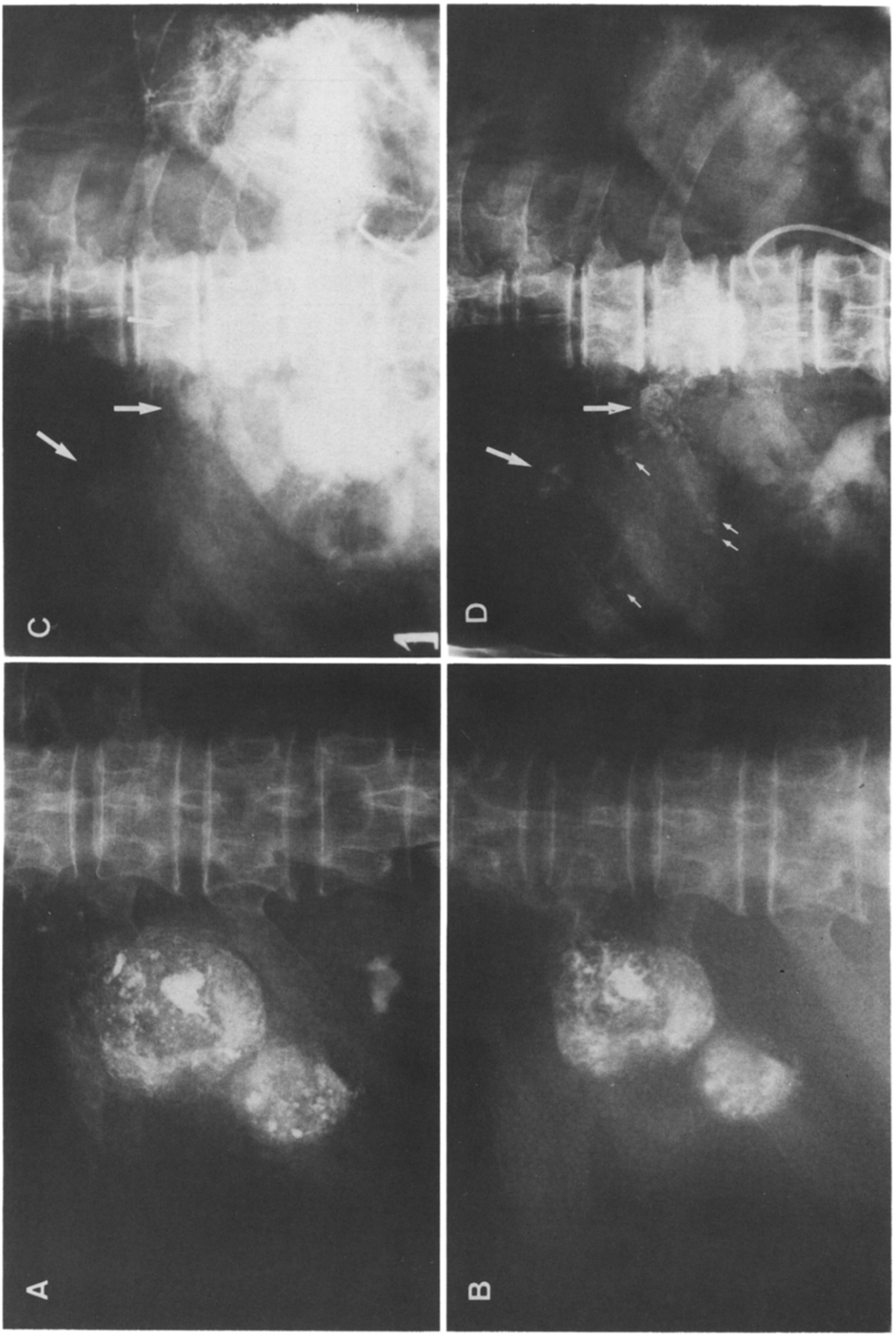

Fig. 3. Abdominal plain X-ray film. (A) Immediately after administration of SMANCS-ethiodol (case 17); (B) 1 month after administration (case 17); (C) capillary phase of common hepatic arteriogram (case 26); (D) immediately after administration of SMANCS-ethiodol a small sized cancer nest of 4 mm in diameter is also clearly detected (see arrows) (case 26).

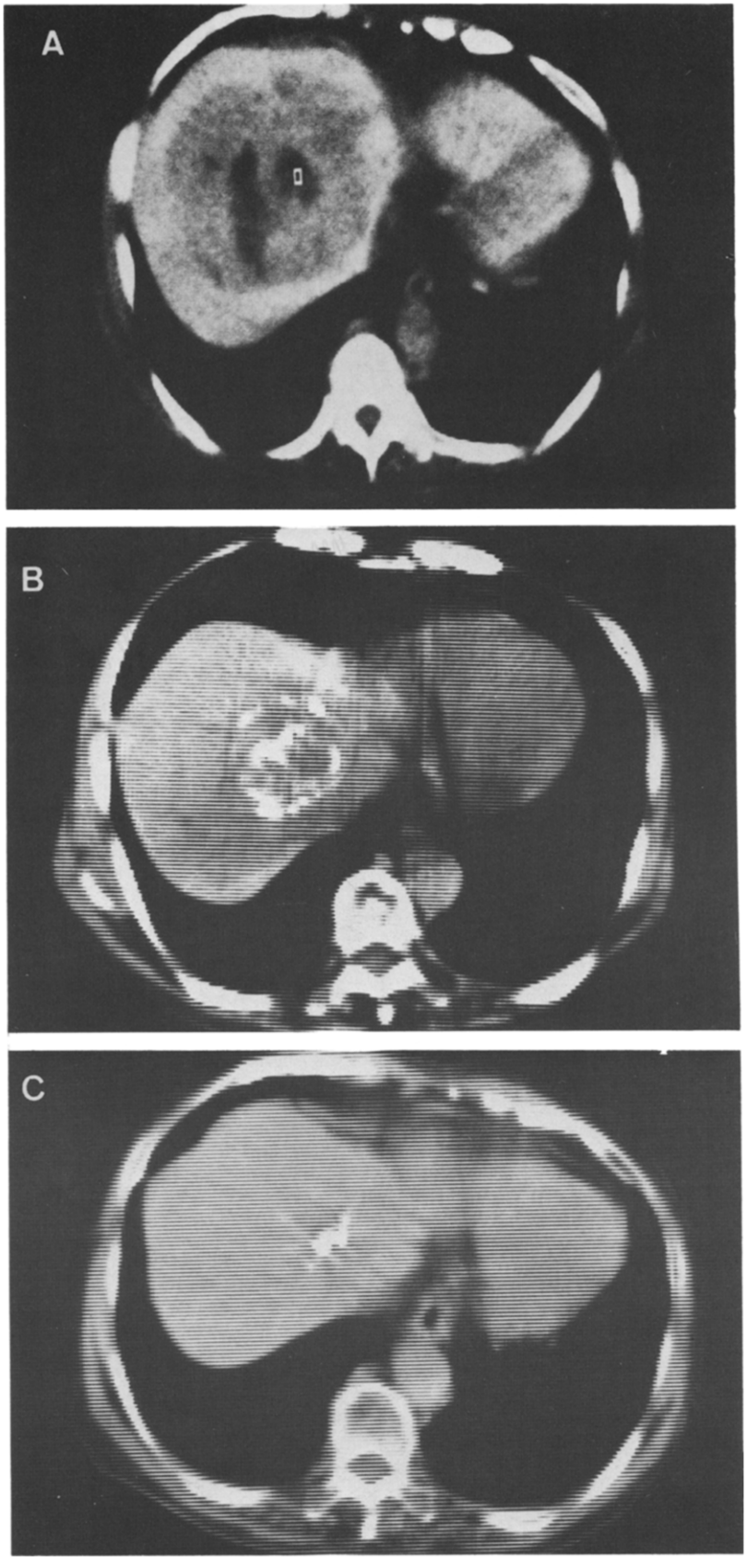

Fig. 4. Demonstration of regression of hepatoma by CT (case 1). (A) Before administration of SMANCS ethiodol; (B) 4 months *after administration; (C)* 17 months *after administration.*

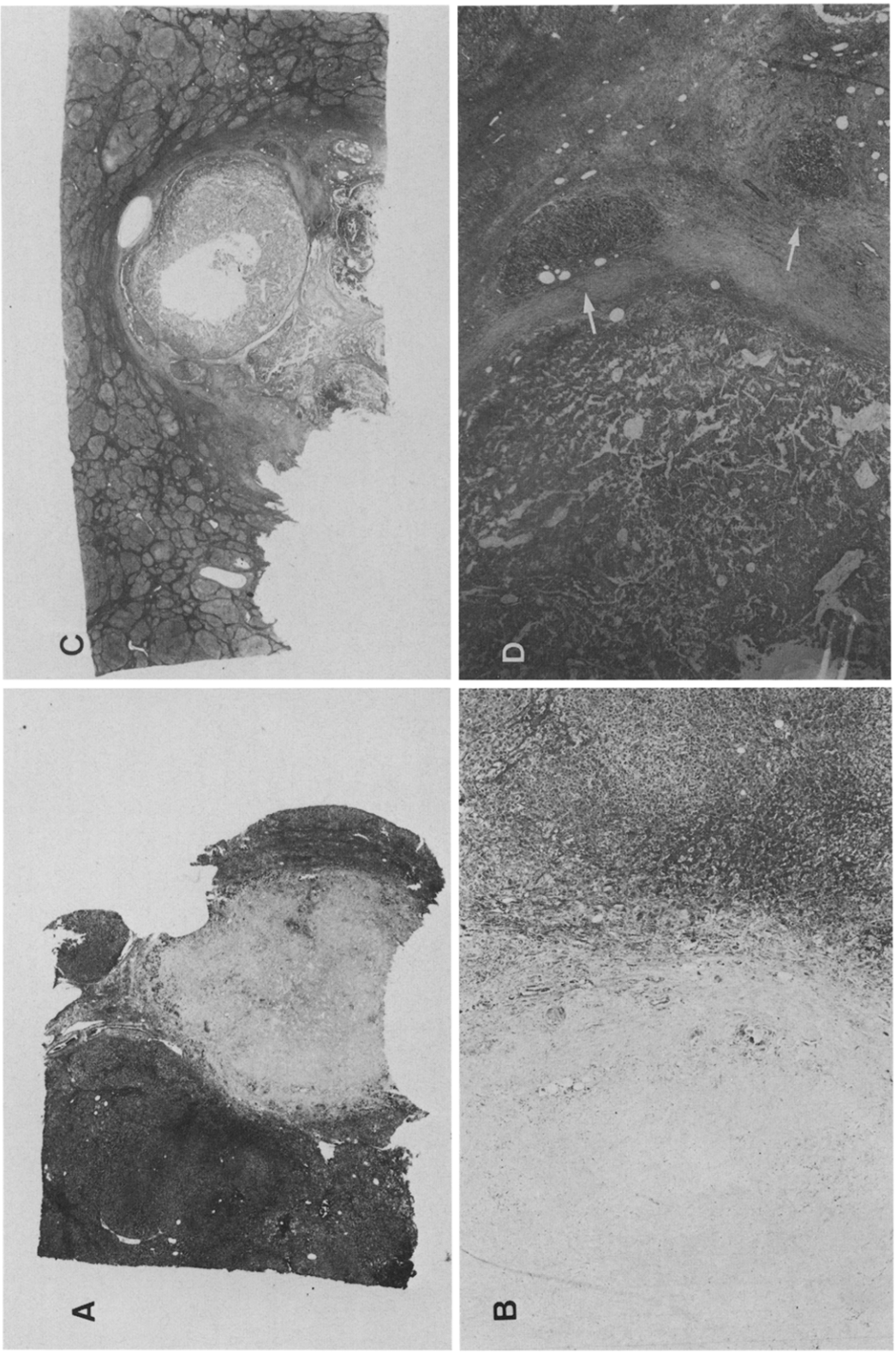

Fig. 7. Resected hepatoma revealed antitumor effect of SMANCS-ethiodol administered preoperatively. (A) and (B) SMANCS-ethiodol (5 mg *in* 5 ml) *had been administered* 17 days *before resectioning; (C) and (D) the drug* (4 mg *in* 4 ml) *has been administered* 22 days *before resectioning. Hepatoma became almost necrotic, but a small cancer nest was still observed at the periphery of the tumor (case in group C).*

The major side-effect observed after arterial infusion of SMANCS-ethiodol was fever of 38–39°C (59%), as shown in Table 3, although most patients became afebrile within a few days. About 20% of the patients experienced dull pain in the upper abdomen which lasted for about 15 min after infusion. Mild and transitory elevations of serum GOT and GPT were seen in about 30%. No hematosuppression was observed. Unexpectedly, moderate leukocytosis was seen in 65% of the patients.

Prognosis

While it is too early to evaluate longer term prognosis at this point, we have compared our present data with a comparison group of patients with advanced hepatomas not treated with this protocol. The present survival data for patients with unresectable hepatoma subjected to SMANCS-ethiodol treatment from February 1981 until June 1982 and observed up to September 1982 are presented in Figure 8. The comparison group of 32 patients with unresectable hepatoma, who were subjected similarly to ligation of the hepatic artery (20 cases) and/or intra-arterial continuous (17 cases) or single (11 cases) administration of mitomycin C and/or 5-fluorouracil, had an average survival period of 2.3 ± 1.6 months after day 1. Clearly, the survival period of patients treated under the present protocol was definitely longer than the comparison group (Fig. 8).

DISCUSSION

Two major advantages of the arterial infusion of SMANCS-ethiodol have been elucidated. Firstly, the selective accumulation of ethiodol in tumor tissue was revealed by X-ray and in resected tumor. This result provides diagnostic advantages with plain X-ray, CT scan or ultrasonography (Figs 1–4). More precise determination of location and size of the tumor using any of these methods has been demonstrated. Furthermore, these data demonstrated that the deposition of ethiodol in

Table 3. Side-effects of arterial administation of SMANCS-ethiodol

1.	Fever 50/85 (59%),	38–39°C,	transitory, 7 days
2.	Pain 19/85 (22%),		transitory, 20 min
3.	Laboratory data		
	GOT: elevation 23/71 (32%)*		
	GPT: elevation 17/71 (24%)*		
	bilirubin: elevation 16/68 (24%)*		
	WBC: increase 35/54 (65%)*,	decrease 6/54 (11%)*	

*All transitory

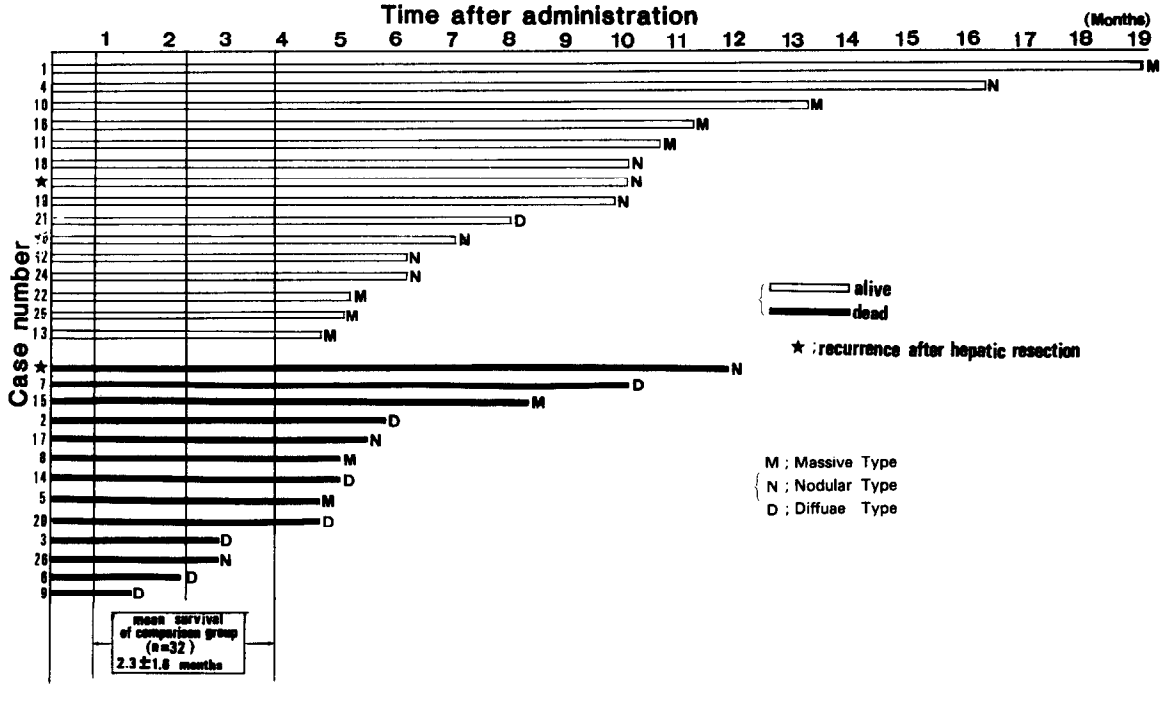

Fig. 8. Prognosis of patients with unresectable hepatoma treated with SMANCS-ethiodol.

the tumor persisted for more than 3 months. The ethiodol deposited in the vasculature of the tumor might also add the effect of embolization of the neovasculature to the effect of the anticancer agent, although we found in the separate study embolization by ethiodol alone was not effective at all [Konno *et al.*, unpublished data]. Secondly, the high-molecular-weight antitumor agent SMANCS can be delivered and deposited more selectively in the tumor tissue for a longer period of time by the present method than any other conventional method and without major technical difficulties or side-effects.

The mechanism of the selective accumulation of ethiodol in hepatomas is not fully understood as yet. We speculate that the combination of the neovascularization with its particular nature, such as vascular structure, mode of blood flow and subsequent increased permeability, and the lack of a reticuloendothelial recovery system in the tumor tissue, may be factors attributing to the observed results. Furthermore, the large molecular nature of SMANCS, particularly in lipid, results in slower diffusion, thus slower clearance than smaller molecules. As a consequence, SMANCS is retained in the tumor for a longer period of time than smaller molecules.

Normal tissues, on the contrary, have well-developed lymphatic systems which effectively recover large proteinaceous molecules such as neocarzinostatin or SMANCS. This efficient and rapid recovery in lymph nodes has been reported after systemic injection [4, 7]. There appears to be fairly effective leakage of SMANCS from tumor capillaries into tumor tissues. In the tumor tissues highly developed neovascularization is known to render substantial leakage of many substances, including macromolecules from the vascular space into the tumor tissue. This assumption is supported by the studies of Peterson *et al.* [8, 9] using [^{132}I]-albumin (mol. wt 6.7×10^4) or fibrinogen (mol. wt 14×10^4) and of Shibata *et al.* [10] using [^{132}I]-α-1-acid glycoprotein (mol. wt 14×10^4). Namely, predominant accumulation of these macromolecules in the tumor tissues or in the exudate was observed. In addition, underdeveloped lymphatics in the cancer tissue will leave the macromolecules *in situ* due to slower diffusion as well as slower recovery. These two aspects, much leakage and little recovery of large molecules, may explain the present results. We have observed in separate experiments that when SMANCS was injected intravenously it accumulated more in the tumor (VX-2) tissue of rabbit than in normal tissues [Maeda *et al.*, unpublished data].

When the bioavailability of SMANCS-ethiodol was measured using resected tumor specimens of the treated patients, it was not surprising that the highest level was found in the tumor tissue followed by adjacent normal tissue. Furthermore, normal liver tissue, obtained distant from the tumor site, exhibited virtually no bioactivity of SMANCS (Table 2), perhaps due to the reasons described above. Localization of drug delivery mainly to the tumor helps to minimize the side-effects. The bioactivity of SMANCS in tumor tissue is concordant with the almost selective presence of ethiodol in the tumor tissue.

We employed two parameters for the assessment of anticancer effect of the present method: serum alpha-fetoprotein (Fig. 5) and tumor size (Fig. 6). The decrease of serum alpha-fetoprotein in 86% of patients was indeed remarkable, and only 2 cases (10%) exhibited continued increase (Fig. 5). A detailed examination of these two cases revealed that one had a distant lung metastasis which was initially unnoticed; the other patient's drug administration was by super-selective catheterization which failed to deliver the drug to the opposite lobe of the liver where a satellite nest resided, and thus its growth continued. These two cases warned us that the super-selective catheterization was unnecessary and perhaps too selective;thus selective arterial infusions such as via the celiac artery or common or proper hepatic arteries was preferable. Of course, such selective catheterization is also much easier to perform.

The tumor size decreased in about 90% of the treated patients to 10–99% of the original size (Fig. 6). This pronounced tumor regression should result in the prolongation of survival of these patients, and our preliminary results support this (Fig. 8). Although most of the patients possessed unresectable advanced hepatoma (Table 1) and whose expected life span was 2.3 ± 1.6 months, the survival period of treated patients far exceeded the comparison group (Fig. 8). Although it is premature to make conclusions at this time, a significant beneficial effect of SMANCS-ethiodol appears evident under the present protocol.

The optimum dose regimen has yet to be established for SMANCS-ethiodol therapy. We have confirmed, however, that an interval of 2–4 weeks with a total of 3–4 doses has proven substantially effective, and for a hepatoma of 10×8 cm more than 15 mg of SMANCS as a total dose is needed for total necrosis of the tumor. The largest volume of SMANCS-ethiodol injected was 6 ml in each hepatic lobe, but this dose was associated with increased abdominal pain. Thompson and Aniyan [11] reported that the average lethal dose of intravenous ethiodol in dogs was 1.58 ml per kg of body weight (94.8 ml/60 kg). Thus our dosage of ethiodol is in a very safe range. Although the LD_{50} of SMANCS

is essentially similar to that of neocarzinostatin [3] on a molar basis, it appears that the above-described local administration of SMANCS-ethiodol may be much less toxic than systemic administration [5]. No severe adverse effects were observed that required discontinuation of drug administration in the present regimen, and the minor systemic side-effects are described in the preceding section (Table 3). A slight fever (37–39°C) may be favorable in view of hyperthermia in the treated patients.

In conclusion, early analysis of the present chemotherapy with SMANCS-ethiodol offers the following advantages. (i) Super-selective catheterization is not necessary, thereby making the procedure amenable to most hospitals. (ii) The anticancer effect was definite in the majority of cases, and tumor regression will continue for a long period without frequent drug administrations. (iii) The side-effects were all transitory and mild, and no severe complications were experienced. Furthermore, there was no hematosuppression or liver toxicity. (iv) The ethiodol was found to be useful for more sensitive and simple radiological diagnosis and the follow-up study of these patients.

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