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Clinicopathological study on combination therapy consisting of arterial infusion of lipiodol-dissolved SMANCS and transcatheter arterial embolization for hepatocellular carcinoma*

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Summary. Combination therapy (LpTAE) consisting of arterial infusion of a lipophilic anticancer drug, SMANCS, dissolved in an oily lymphographic agent, lipiodol (LPD), and transcatheter arterial embolization (TAE) for hepatocellular carcinoma (HCC) was studied with special reference to the pathological findings. A total of 32 patients were subjected to surgical resection after LpTAE. The pattern of LPD deposition in the tumor was examined by CT scan (Lipiodol CT, LpCT) at 7 days and/or 1 month after LpTAE. The resected materials were examined radiographically with soft X-rays and histologically. LPD was deposited in tiny daughter nodules with a diameter of less than 5 mm and in tumor thrombi as well as in the main tumors, which showed necrotic change. Part of the LPD flowed out from the main tumor via the drainage vein and was deposited in the capsular invasion, resulting in necrosis. LPD accumulated almost exclusively within the blood spaces of trabecular-type HCC, creating a pattern corresponding to a cast of the tumor vessels, which showed prominent necrosis. On the other hand, LPD was not deposited in scirrhous, compact, or well-differentiated HCC, which showed little or no necrosis. It was demonstrated that LpCT images, which accurately depicted the existence and the extent of LPD deposition and necrosis in the tumor, were useful for precise evaluation of the therapeutic effect. Our findings indicate that LpTAE and LpCT are valuable for the diagnosis and treatment of HCC and should play a central role in systemic therapeutic approaches to this disease.

Introduction

Surgical resection remains the most reliable treatment for localized hepatocellular carcinoma (HCC). However, it is not necessarily the therapy of first choice because HCC is often associated with liver cirrhosis, seems to be of multicentric origin, or shows early intrahepatic metastasis and because many cases are diagnosed as being too advanced for surgery. Transcatheter arterial embolization (TAE) has been proven to be one of the most effective nonsurgical therapies, achieving survival values comparable with those obtained using surgical resection [15].

A lipid lymphographic agent, lipiodol (LPD), shows a peculiar property of being selectively deposited in HCC following its arterial administration. Using this property of LPD, a new therapeutic approach for delivering an anticancer drug selectively into HCC has been developed. A lipophilic anticancer drug, SMANCS [11], dissolved in LPD is selectively deposited within HCC and is then gradually and continuously released from the trapped LPD into the tumor tissues. The clinical validity of this therapeutic approach has been clearly demonstrated [9]. Other water-soluble chemotherapeutic agents, e.g., doxorubicin and cisplatin, have also been shown to be effective when emulsified or suspended in LPD for arterial infusion [7, 13].

In 1983, we combined TAE and LPD-mediated delivery of an anticancer agent (LpTAE) for the treatment of HCC for the first time and reported its clinical advantages [3, 4]. In the present investigation, the characteristics of this combination therapy were studied with special reference to the pathological findings.

Patients and methods

Patients. A total of 180 individuals diagnosed as having HCC on the basis of high AFP levels, various imaging methods, and/or histological examination were treated with LpTAE. In all, 32 patients (37 HCC nodules) were operated on at 26–235 (mean, 81) days after LpTAE. Table 1 shows their clinical features. HCCs with a diameter of less than

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Table 1. Clinical background of patients

Number of patients	32 (37 nodules)
Sex (M/F)	28/4
Age (years)	42–68 (mean, 54.9)
Size of tumor (cm)	1.2–9.3 (mean, 3.4)
Number of nodules <5 cm	30
Number of nodules ≥5 cm	7
Coexistent liver disease:	
Liver cirrhosis (+)	28 (87%)
Chronic hepatitis (+)	4 (13%)
HBsAg (+)	12 (38%)
AFP (≥400 ng/ml)	13 (41%)
Period from LpTAE to operation (days)	26–235 (mean: 81)
Stage[a] I	6 (19%)
II	12 (38%)
III	8 (25%)
IV	6 (19%)

[a] TNM classification according to the general rules for primary liver cancer

5 cm were designated as being small, whereas those with a diameter of 5 cm or more were designated as being large.

Treatment. SMANCS dissolved in LPD at a concentration of 1 mg/ml was obtained from the Department of Microbiology, Kumamoto University, Japan. In all, 3–5 ml of the solution was infused via the proper hepatic artery. After the selective deposition of LPD in the tumors had been confirmed by fluoroscopy, TAE was superimposed by intra-arterial injection of small pieces of gelatin sponge containing 20–40 mg doxorubicin. CT scans (Lipiodol CT, LpCT) were performed at 7 days and/or 1 month after LpTAE to classify the pattern of LPD deposition in the tumor.

Histological investigation. The surgically resected materials were cut into 5-mm thin slices. Each slice was radiographed with soft X-rays for macroscopic observation of LPD deposition. The tissues were fixed with formalin. A histopathology study was performed on each 5-mm slice. Slices were stained with hematoxylin-eosin, silver impregnation, Azan, elastica van Gieson, and Sudan III. Each HCC was histologically classified according to the WHO classification [1]. Well-differentiated HCC was classified separate from the WHO classification.

LpCT images were compared with the soft X-ray radiographs and histological findings, with special attention being paid to the therapeutic effectiveness of LpTAE, i.e., the rate of tumor cell necrosis.

Results

Correlation between LpCT images and tumor cell necrosis

LPD deposited in HCC tissues appeared as very high-density areas (HDA) on LpCT images. The patterns of LPD deposition in small HCCs were classified into four basic types and an intermediate type as schematically illustrated in Fig. 1. Of 30 small HCC nodules, 9 (30%) showed the type 1 pattern. Types 2, 3, and 4 were observed in 4 (13%), 5 (17%), and 10 (33%) nodules, respectively; 2 HCC nodules (7%) displayed a mixed pattern of types 1 and 2, i.e., intermediate type 1+2.

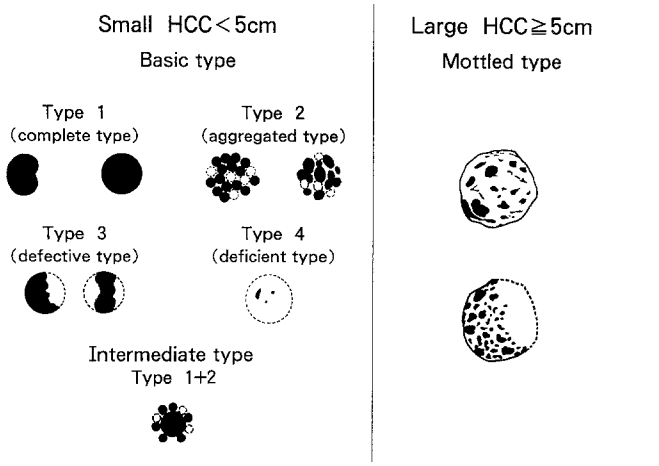

Fig. 1. Classification of lipiodol CT patterns. *Basic type:* This type is seen mainly in HCCs measuring <5 cm in diameter and is subclassified into 4 types. *Type 1 (complete type)* – these tumors are seen as almost round and homogeneous HDAs, with LPD being deposited throughout the lesion. *Type 2 (aggregated type)* – these tumors display an aggregation of small HDAs. *Type 3 (defective type)* – these tumors exhibit an HDA with a defect, i.e., LPD is not deposited in some parts of the lesion. *Type 4 (deficient type)* – these tumors are not seen as HDAs, i.e., little or no LPD is deposited in the lesion. *Intermediate type:* This type shows a pattern intermediate between those of types 1 and 2, i.e., some small HDAs appear as buds (*type 2*) adjacent to the main tumor of the complete type (*type 1*). *Mottled type:* This type is seen in HCC nodules with a diameter of >5 cm. An imbalance between the volume of HCC nodules and the amount of LPD injected may be a cause of these irregular and dispersed LpCT images

Figure 2 shows the correlation between the type of LPD deposition and tumor cell necrosis. In type 1, total cell necrosis was seen in eight of nine small nodules. In contrast, tumor cell necrosis was almost never seen in small HCC nodules with type 4 LPD deposition, i.e., little or no deposition of LPD. In HCC nodules of types 2 and 3, the tumor cells in the area of positive LPD deposition were necrotic, whereas those in the area showing no LPD deposition remained viable. Tumor cell necrosis seemed to be directly proportional to the amount of LPD deposited.

Seven large HCCs were resected, and five showed a mottled type of LPD deposition in the presence or absence of a defect. An imbalance between the volume of the HCC and the amount of LPD injected may have been a cause of these irregular LpCT images. Type 1- and type 4-like depositions were seen in one case each.

Blood vessels in HCC and LPD deposition

The soft X-ray radiographs revealed selective deposition of LPD in the HCC nodules (Fig. 3). On close observation, LPD was seen as narrow and uneven linear structures arranged in an irregular and complex pattern. These structures were histologically confirmed to be the blood spaces of HCC. Sudan III stained LPD as a red substance in these blood spaces. Because LPD was eluted with ethanol during dehydration, the blood spaces were found not to contain LPD on hematoxylin-eosin staining (Fig. 4).

Type of Lipiodol CT		No. of nodules(%)	Rate of necrosis: 0%	~10%	~50%	50%~	90%~	100%
Basic type	Type 1 (Complete type)	10(27.0)					●	●●●●●○
	Type 2 (Aggregated type)	4(10.8)					●●●	
	Type 3 (Defective type)	5(13.5)			●	●●	●	
	Type 4 (Deficient type)	11(29.7)	●●●●●	●●●	○			
Intermediate type	Type 1 + 2	2(5.4)				●	●	
Mottled type		5(13.8)				○○○○	○	
Total no. of nodules (%)		37(100)	7(18.9)	3(8.1)	2(5.4)	8(21.6)	8(21.6)	9(24.3)

●: nodule with a diameter of less than 5 cm. ○: nodules with a diameter of more than 5 cm.

Fig. 2. Schema showing the relationship between the type of lipiodol CT and the rate of tumor necrosis

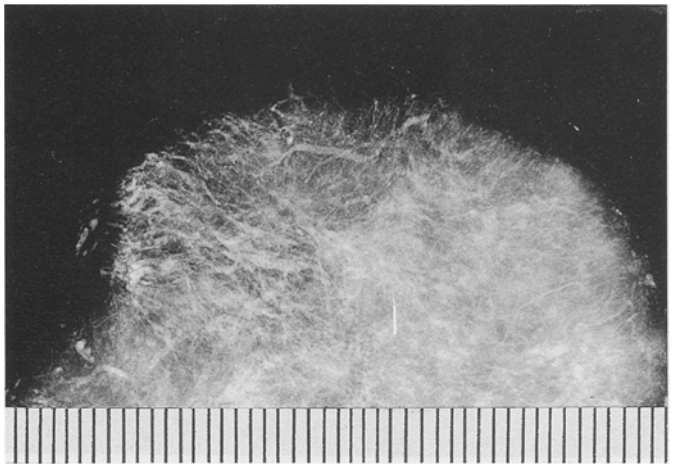

Fig. 3. Findings on soft X-ray radiographs. LPD, which is deposited selectively within the tumor, appears as fine linear structures arranged in irregular and complex patterns corresponding to a cast of the tumor blood vessels

Histologically, it was seen in HCCs displaying type 1 LPD deposition that part of the LPD trapped in the main tumor had flowed out via the drainage vein and had deposited in the tumor tissue in the capsular invasion and in the surrounding small daughter nodules, resulting in necrotic change in these parts (Fig. 5). Even small daughter nodules situated far from the main tumor showed LPD deposition and necrosis.

LPD deposition, architecture of tumor blood vessels, histological classification, and therapeutic effects of LpTAE

As can be seen from Table 2, the deposition of LPD in the tumor showed a close correlation with the tumor blood vessels, the histological classification, and the therapeutic effects. The trabecular type, in which broad blood spaces were well developed, showed high LPD deposition and striking necrotic change. This histological type was seen in all nodules exhibiting type 1 LPD deposition.

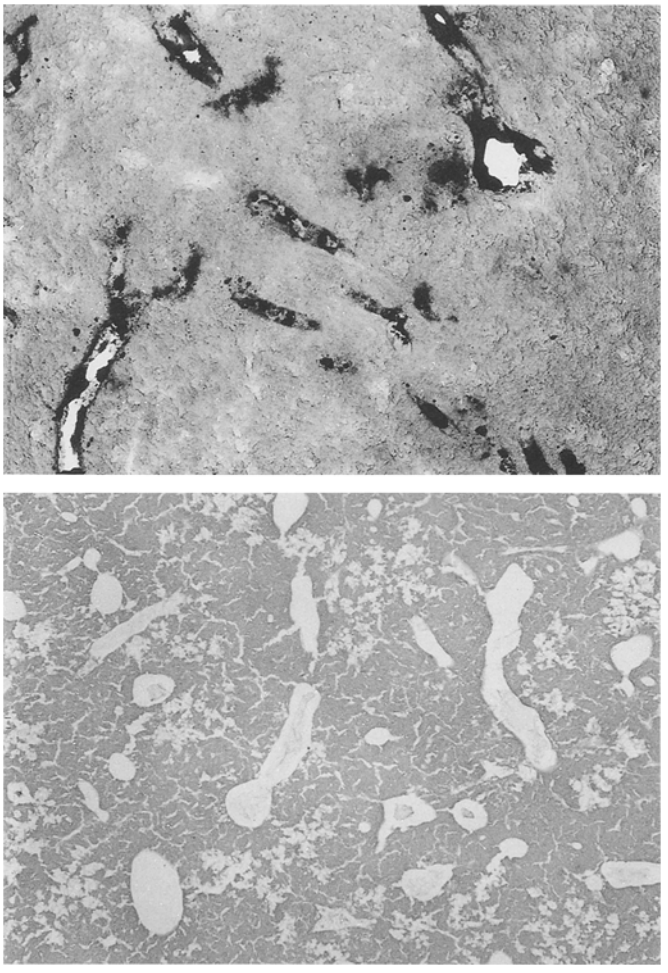

Fig. 4. Histological findings in LPD-deposited HCC. LPD is observed as a red (black on the photo) substance within the blood spaces of the tumor, positively stained by Sudan III (*top*, ×100). The blood spaces appear empty on hematoxylin-eosin staining (*bottom*, ×100). Note the striking necrosis of the tumor cells

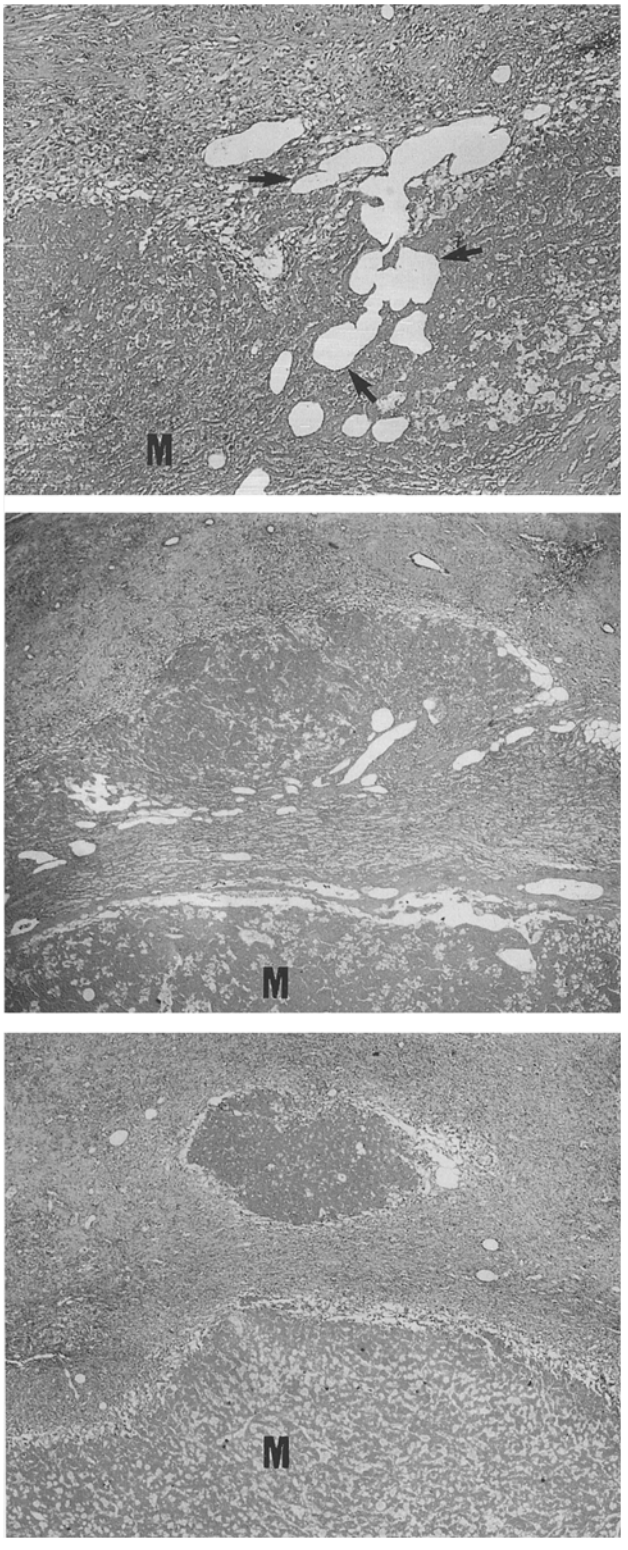

Fig. 5. Part of the LPD trapped in the main tumor (**M**) leaks from it through the drainage vein (*arrow*; *top*, H&E, ×100) and is deposited in the tumor tissue invading the capsule (*middle*, H&E, ×20) and in the surrounding tiny daughter nodules (*bottom*, H&E, ×20)

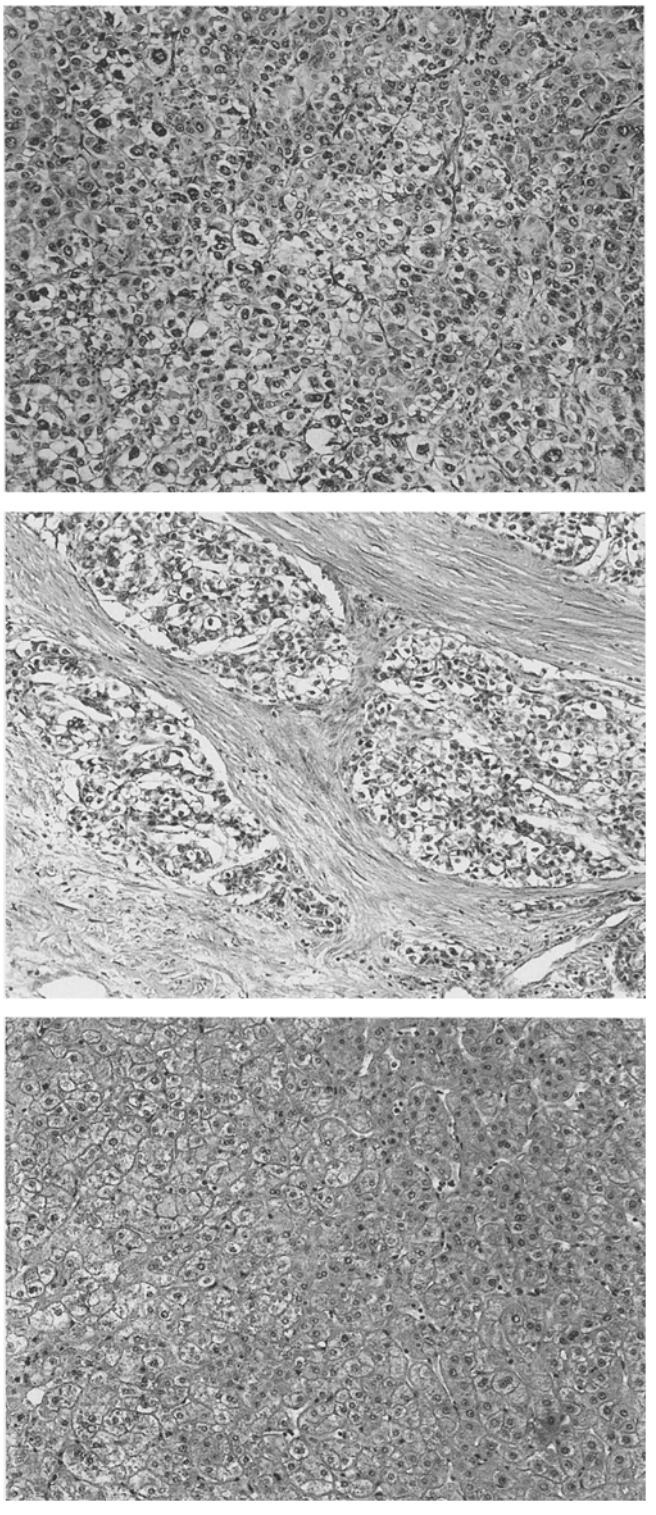

Fig. 6. Histological findings in HCC showing no LPD deposition. The compact-type HCC (*top*, H&E, ×100) has well-developed blood spaces, which seem to be too narrow for LPD deposition due to compression of the thick trabecular growth of the tumor cells. The scirrhous-type HCC (*middle*, H&E, ×40) contains a small number of blood spaces. The number and width of the blood spaces in well-differentiated HCC (*bottom*, H&E, ×100) does not differ from that in the nontumorous portion; LPD deposited in the tumor is eluted in the same way as that in the nontumorous portion

Table 2. Relationship between lipiodol deposition and the architecture of tumor blood vessels, histological picture, and therapeutic effect

Lipiodol desposition in tumor tissue	Tumor blood vessel		Histological picture (WHO)	Therapeutic effect
	Quantity	Caliber		
+	High	Broad	Trabecular type	+
–	High Almost normal	Narrow	Compact type Well-differentiated type[a]	–
–	Low	Narrow	Scirrhous type Combined type, rare Sarcomatous change, rare	–

[a] Corresponding approximately to Edmondson-Steiner's grade I

The predominant histological types of HCCs showing type 4 LPD deposition were compact (4 of 10 nodules), scirrhous (2 nodules), and well-differentiated (4 nodules; Fig. 6). LPD could almost never be detected in these nodules on soft X-ray radiographs. Histological examination revealed that the blood spaces of these tumors, scanty in number and/or narrow in diameter, did not contain LPD. Tumor cell necrosis was negligible, involving less than 10% of the whole nodule.

The deposition of LPD and the presence of tumor cell necrosis in HCC nodules displaying type 2 or 3 LPD deposition reflected the architecture of the blood vessels as described above, i.e., areas with well-developed, broad blood spaces retained LPD and showed tumor cell necrosis.

Discussion

TAE is a powerful nonsurgical treatment for HCC [15]. However, histological evaluation of TAE-treated tumor tissues has revealed that the capsular invasion, the small daughter nodules, and the tumor thrombi are resistant to TAE [2, 12]. Because the effect of TAE depends mainly on occlusion of the feeding artery of HCC with small particles of gelatin, tumor necrosis is extensive, but tumor tissues directly under or invading the capsule are thought to escape from the ischemic attack. It is presumed that TAE has little ischemic effect on small daughter nodules since these nodules seem to receive a dual supply of blood via arteries and portal veins and because solid particles of gelatin are not uniformly distributed throughout the liver.

As compared with gelatin particles, LPD can be delivered into the tumor itself and is more diffusely distributed throughout the liver. LPD is trapped even in small daughter nodules since it is a fluid with the ability to be finely dispersed. We have previously shown that LPD is trapped and accumulated in irregularly developed tumor blood vessels with abnormal architecture [5].

LPD must be mixed with an anticancer agent to provide a therapeutic effect because LPD itself does not have the embolic effect of gelatin sponge. The anticancer agent aclacinomycin A (ACA) contained in LPD (ACA/LPD) is gradually released from LPD into the tumor tissue. For the arterial administration of ACA/LPD to be effective, the HCC cells must be sensitive to the anticancer agent mixed in the LPD. Because HCC cells are not always sensitive to an anticancer agent, this therapy has a limited effect. Since the volume of LPD injected is usually 3–5 ml per dose, arterial administration of ACA/LPD has to be carried out repeatedly to achieve a better therapeutic effect when the volume of the tumor is large.

We have introduced LpTAE, i.e., TAE supplemented with LPD-mediated delivery of an anticancer agent for promotion of the therapeutic effect [3, 4]. This combination therapy has merits and overcomes the above-described inadequacies of both TAE and LPD.

In the present study, we analyzed the histological basis for the clinical effects of LpTAE. We found that the tumor tissues invading the capsule of the main tumor and the tiny daughter nodules, which are difficult to treat with TAE alone, showed necrotic changes. We also frequently observed LPD retention in tumor thrombi, which was associated with a therapeutic effect. These findings indicate that LpTAE is more effective than TAE alone. A modified method of LpTAE has also been described by other investigators [8, 14].

In addition to its therapeutic merit, LpCT was useful for the diagnosis of intrahepatic spread of HCC since small daughter nodules could be detected as clear HDAs [16]. Furthermore, we demonstrated that LpCT was useful for predicting the therapeutic effect of LpTAE [6]. HCC displaying type 1 LPD deposition can be expected to undergo total necrosis. Type 2 deposition suggests that more than 90% of the tumor tissues will undergo necrosis. In type 3 deposition, the LPD-deposited area would be necrotic, whereas the area showing no LPD deposition would remain viable. In HCC exhibiting type 4 deposition, no therapeutic effect can be expected.

In the histological classification, HCCs displaying type 1 LPD deposition and the LPD-positive areas in HCCs showing types 2 and 3 deposition were trabecular. LPD trapped in the blood spaces of this histological type was hardly eluted. One-third of our cases did not retain LPD, i.e., they showed type 4 LPD deposition. Their histological classification was the compact, scirrhous, or well-differentiated type. The blood spaces seemed to be too narrow in diameter for deposition of LPD in the compact type and too few in number in the scirrhous type. It was presumed that LPD was indeed deposited in the well-differentiated type of HCC but was as rapidly washed from the tumorous part as it was from the nontumorous portion.

On the other hand, these observations also suggest a therapeutic limitation for LpTAE in HCCs of the scirrhous, compact, and/or well-differentiated type. It should be kept in mind that LpTAE does not always produce a therapeutic effect in small HCCs with a diameter of less than 2 cm; small HCCs are often well-differentiated or are composed of various histological types, in which LPD is not deposited [6].

Since precise evaluation of the therapeutic effect of LpTAE is possible with LpCT, a more effective treatment modality for the next step can be appropriately selected. For example, in the case of type 4 LPD deposition, operative resection or percutaneous ethanol injection therapy [10] should be considered.

In conclusion, the structure of the tumor blood vessels affects the sensitivity of HCCs to LpTAE. We can expect total tumor cell death in HCCs, including the capsular invasion and the daughter nodules, if they show type 1 LPD deposition. These HCCs are histologically of the trabecular type and have broad blood spaces. LpTAE and LpCT are useful procedures for the diagnosis and treatment of HCC and are valuable in the design of therapeutic strategies for the management of patients with this disease.

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