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Anticancer Effects of Local Administration of Mitomycin C via the Hepatic Artery or Portal Vein on Implantation and Growth of VX_2 Cancer Injected into Rabbit Liver[1]

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ABSTRACT

Rabbits were inoculated with a suspension of VX_2 carcinoma cells in the liver, and mitomycin C was given via the hepatic artery or the portal vein for a study of the anticancer effects.

Twenty-eight rabbits were killed for preliminary study at 1 h or 1, 3, 7, 9, 12, or 14 days after the inoculation. Another 36 rabbits were divided into three groups. Groups A and B were given the agent (0.5 mg/kg), 1 h after the inoculation and on Days 2, 4, 6, and 8, into the common hepatic artery or the splenic vein, respectively. Group C was not treated after inoculation. The mean numbers of cancer nodules per rabbit in Groups A, B, and C were 11.9, 36.4, and 83.4, respectively, at 12 days after inoculation. The number of cancer nodules of Group A was smallest ($P < 0.025$, F test). The means of the total cross-sectional area of tumor nodules in Groups A, B, and C were 32.7, 79.7, and 217.3 mm^2, respectively. The total cross-sectional area of the cancer nodules of Group A was smallest ($P < 0.05$, F test). These results suggest that the anticancer agents given via the hepatic artery had better effects on early (small) metastatic liver tumor than those via the portal vein.

INTRODUCTION

Postoperative results for cancers of the digestive organs are improving, but death still occurs following removal of primary lesions, especially from the pancreas or colon, due to liver metastases that form during the early postoperative period. Further improvement of results depends, in part, on finding a countermeasure to liver metastases. Currently, embolization of the intrahepatic artery and administration of anticancer agents via the hepatic artery are done for unresectable metastatic liver tumors (1–3). However, small metastatic cancer nodules in the liver are nourished by both hepatoarterial and portal blood (1, 4–6), and it is not known whether the hepatic artery or portal vein is the better route for prophylactic administration of the appropriate anticancer agents if we select one route, although treatment through both routes might be the best. This study was designed to investigate this problem preceding the development of feeding vessels for intrahepatic cancer nodules that had metastasized via the portal vein.

MATERIALS AND METHODS

Sixty-four New Zealand White rabbits, weighing 2–3 kg each, were used in the following experiments.

MMC,[3] characterized by a level dependency (MMC is effective when its level in the blood is high), rapid disappearance from blood (7, 8), and rapid fall in intrahepatic activity (8), was used as the anticancer agent.

The VX_2 tumor cells (9) were injected into the liver in all rabbits as follows. The tumor strain was maintained by successive transplantation into the hind leg muscle of rabbits. Extirpated VX_2 tumor from the muscle was minced in Hanks' solution and filtered through four sheets of gauze. The filtrate was adjusted to a concentration of $10^7 \times 1.0$–2.0 cells per ml. Under general anesthesia with 25.6 mg of sodium pentobarbital per kg i.v., laparatomy was done through a midline abdominal incision, and the cell suspension in a volume of 0.25–2.0 ml was injected into the portal vein through the short gastric vein with a 27.5-gauge needle.

Implantation and Growth of VX_2 Cancer Cells

We wanted to establish the term of administration of the anticancer agent and also the period needed between the inoculation to the killing of the rabbits in the later study. Twenty-eight rabbits were divided into 7 groups.

Rabbits in Group 1 ($n = 2$) were killed 1 h after the inoculation, which in this group was of 2.0 ml of the suspension, to facilitate the histological investigation.

Rabbits in Group 2 ($n = 2$) were inoculated with 1.0 ml of the cell suspension and killed 1 day later.

Rabbits belonging to Groups 3–7 were given injections of 0.25 ml of the cell suspension. In Groups 3, 4, and 5, each containing 4 rabbits, all were killed on Days 3, 7, or 9, respectively. In Groups 6 and 7, each containing 6 rabbits, all were killed on Days 12 or 14, respectively.

Livers were removed from the animals, sliced 5 mm thick, stained with hematoxylin-eosin, and examined under a microscope.

Evaluation of Anticancer Effects of MMC Given via Hepatic Artery or Portal Vein

Thirty-six rabbits were given injections of 0.25 ml of the cell suspension and divided into the following three groups.

Group A (Ten Rabbits). Immediately after the cell injection, an indwelling catheter (outer diameter, 0.2 mm) was inserted into the common hepatic artery via the left gastric artery. MMC (0.5 mg/kg) was injected 1 h after the inoculation and again on Days 2, 4, 6, and 8.

Group B (Eleven Rabbits). Immediately after the cell injection, a catheter (outer diameter, 1.0 mm) was inserted into the splenic vein. MMC was injected as in Group A.

Group C (Fifteen Rabbits). These rabbits were not treated after the injection.

One rabbit from each of these three groups was killed on Day 7 after the inoculation, and the others were killed on Day 12. The liver was removed and sliced 5 mm thick. The numbers and the diameter of cancer nodules on cut surfaces were recorded, and the nodules were histologically investigated.

The numbers and the total cross-sectional areas of cancer nodules of the rabbits killed on Day 12 were compared by the following method. Tumor nodules were categorized by their diameter into Grades 1 (1–2 mm), 2 (2–3 mm), 3 (3–4 mm), or 4 (4 mm or more). Nodules smaller than 1 mm in diameter were ignored. The diameters of tumor nodules of Grades 1, 2, and 3 were assigned values of 1.5, 2.5, and 3.5 mm, respectively, and the actual diameters of tumor nodules of Grade 4 were measured individually. From this, the cross-sectional area of tumor nodules (mm^2; X) from each rabbit was calculated. The value of Y transformed as $Y = \ln(X + 10)$ was used to approximate the distribution curve of X to a probability curve for the statistical analysis of the cross-sectional areas (10). For the same reason, the number of cancer nodules (M) was transformed to Z as $Z = \ln(M + e)$ ($e = 2.718$).

RESULTS

Implantation and Growth of VX_2 Cancer Cells. No tumor nodules were seen macroscopically in Groups 1, 2, or 3. Tumor nodules were seen macroscopically in one of the 4 rabbits of Group 4 and in all rabbits of Groups 5, 6, and 7. Most nodules

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[3] The abbreviation used is: MMC, mitomycin C (Kyowa Hakko Kogyo Co., Japan).

were punctate in Group 4, smaller than 1 mm in Group 5, 1–2 mm in Group 6, and 3–5 mm in Group 7. Cancer nodules were distributed among all lobules of the liver almost evenly.

In Group 1, cancer cells were found in the interlobular portal branches with a number of small inflammatory cells (Fig. 1). In Groups 2 and 3, cancer cells in the interlobular portal branches collected and formed embolic conditions with fibrin sediment (Fig. 2). In addition, solitary cancer cells were dispersed in the sinusoids, which had regions of infiltration as well (Fig. 3). In Group 4, VX_2 cellular foci centered around the interlobular region; they proliferated and grew toward the center of the lobule (Fig. 4). In Groups 5, 6, and 7, cancer regions showed more proliferation of cancer cellular foci that proceeded to the extent of destruction of lobular structures (Fig. 5).

Evaluation of Anticancer Effects of MMC Given via Hepatic Artery or Portal Vein. The mean numbers of cancer nodules in Groups A, B, and C were 11.9 ($Z = 2.13 \pm 1.05$), 36.4 ($Z = 3.30 \pm 1.03$), and 83.4 ($Z = 3.98 \pm 1.09$), respectively. More than 80% of the cancer nodules were smaller than 2 mm across in each group (Tables 1 to 3).

The averages of the total cross-sectional area of cancer nodules in Groups A, B, and C were 32.7 mm^2 ($Y = 3.22 \pm 1.00$), 79.7 mm^2 ($Y = 4.16 \pm 0.94$), and 217.3 mm^2 ($Y = 4.93 \pm 1.05$), respectively (Tables 1 to 3).

The F test showed that the number of cancer nodules in Group A was fewer than in Groups B or C ($P < 0.025$, $P < 0.005$, respectively; Fig. 6), and that the total cross-sectional area of the cancer nodules in Group A was smaller than in Groups B or C ($P < 0.05$, $P < 0.005$, respectively; Fig. 7).

Histological studies showed necrosed cells and empty spaces in the foci in Groups A and B on Day 7 (Fig. 8). In these groups, several cancer lesions showed marked fibrosis (Fig. 9),

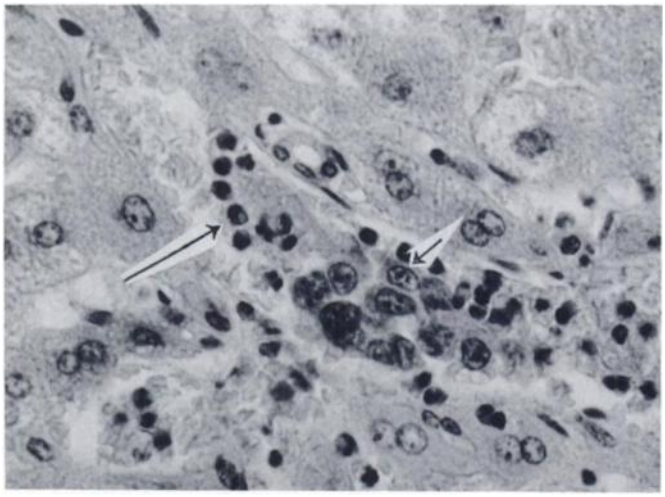

Fig. 1. Liver specimen 1 h after injection of VX_2 cancer cells. The *short arrow* points to a VX_2 cancer cell; the *long arrow*, to a small inflammatory cell in the interlobular portal branch. H & E, × 500.

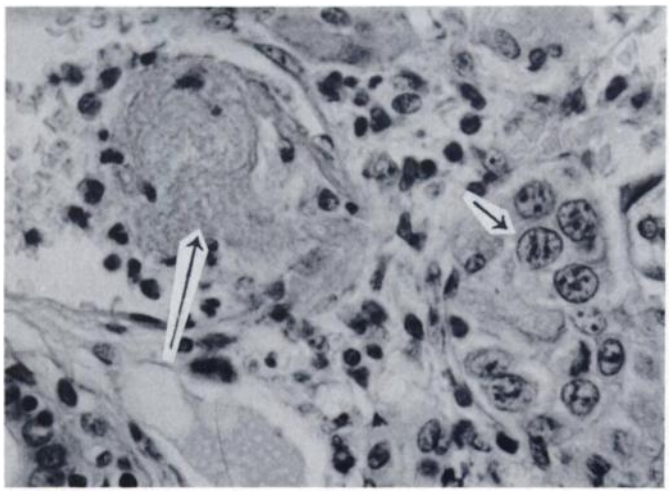

Fig. 2. Liver specimen 1 day after injection of VX_2 cancer cells. The *short arrow* points to a VX_2 cancer cell; the *long arrow*, to fibrin sediment in the portal branch. H & E, × 500.

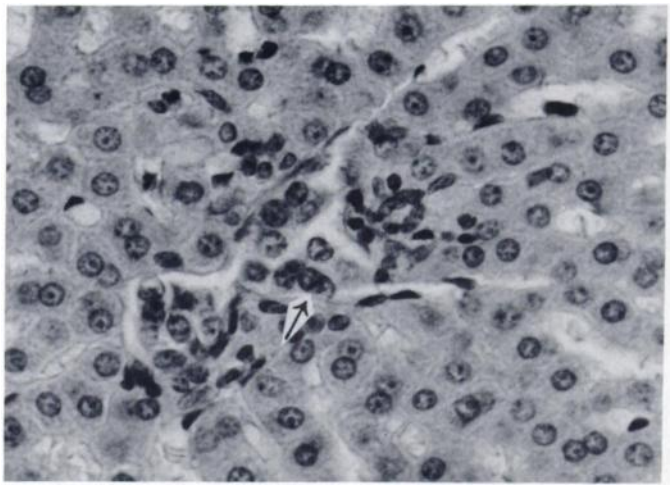

Fig. 3. Liver specimen 3 days after injection of VX_2 cancer cells. The *arrow* points to a VX_2 cancer cell infiltrating the sinusoid. H & E, × 500.

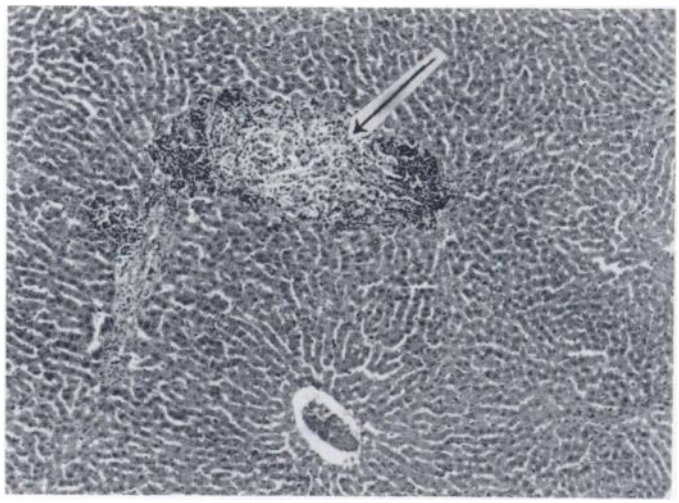

Fig. 4. Liver specimen 7 days after injection of VX_2 cancer cells. The cancer focus is shown with an *arrow*. H & E, × 20.

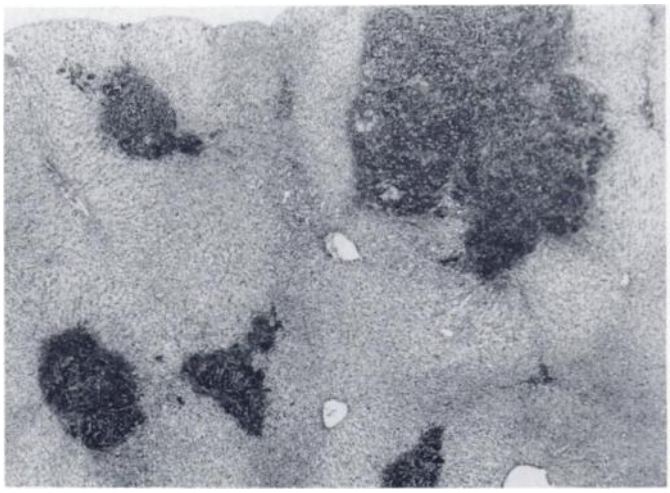

Fig. 5. Liver specimen 12 days after injection of VX_2 cancer cells. Cancer foci are seen as *dark regions*. H & E, × 10.

Table 1 *Numbers and total cross-sectional areas of VX_2 cancer nodules in Group A inoculated with MMC via the hepatic artery and killed 12 days after inoculation (n = 9)*

Rabbit	1[a]	2	3	4	M[b]	Z	X	Y
A-1	0	0	0	0	0	1.0	0	2.30
A-2	2	0	0	0	2	1.55	3.5	2.61
A-3	0	0	0	0	0	1.0	0	2.30
A-4	2	0	0	0	2	1.55	3.5	2.60
A-5	5	1	0	0	6	2.27	13.7	3.17
A-6	7	0	0	0	7	2.17	12.4	3.11
A-7	43	7	2	2	54	4.04	161.3	5.14
A-8	25	5	0	1	31	3.52	84.6	4.55
A-9	3	2	0	0	5	2.04	15.1	3.22
	9.7 ±14.7[c]	1.7 ±2.6	0.2 ±0.7	0.3 ±0.7	11.9 ±18.5	2.13 ±1.05	32.7 ±55.0	3.22 ±1.0

[a] 1–4, numbers of cancer nodules in Grades 1 (1–2 mm), 2 (2–3 mm), 3 (3–4 mm), and 4 (4 mm or more), respectively.
[b] *M*, total number of cancer nodules per rabbit; *Z*, $\ln(M + e)$; $e = 2.718$; *X*, total cross-sectional area of cancer nodules per rabbit (mm^2); *Y*, $\ln(X + 10)$.
[c] Mean ± SD.

Table 2 *Numbers and total cross-sectional areas of VX_2 cancer nodules in Group B inoculated with MMC via the portal vein and killed 12 days after inoculation (n = 10)*

Rabbit	1[a]	2	3	4	M[b]	Z	X	Y
B-1	30	2	0	0	32	3.55	62.8	4.29
B-2	99	12	2	0	113	4.75	252.9	5.57
B-3	10	0	0	0	10	2.54	59.6	4.24
B-4	31	1	0	0	32	3.55	17.6	3.32
B-5	0	0	0	0	0	1.0	0	2.30
B-6	24	1	0	0	25	3.32	47.3	4.05
B-7	45	11	1	0	57	4.07	143.1	5.03
B-8	42	6	0	0	48	3.93	103.6	6.73
B-9	12	0	0	0	12	2.69	88.5	4.60
B-10	28	6	1	0	35	3.63	21.2	3.44
	32.1 ±27.4[c]	3.9 ±4.6	0.4 ±0.7	0	36.4 ±32.0	3.30 ±1.03	79.7 ±74.6	4.16 ±0.94

[a] 1–4, numbers of cancer nodules in Grades 1 (1–2 mm), 2 (2–3 mm), 3 (3–4 mm), and 4 (4 mm or more), respectively.
[b] *M*, total number of cancer nodules per rabbit; *Z*, $\ln(M + e)$; $e = 2.718$; *X*, total cross-sectional area of cancer nodules per rabbit (mm^2); *Y*, $\ln(X + 10)$.
[c] Mean ± SD.

Table 3 *Numbers and total cross-sectional areas of VX_2 cancer nodules in Group C not given MMC injection (n = 14)*

Rabbit	1[a]	2	3	4	M[b]	Z	X	Y
C-1	80	25	3	0	108	4.71	292.8	5.71
C-2	35	11	0	0	46	3.89	115.8	4.84
C-3	24	4	0	0	28	3.42	62.0	4.28
C-4	32	9	0	0	41	3.78	100.6	4.71
C-5	113	52	13	0	178	5.20	579.7	6.38
C-6	5	1	0	0	6	2.17	13.7	3.17
C-7	33	1	1	1	36	3.66	71.0	4.40
C-8	64	9	0	0	73	4.33	132.0	4.96
C-9	168	16	0	0	184	5.23	375.0	5.95
C-10	57	3	2	0	62	4.17	185.8	5.23
C-11	45	7	2	0	54	4.04	133.1	4.96
C-12	241	62	14	2	319	5.77	896.3	6.81
C-13	22	6	0	0	28	3.42	68.3	4.36
C-14	1	3	0	0	4	1.90	16.5	3.28
	65.7 ±67.3[c]	14.9 ±19.0	2.5 ±4.8	0.2 ±0.6	83.4 ±87.9	3.98 ±1.09	217.3 ±249.7	4.93 ±1.05

[a] 1–4, numbers of cancer nodules in Grades 1 (1–2 mm), 2 (2–3 mm), 3 (3–4 mm), and 4 (4 mm or more), respectively.
[b] *M*, total number of cancer nodules per rabbit; *Z*, $\ln(M + e)$; $e = 2.718$; *X*, total cross-sectional area of cancer nodules per rabbit (mm^2); *Y*, $\ln(X + 10)$.
[c] Mean ± SD.

and karyopyknosis of cancer cells with wide intercellar space on Day 12 (Fig. 10). Such histological changes were greater in rabbits killed on Day 7 than on Day 12, and they were not found in Group C on Days 7 and 12. No histological difference in the site or form of growth was found in Groups A and B.

DISCUSSION

Results from our first series of experiments showed that VX_2 cancer cells injected via the portal vein into the liver could be seen as cancer foci after 3 days, and that cancer nodules were smaller than 1 mm on Day 9, growing to over 3 mm on Day 14. Based on these findings, we decided to give the anticancer agent up to Day 8 and to kill the rabbits on Day 12 in the second series of experiments.

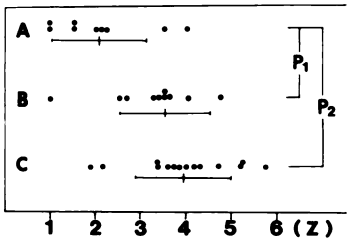

Fig. 6. Numbers of VX_2 cancer nodules 12 days after injection. *A*, rabbits inoculated with MMC via the hepatic artery ($n = 9$). *B*, rabbits inoculated with MMC via the portal vein ($n = 10$). *C*, rabbits not given MMC ($n = 14$). *Z*, $\ln(M + e)$; *M* is the number of cancer nodules per rabbit; *e* is 2.718. *Points*, mean; *bars*, SD. P_1, $P < 0.005$. P_2, $P < 0.025$.

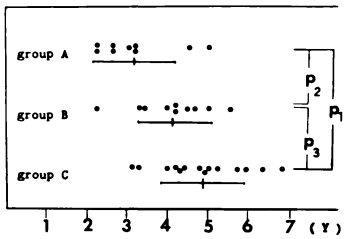

Fig. 7. Total cross-sectional areas of VX_2 cancer nodules 12 days after injection. *Group A*, rabbits given injections of MMC via the hepatic artery ($n = 9$). *Group B*, rabbits given injections of MMC via the portal vein ($n = 10$). *Group C*, rabbits without MMC injection ($n = 14$). *Y*, $\ln(X + 10)$; *X* is cross-sectional area of cancer nodules per rabbit (mm^2). *Points*, mean; *bars*, SD. P_1, $P < 0.005$. P_2, $P < 0.05$. P_3, $P < 0.1$.

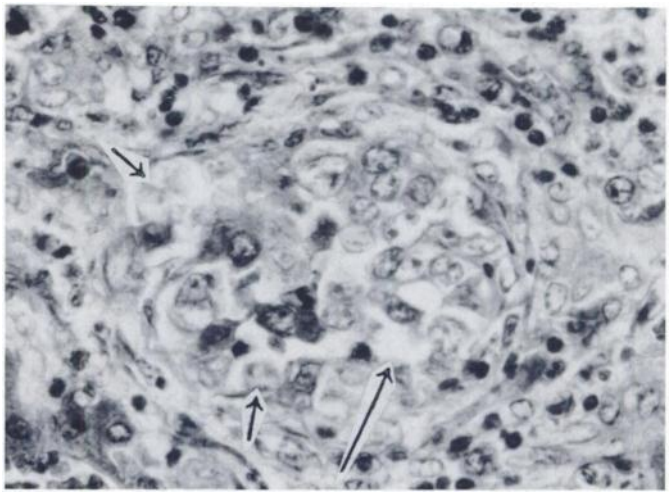

Fig. 8. Liver specimen of rabbits given injections of MMC via the hepatic artery and killed 7 days after injection of VX_2 cancer cells. The *short arrows* point to a necrosed cell; the *long arrow*, to an empty space. H & E, × 500.

Many reports have suggested that the hepatic artery is the main feeding vessel of metastatic cancer of the liver (1–5, 11, 12). However, for micronodules smaller than 2–3 mm across, the portal vein might be more important (1, 5). Nodules of 1 mm or less have no nutrient blood vessels; there is an avascular stage during which the nodules are nourished only by diffusion (4, 13).

Concerning routes for prophylactic or early administration of anticancer agents to liver metastases, a few reports state the effectiveness of administration via the hepatic artery or the portal vein. Taylor (14) gave 5-fluorouracil directly into the

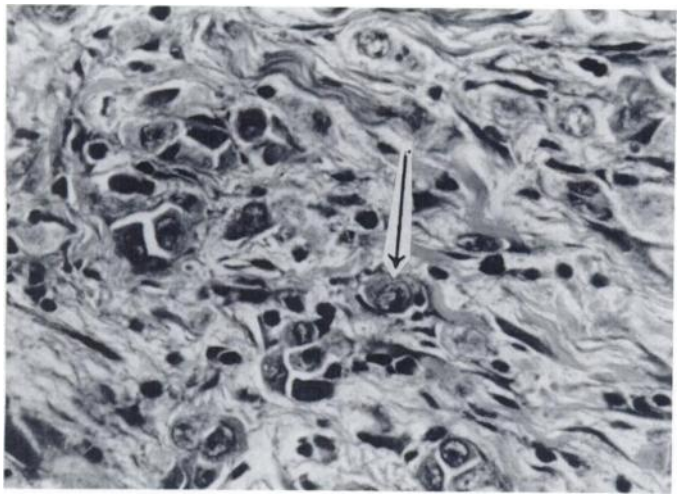

Fig. 9. Liver specimen of rabbits given injections of MMC via the hepatic artery and killed 12 days after injection of VX_2 cancer cells. This lesion showed marked fibrosis. *Arrow*, VX_2 cancer cells. H & E, × 500.

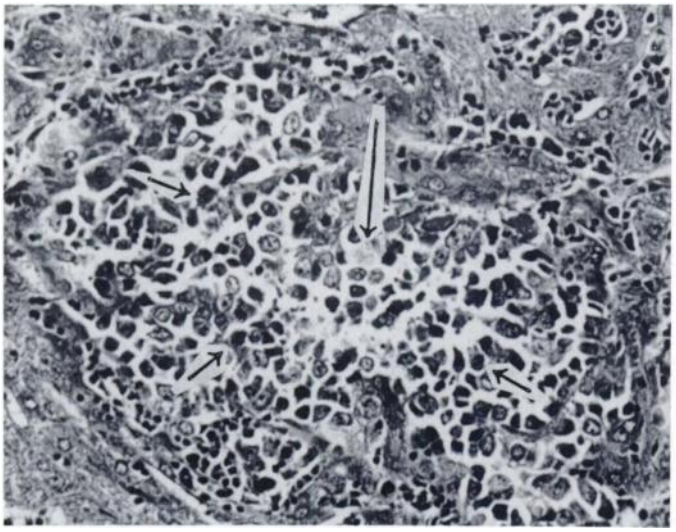

Fig. 10. Liver specimen of rabbits given injections of MMC via the portal vein and killed 12 days after injection of VX_2 cancer cells. The *short arrows* point to VX_2 cancer cells with karyopyknosis; the *long arrow*, to a necrosed cell. Intercellular space is wide. H & E, × 500.

portal vein of patients without macroscopic liver metastases after surgery for colorectal cancer; the incidence of liver metastases was reduced by this technique. He did not try administration via the hepatic artery. Hisazaki (1) gave MMC via the hepatic artery or portal vein 7 days after injection of Yoshida sarcoma cells into the portal vein of rats, and he reported that this agent had an antitumoral effect with no significant difference between the routes of administration. This worker thought that the portal vein would probably be the better route at the early stage before the cancer nodules were established.

In our experiments, MMC given via the hepatic artery gave higher anticancer efficacy according to both the index of total cross-sectional area of the tumor nodules and the number of tumor nodules. The number of tumor nodules 1–2 mm across was smallest in the group given MMC via the hepatic artery. This suggests that administration via this artery is effective also at the avascular stage when tumor nodules are 1 mm or less in diameter. One of the possible reasons for the weaker antitumoral effect of MMC given via the portal vein might be as follows. The formation of metastatic lesions centers around the interlobular space, so blood from the hepatic artery that flows into the portal branches or the sinusoids after nourishing the interstices possibly has a greater chance of reaching the tumors than blood from the portal vein, which mostly flows directly into the sinusoids (15).

Only slight antitumor effects of MMC in the rabbits killed on Day 12 were found histologically; we think the reason was that MMC was given during the process of proliferation. VX_2 cancer grows very rapidly, so surviving cancer cells might displace the necrosed cells and promote formation of large nodules (16).

These experiments showed that MMC had more effect when it was given via the hepatic artery than via the portal vein, at the stage before tumor angiogenesis in rabbits inoculated with VX_2 cancer cells. Our findings suggest that the hepatic artery route may be more effective than the portal vein route for local chemotherapy with MMC of macroscopic liver metastases or of potential microscopic disease for patients at high risk of developing liver metastases.

In this paper we have discussed the routes for local chemotherapy for liver metastases, but as Taylor described (14), this local chemotherapy might not have merit for other recurrence factors, such as peritoneal dissemination, lymph node metastases, and other remote metastases. Then it may be better to use the local chemotherapy in combination with other systemic regimens in clinical applications.

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