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A Study on Chemotherapy of Experimental Liver Cancer with Mitomycin C : Comparion between the Effect of Intra-arterial and Intra-portal Injection

by

HIROJI KATO

From the 2nd Surgical Division, Kyoto University Medical School
(Director : Prof. Dr. CHUJI KIMURA)

the Department of Surgery, Wakayama Red Cross Hospital
(Director : Prof. Dr. YUKIO NAITO)

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I. INTRODUCTION

In spite of recent advances in liver surgery, there are few resectable cases of primary and secondary liver cancer. It is presently thought that the most effective treatment of non-resectable liver cancer is the local administration of a carcinostatic agent. Since the report by MILLER[14] of a carcinostatic agent administered into the hepatic artery, this method has been employed almost exclusively for local administration of the agents. The liver receives a dual blood supply from the hepatic artery and the portal vein ; the latter also plays an important role in the hemodynamics of the liver, although it is a vein. Many studies of the livers of both animals and human subjects have been reported concerning the blood supply of liver tumors. However, no experimental study has been reported comparing the effect of the injection of carcinostatic agents into the proper hepatic artery with the injection into the portal vein. In the present experiment, mitomycin C was injected into the proper hepatic artery or the portal vein of rabbits inoculated with Brown-Pearce carcinoma into the liver in order to compare the effect of the two routes of administration. Colored gelatin solutions were then infused into both vessels to determine the cause of effectiveness.

II. MATERIALS AND METHODS

1. *Materials*

a) The animals used were rabbits of mixed breed. For serial transplantation male rabbits weighing 1.5 to 2.5 kg, and for intra-hepatic inoculation male and female rabbits weighing 2.3 to 2.8 kg were used.

b) The tumor used was Brown-Pearce carcinoma[3], which had originally appeared as a spontaneous testicular tumor in the rabbit. Morphologically it has the appearance of undifferentiated squamous epithelium carcinoma.

The tumor was produced by serial transplantation into the testicles of rabbits. Two

to three weeks after transplantation, the testicular tumor or metastatic omental tumor was extirpated, and after removal of the central necrotic portion and fibrous tissue, an equal volume of the saline solution containing penicillin and streptomycin was added. The tumor was reduced to a cell suspension in the glass homogenizer. This suspension was then kept in a small flask in an ice box and used within 2 hours. As malignity of the tumor altered periodically during serial transplantation, the intra-hepatic inoculation of the tumor suspension was performed during one of the highly malignant periods.

c) The carcinostatic agent used was mitomycin C, 1 mg of which was dissolved in 1 ml of 5% dextrose solution.

d) The method used for preparing the colored gelatin solutions was essentially the same as that described by BREEDIS and YOUNG[2]. The solution for the intra-arterial infusion was colored with carmine and that for the intra-portal infusion with Prussian blue. Carmine (Chroma) 25 g or Prussian blue (Chroma) 15 g in 150 ml of distilled water was agitated for 30 minutes in a shaking machine to form a colloidal solution, which was then mixed with 300 ml of boiling distilled water containing 24 g of gelatin (Bacto-gelatin : Difco). After the solution was preserved with a small amount of crystalline thymol at 37°C for a few days, the sediment was discarded. Prior to use, the solutions were heated to 60°—80°C and then cooled to 40°C.

2. *Methods*

a) Intra-hepatic Inoculation of Brown-Pearce Carcinoma

The abdomens of the rabbits were opened following intravenous pentobarbital sodium anesthesia and a local injection of procaine hydrochloride. A 25 guage needle was used to inject 0.3 ml of the tumor suspension into the left anterior lobe of the liver. After the injection the punctured point was lightly compressed with the finger for one or two minutes to prevent leakage of the suspension.

b) Injection of Mitomycin C into the Hepatic Artery and the Portal Vein

Development of liver tumor was observed during laparotomy performed 7 days or in some cases 9 days, after intra-hepatic inoculation of the tumor. In the group receiving intra-arterial injection 2 mg/kg of mitomycin C was injected into the proper hepatic artery, or in the group receiving intra-portal injection, into a branch of the superior mesenteric vein. In the control group only simple laparotomy was performed.

For the intra-arterial injection the portion of the proper hepatic artery distal to the branch supplying the right posterior lobe was exposed, and a 27 guage lymphography needle inserted (Fig. 1). The period of injection was about one minute. Bleeding after puncture of the artery was controlled by compression with a piece of autogenous muscle.

c) Intravascular Infusion of the Colored Gelatin Solutions

The animals were laparotomized and changes of the tumor were observed 3 to 6 days after the injection of mitomycin C. Thereafter, colored gelatin solutions were infused into the hepatic artery and the portal vein. A polyethylene catheter was inserted into the aorta with its tip at the opening of the celiac artery. Heparin solution was injected through the catheter. The stomach was removed with ligation of all branches of the celiac artery except the proper hepatic, and followed by ligation of the aorta above and below the celiac artery. Another catheter was inserted into the portal vein and fixed. The thoracic cavity was opened and the inferior vena cava was cut so that the infused solutions might

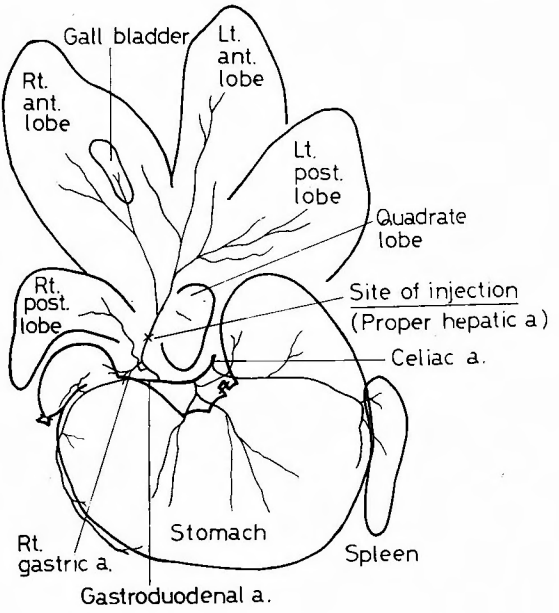


Fig. 1. Anatomy of the celiac artery of the rabbit illustrating the site of intra-arterial injection of mitomycin C. Caudal aspect of the liver is shown.

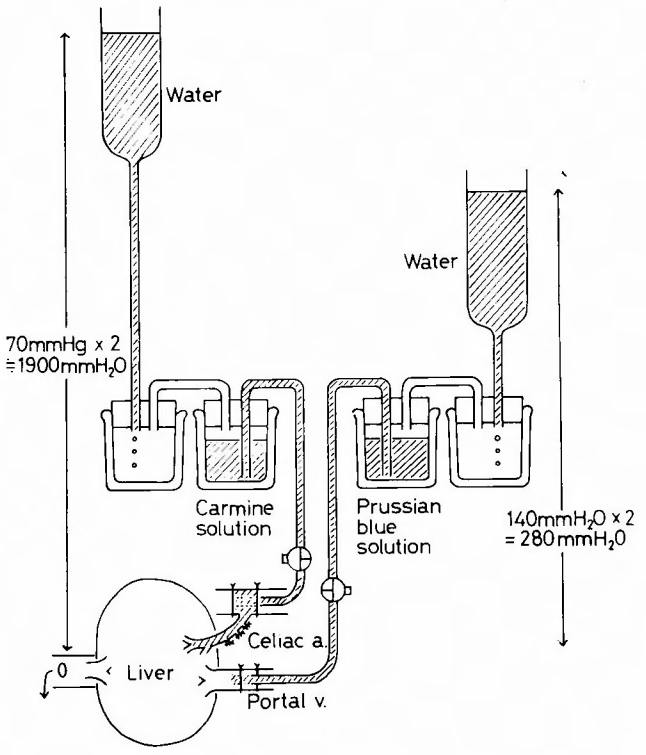


Fig. 2. Apparatus for infusion of colored gelatin solutions into the hepatic artery and the portal vein under different pressures.

flow out. The solutions were infused with the apparatus as shown in Fig. 2. Infusion was performed at two times the mean aortic pressure and the portal pressure in normal rabbits. Since in 4 normal rabbits the average mean aortic pressure was 70 mmHg and the average portal pressure 140 mm H_2O, intra-arterial and intra-portal infusions were carried out respectively under a pressure of

$$70 \times 13.6 \times 2 = 1900 \text{ mm } H_2O$$

and

$$140 \times 1.0 \times 2 = 280 \text{ mm } H_2O$$

The period of infusion was about 5 minutes.

After finishing the infusion, all the vessels from and to the liver were ligated and the liver was removed and placed in ice-cold 20% formalin for 24 hours in order to gel the injection masses completely. It was then allowed to fix at room temperature. The microscopic sections were stained with hematoxylin and eosin.

III. RESULTS

1. *Anti-tumor Effects of Mitomycin C on the Liver Inoculated with Brown-Pearce Carcinoma in Various Groups*

Evaluation of the anti-tumor effect of carcinostatic agents on transplantable cancer is a very difficult problem. The author took as criteria of the effect of the carcinostatic agent (1) change in gross tumor size, (2) inhibition of widespread multiple hepatic metastases and (3) histological changes. In the present experiment it was impossible to produce identical sized tumors at the time of injection of the carcinostatic agent. Therefore, the approximate size of the tumor was estimated by inspection and palpation prior to injection of mitomycin C, and the size after the treatment measured by serial slices of the fixed liver. The ratio of the cubic measure (length × width × thickness) of the latter to that of the former was calculated, and it was termed "tumor growth index". Widespread multiple hepatic metastases were examined macroscopically and histologically. As to the histological findings, degeneration or necrosis of the tumor cells except for spontaneous regressive degeneration caused by circulatory disturbance observed even in the control group and fibrosis around the tumor tissue were classified respectively into 4 grades of (−), (+), (++), (+++).

When the tumors had already developed largar than half of one lobe of the liver at the time of the injection of mitomycin C, despite administration of the agent, the animals soon died of marked enlargement of the inoculated tumors and extensive hepatic metastases. These cases, therefore, were excluded from the investigation evaluating the effect of the carcinostatic agent. The anti-tumor effects were evaluated in 6 rabbits in the control group, 7 in the intra-arterial and 8 in the intra-poral group. The results are tabulated in Table 1.

i) Tumor Growth Index

Comparison of the tumor size before and after the treatment is shown in Chart 1. Average and range of tumor growth index are shown in Table 2. Tumor growth was obviously inhibited in the treated rabbits, more markedly so in the intra-arterial group than in the intra-portal group. In 2 out of the 7 rabbits in the intra-arterial group, the liver tumor appeared to have diminished slightly.

Table 1 Anti-tumor effects in various groups

Group	Rabbit No.	Size of tumors: Before treatment — Days after inoculation	Size of tumors: Before treatment — Size (cm³)	Size of tumors: After treatment — Days after treatment	Size of tumors: After treatment — Size (cm³)	Tumor growth index	Histological changes: Degeneration	Histological changes: Fibrosis	Widespread multiple hepatic metastases	Metastases to other organs
Controls	108	9	1.5×1.0×1.8	5	6.0×4.5×1.0	22.5	−	−	+	−
	178	7	1.0×1.0×1.0	5	3.0×3.0×1.0	9.0	−	−	+	Lungs
	201	7	0.5×0.5×0.5	4	2.0×2.0×1.0	32.0	−	−	−	Omentum
	220	7	0.8×0.8×0.8	5	4.0×4.0×1.5	48.0	−	−	+	−
	238	7	2.0×2.0×1.0	5	4.0×2.0×1.0	2.0	−	−	+	Omentum
	242	7	1.5×1.5×0.8	5	5.0×4.0×2.0	9.0	−	−	+	Lungs
Intra-arterially treated	229	9	2.0×2.0×1.0	6	2.5×1.5×1.0	0.9	##	##	−	Lungs & retroperitoneum
	202	7	5.0×4.0×1.5	4	4.0×4.0×2.0	1.1	##	+	−	Omentum
	218	9	0.8×0.8×0.8	4	1.0×0.6×0.6 0.7×0.5×0.5 0.3×0.3×0.3	1.1	##	##	−	−
	215	9	0.7×0.7×0.7	4	0.6×0.4×0.4	0.3	+	##	−	−
	245	7	2.0×1.0×1.0	5	2.5×1.2×1.0	1.5	−	##	−	Lungs
	216	9	2.0×1.0×1.0	3	4.0×2.5×1.5	7.5	−	−	−	−
	208	7	0.8×0.8×0.8	3	0.8×0.8×0.8	1.0	−	−	+	−
Intra-portally treated	227	9	0.8×0.8×0.8	6	0.8×0.8×0.8	1.0	+	##	−	Lungs
	239	7	1.6×1.2×0.8	5	1.5×1.3×1.2	1.5	+	##	−	−
	221	9	1.5×0.6×0.6 1.0×0.3×0.3	3	3.0×1.0×1.0	4.8	−	##	−	−
	214	9	1.0×1.0×1.0	4	2.0×2.0×1.5	6.0	−	+	−	Retroperitoneum
	244	7	3.0×0.7×0.7	5	4.5×1.3×1.0	3.8	−	+	−	Lungs
	232	7	1.5×1.0×0.8	4	3.0×2.0×1.0	5.0	−	−	−	Lungs
	211	7	0.8×0.8×0.8	3	2.5×1.5×1.2	8.7	−	−	+	−
	199	7	1.5×1.2×0.8	4	3.0×2.0×1.5	6.2	−	−	+	−

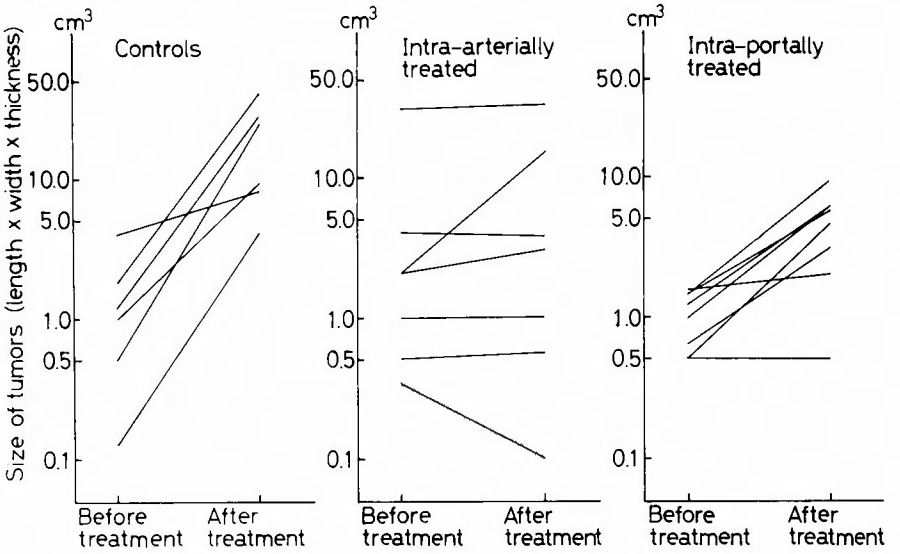


Chart 1. Size of tumors before and after injection of mitomicin C

ii) Widespread Multiple Hepatic Metastases

In the majority of the control group there were observed widespread multiple hepatic metastases generally smaller than 2 mm in diameter distributed throughout the liver. In both treated groups, however, these metastases seem to have been markedly inhibited (Table 3).

Table 2 Average and range of tumor growth index

Controls	20.4 (2.0～48.0)
Intra-arterially treated	1.9 (0.3～7.5)
Intra-portally treated	4.7 (1.0～8.7)

Table 3 Widespread multiple hepatic metastases

	−	+
Controls	1	5
Intra-arterially treated	6	1
Intra-portally treated	6	2

iii) Histological Changes

a) Control Group

Although the morphological appearance of the tumor cells was fairly varied depending on the rabbit or the part of the tumor examined, in general they were polymorphous and slightly basophilic. The nuclei were relatively large and round or oval in shape with relatively scanty chromatin. Numerous mitotic figures were observed. In the internal part of large tumor foci, irregular areas of spontaneous regressive degeneration or necrosis due to circulatory disturbances were found (Figs. 3, 4). There was no degeneration of tumor cells such as was found in the treated groups. On the whole tumor tissue showed alveolar arrangement resembling basal cell carcinoma. Scant fibrous connective tissue was found around the tumor tissue. Numerous small metastatic tumor foci were observed in practically all animals.

b) Intra-arterial Injection Group

In 4 of the 7 animals degeneration of tumor cells was recognized. In one animal degeneration was marked and extensive necrosis was seen. However, near the main tumor a few non-degenerative tumor foci were found. The degenerated tumor cells varied from

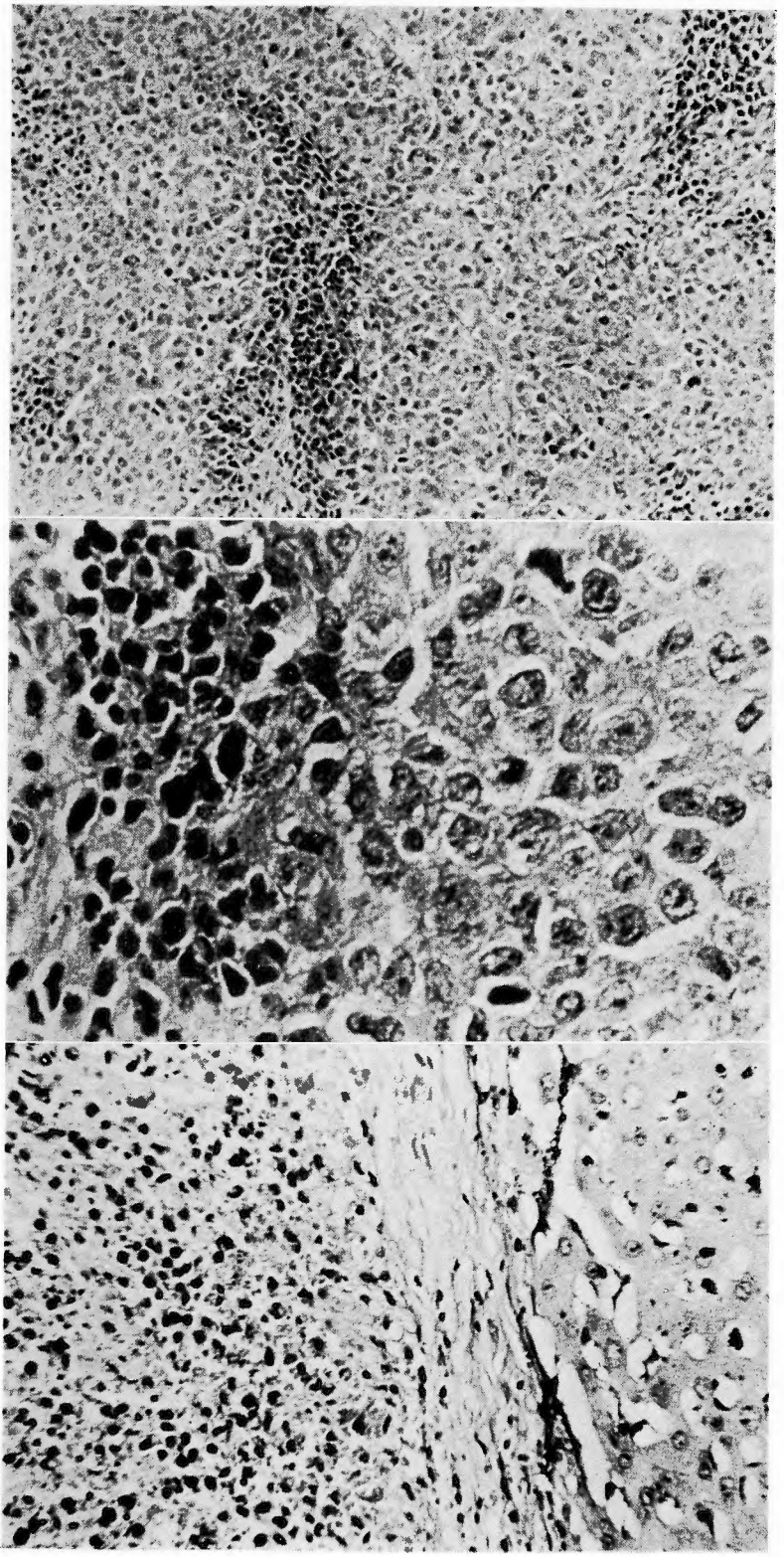


Fig. 3. Brown-Pearce carcinoma inoculated into the rabbit liver of the control group. Actively growing tumor tissue with numerous mitotic figures and spontaneous regressive degeneration scattered in stripe-pattern are shown. ×100.

Fig. 4. High magnification of Fig. 3. In the left one third degenerated tumor cells with pycnotic nuclei are shown. ×400.

Fig. 5. Brown-Pearce carcinoma of the liver treated with mitomycin C intra-arterially. Degenerated tumor tissue with pycnotic nuclei in the left half, uninvolved liver tissue in the right one third and fibrous connective tissue in the intermediate part are shown, ×200.

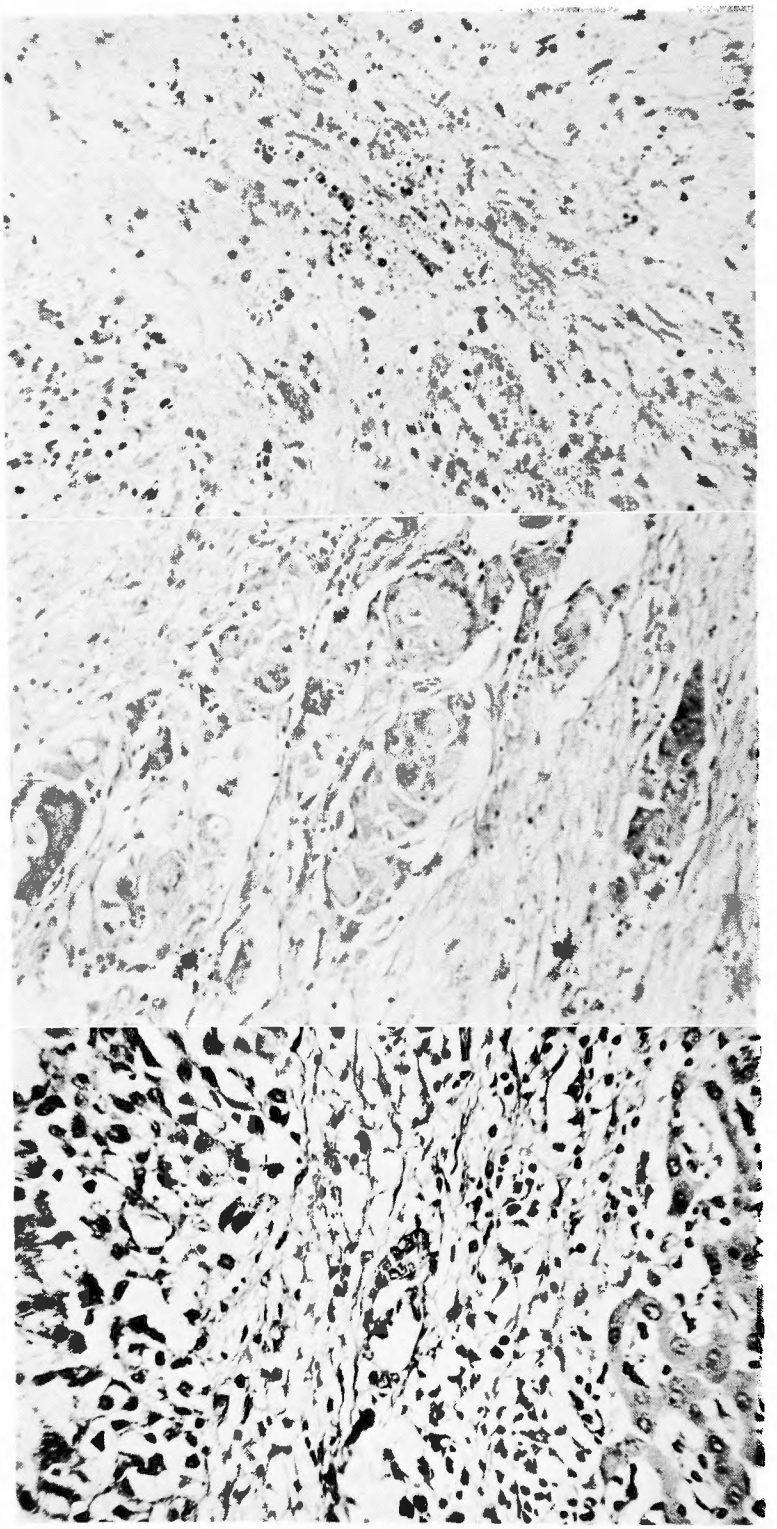
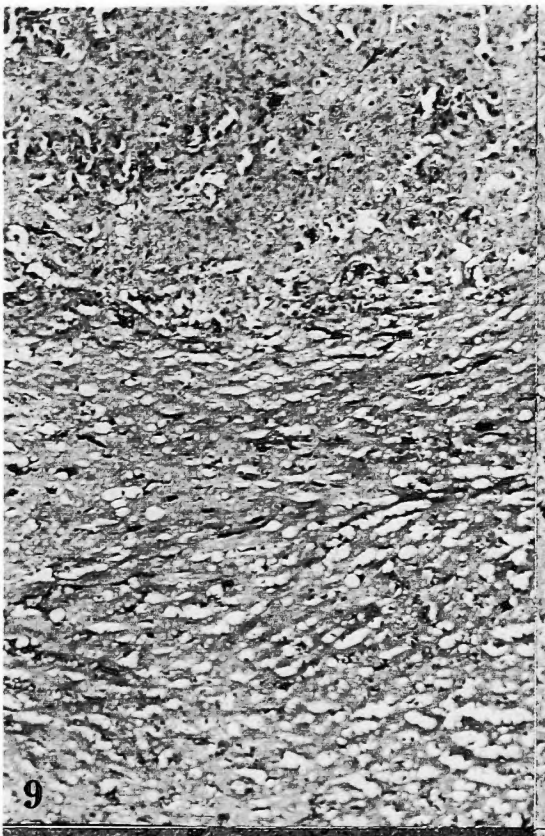


Fig. 6. Internal part of the same tumor tissue as Fig. 5. Disrupted tumor cells and fragmentation of nuclei with infiltration of small round cells and increasing stromal tissue are seen in the necrotic tumor tissue. ×200.

Fig. 7. Brown-Pearce carcinoma of the liver treated with mitomycin C intra-arterially. Syncytium like giant tumor cells in the fibrous connective tissue surrounding the tumor tissue are shown. ×200.

Fig. 8. Brown-Pearce carcinoma of the liver treated with mitomycin C intra-portally. In the left one third of the figure tumor cells with eosinophilic and atrophic cytoplasm with slightly pycnotic nuclei are seen in the somewhat degenerated tumor tissue which is surrounded with dense connective tissue as seen in the middle portion. ×200.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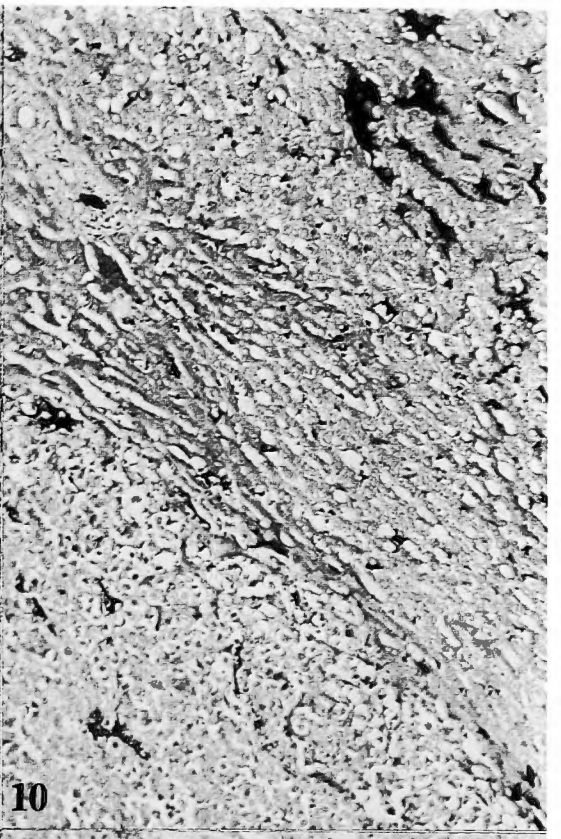
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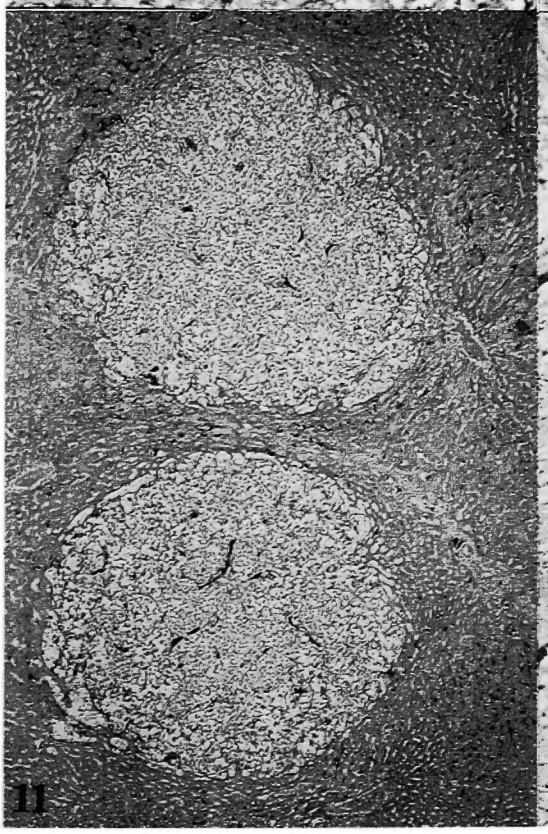
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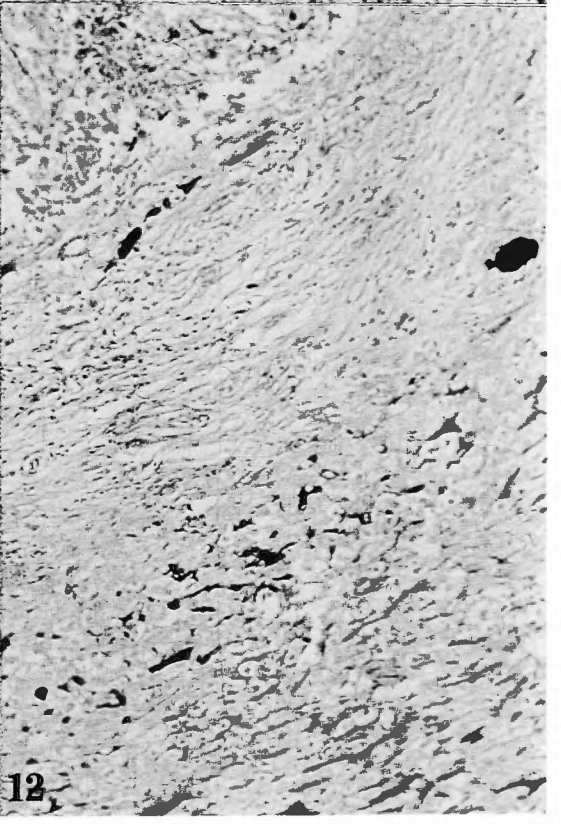
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Fig. 9. Brown-Pearce carcinoma surrounded with liver tissue in an untreated animal. Carmine was infused into the hepatic artery and prussian blue into the portal vein using the the apparatus as illustrated in Fig. 2. In tumor tissue in the upper one third and surronding liver tissue carmine alone is obrerved, while in the unaffected liver tissue apart from the tumor tissue Prussion blue is predominantly observed. × 70.

Fig. 10. Brown-Pearce carcinoma tissue in the left lower one third surrounded with liver tissue in an untreated animal. Dyes were infused as described in the legend for Fig. 9. Both the dyes are almost equally observed in the marginal area of tumor tissue and surrounding liver tissue, while Prussion blue is predominant in the liver tissue apart from the tumor. × 70.

Fig. 11. Two small tumor foci of Brown-Pearce carcinoma of the liver in an untreated animal. Dyes were infused as described in the legend for Fig. 9. Prussian blue is observed in the upper tumor focus and carmine in the lower one. × 28.

Fig. 12. Brown-Pearce carcinoma of the liver treated with mitomycin C intraportally. Dyes were infused as described in the legend for Fig. 9. In the slightly degenerated tumor focus in the left upper corner no dye is found. In the surrounding fibrous connective tissue and liver tissue close to it carmine alone is observed. × 70.

those showing eosinophilic staining of the cytoplasm, indefinite and irregular cell outlines and pycnotic nuclei to distruption of the cytoplasm and fragmentation of nuclei. Fibrosis was observed in 5 animals (Figs. 5, 6). In one animal in this group giant tumor cells resembling syncytium were observed in the fibrous connective tissue around the degenerated tumor tissue (Fig. 7).

As a rule the more pronounced the degeneration of tumor foci, the more dense is the fibrous connective tissue (Chart 2). That is to say, fibrosis is roughly in proportion to the degeneration of tumor tissue.

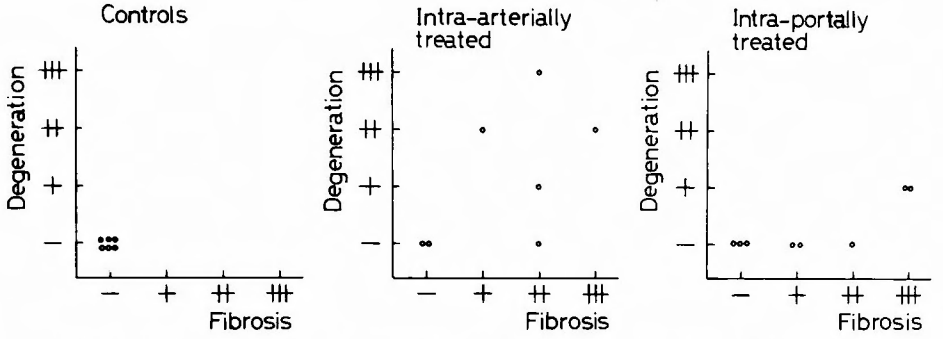

Chart 2. Degenneration of tumor cells and fibrosis after injection of mitomycin C

c) Intra-portal Injection Group

Mild degeneration of tumor cells were recognized in 2 out of 8 animals. In these two cases almost all the tumor cells had eosinophilic and atrophic cytoplasm with slightly pycnotic nuclei (Fig. 8). In one animal a small tumor focus surrounded with dense fibrous connective tissue was found and in the other a larger tumor focus divided by fibrous connective tissue. No giant tumor cells were found in either the tumor or the connective tissue. In the other 6 animals there was no degeneration of tumor cells except for spontaneous regressive degeneration, but in 3 out of 6 animals fibrosis around the tumor was observed. Growth of the tumor surrounded by fibrous connective tissue was not so marked as in the metastatic tumor tissue elsewhere in the liver tumor. Compared with the intra-arterial injection group degeneration of tumor cells in this group was much less marked but the degree of fibrosis was the same in both groups. That is, fibrosis in this group is more marked than degeneration of tumor cells.

2. *Intravascular Distribution of Colored Gelatin Solutions in the Liver Inoculated with Brown-Pearce Carcinoma*

Distribution of the dye in the liver and tumor tissue was investigated histologically and the results are tabulated in Table 4.

The summary of the findings is as follows:

i) In the internal area of large tumor foci only the arterial dye was observed, while in the marginal area surrounded by liver parenchyma small amounts of portal dye were found in a few animals. Frequently enlarged portal veins were observed between the tumor tissue and surrounding liver parenchyma (Figs. 9, 10). In the portions of degeneration or necrosis no dye was found.

ii) In the small metastatic tumor foci either or both dyes were usually observed in

Table 4 Distribution of the colored gelatin solutions infused into the hepatic artery and the portal vein

Group	Rabbit No.	Anti-tumor effect			Large tumor foci						Liver parenchyma					
		Degeneration	Fibrosis	Widespread hepatic metastases	Internal area		Marginal area		Small tumor foci		Area in contact with large tumor foci***		Area near large tumor foci		Area apart from tumor foci	
					A*	P**	A	P	A	P	A	P	A	P	A	P
Controls	96	−	−	+	+	−	+	+	+	+	+	++	+	+++	+	+++
	105	−	−	−	+	−	+	+			+	++	+	++	+	+++
	108	−	−	+	+	−	++	−	++	+	+	+	+	++	+	+++
	178	−	−	+	+	−	+	−	+	++	+	+	+	++	+	++
	220	−	−	+	+	−	+	−	+	++	++	+	+	+	+	++
Intra-arterially treated	229	+++	++	−	−	−	−	−	+	+	++	+	++	+	+	++
	245	+	++	−	+	−	+	−			++	+	+	++	+	++
Intra-portally treated	239	−	+++	−	+	−	+	−			+	−	++	+	+	+++
	221	−	++	−	+	−	+	−			+	++	+	++	+	++
	244	−	−	−	+	−	+	−			+	+	+	+++	+	+++
	232	−	−	−	++	−	++	−			++	++	++	++	+	++

* : The dye infused into the hepatic artery
** : The dye infused into the portal vein
*** : Contains the connective tissue surrounding tumor tissue

a single liver specimen. On the whole the amounts of both dyes were almost equal (Fig. 11).

iii) In the liver parenchyma the portal dye was predominant, but around the tumor growing by expansion, the arterial dye increased in relation to the nearness to the larger tumor foci. The portal dye was relatively predominant in the liver tissue invaded by the tumor growing by infiltration. On the whole in the liver tissue surrounding the large tumor foci the two dyes were found in approximately equal amounts. However, in the area surrounding small tumor foci the portal dye was predominant as well as in the area apart from tumor foci.

iv) Around the tumor foci subject to degeneration or fibrosis following the administration of mitomycin C, the tendency for the dye infused into the artery to predominate became more marked. In fibrous connective tissue and only slightly degenerative tissue surrounded by fibrous tissue only the arterial dye was observed (Fig. 12).

v) Distribution of the dyes appeared to depend upon the anti-tumor effect of mitomycin C, rather than upon the route of administration.

IV. DISCUSSION

Since KLOPP[9][10], in 1950 reported excellent results with intermittent intra-arterial injection of nitrogen mustard by canulation in the therapy of malignant tumors, intra-arterial administration of carcinostatic agents has been frequently employed for malignant tumors of the head and neck, the pelvic viscera and the extremities. For the local chemo-

therapy of liver cancer, intra-arterial administration of carcinostatic agents has been employed almost exclusively since MILLER[14], in 1961, administered them by canulation into the hepatic artery. Although in addition to the hepatic artery the portal vein is also an important afferent vessel of the liver, the opinion has prevailed that primary and secondary carcinoma of the liver receives its blood supply exclusively from the hepatic artery and the portal blood does not enter the tumor tissue.

In 1937, WRIGHT[20], who injected colored gelatin solution or oil emulsion into both the hepatic artery and the portal vein of livers removed from human cadavers, insisted that secondary liver cancer was supplied from arterial blood and drained from the portal vein. MANN et al.[12] studied the human liver by the injection-cast technique, and reported that in hemochromatosis with cirrhosis and hepatoma the neoplastic nodules derived their supply of blood only from the hepatic artery. BREEDIS and YOUNG[2] reported that malignant neoplasms growing in the liver tended to acquire an exclusively arterial blood supply based on studies of intravascular injection with India ink or colored gelatin solution in the metastatic cancer in men, DAB hepatoma in rats, and V_{x2} carcinoma inoculated in the liver of rabbits. MURTHY[17], who infused India ink through the portal vein of rats with DAB hepatoma and failed to recover the dye in the tumor, concluded that liver tumor depended on the hepatic artery for its blood supply and not on the portal vein. MIYAKE and OKUHIRA[15] reported following infusion-cast technique or dye infusion method in cases of hepatoma with liver cirrhosis, cholangioma and liver metastases of esophageal carcinoma, that liver tumor received arterial blood almost exclusively. MORITA[16] decided by infusion of India ink or resin cast method in DAB hepatoma, that the arterial blood supply was predominant in tumor tissue. ANDO[1], in view of actively growing arterial vessels in metastatic liver cancer found on necropsy using vascular cast method supposed that the predominant blood supply was arterial.

MATSUMURA[13] insisted that in the experiment above described little attention had been paid to the pressure of infusion of dye solution or resin. In his experiment, portal and arterial pressures were measured in each experimental animal immediately after laparotomy, and colored solutions were infused simultaneously into both vessels with the pressure the same as, or in proportion to, those measured. According to him, in a DAB hepatoma tumor presenting a typical picture of cholangioma type the blood supply was exclusively arterial, while in tumors of hepatoma type portal blood predominated. In an infusion experiment with a liver tumor transplanted with Walker tumor 256 via the portal vein, only dye from the hepatic artery was found in the internal area of large tumor foci, while dye from the portal vein was found in the marginal area and also was predominant in small tumor foci.

TORII[18] carried out an experiment to compare the effects of a carcinostatic agent by various administration routes on liver cancer. Ascites hepatoma AH 7974 was inoculated into the liver of rats via the portal vein and one week later 5 mg of nitrogen mustard-N-oxide was administered via various routes to the animals for 5 successive days. He reported that the most effective route was the intra-arterial, next the intra-portal, third the intravenous, and the least effective the intraperitoneal. In this experiment, however, intra-arterial administration was performed via an indwelling polyethylene catheter in the abdominal aorta with the tip at the opening of the celiac artery. It can be presumed that

only a small portion of the agent administered reached the liver via the proper hepatic artery and the larger remaining portion entered the portal vein or systemic circulation. Therefore, the effect of this method would differ considerably from that produced by injection of the carcinostatic agent directly into the proper hepatic artery.

In order to study more precisely the effect of intra-arterial and intra-portal injection on liver cancer, Brown-Pearce carcinoma was inoculated into the liver of rabbits and then mitomycin C was injected directly into the proper hepatic artery and a branch of the portal vein. The former was so small that continuous infusion or repeated injection was not possible.

In the present experiment, tumor growth index, widespread hepatic multiple metastases, and histological changes were used as the criteria of anti-tumor effects. Both the intra-arterial and the intra-portal injection groups showed evident effects as compared with the control group in all the criteria. Comparing the intra-arterial with the intra-portal injection group, as to tumor growth index and degeneration of tumor cells the former was superior to the latter. There was no significant difference in the amount of fibrosis around the tumor tissue or in intra-hepatic dissemination. From this the author concludes that while intra-arterial injection was somewhat more effective than intra-portal injection, the effects of the latter were also significant when compared to the control.

The underastanding of the mechanism of the considerable anti-tumor effects of the intra-portal injection method is dependent on a thorough understanding of the blood supply of the liver.

LUCKÉ[11], in order to compare the ability of the liver and the lung to support growth of cancer, simultaneously injected suspensions of V_{x2} rabbit carcinoma cells into a systemic vein and into either the portal vein or the hepatic artery. It was found that the average size of liver tumors invariably exceeded that of lung tumors. According to this experiment, tumors once established, grow faster in the liver than in the lung. WILLIS[19] compared mitotic activity in primary malignant tumors of various nature and origin with their hepatic metastases. He found that mitotic activity of hepatic tumors decidedly exceeded that of the primary growths. These studies support the hypothesis that the liver affords a highly favorable environment for the growth of malignant tumors. Portal blood contains a high concentration of carbohydrate and amino acids but relatively low oxygen tension. According to GRINDLAY[5] portal blood accounts for 60 to 80 percent of all blood flow through the liver. These factors suggest that the high carbohydrate content and relatively low oxygenation of liver tissue may play an important role in providing a highly favorable medium for the growth of the highly glycolytic, relatively anerobic malignant cells. HIRONO[6] observed that tumor growth and metastatic spread were inhibited by segmental interruption of portal blood supply to the region of implanted tumors. This result can perhaps be attributed mainly to the consequent change in the normally favorable environment for the growth of malignant tumors. FISHER[4] conducted electron microscopic studies of Walker 256 tumors inoculated in the liver and observed pseudopodal cytoplasmic extension of the tumor cells, from which he concluded that some tumor cells in the marginal area of hepatic tumor may directly receive nutritional supply from the hepatic cells around the tumor. These studies would account for the considerable inhibition of tumor growth and for the fibrosis formation following intra-portal injection of the carcinostatic agent.

It may be assumed that the most actively growing tumor cells in the marginal area of tumor foci were subject to intense anti-tumor activity by being exposed to the carcinostatic agent contained in the portal blood even though the tumor cells in the internal area had no contact with it. It is thought that intra-portal injection of the carcinostatic agent is particularly effective in tumor tissue growing by infiltration, as the tumor cells in the marginal area are more dependent upon the portal blood for their nutrients. Not infrequently the growing tumor may spread small tumor foci in the liver parenchyma around the main tumor foci. It is reasonable to assume then that these small foci are subject to more intense anti-tumor effect through intra-portal than intra-arterial injection, since they are surrounded by liver tissue supplied mainly with portal blood, even though they are themselves supplied equally with arterial and portal blood.

INOUYE[7] and ITO[8] respectively reported some cases of primary and secondary liver cancer showing marked effects following intra-arterial injection of mitomycin C. However, in spite of extensive necrosis of the principal tumors and proliferation of the surrounding connective tissue, a few live tumor cells were found in the marginal area of the tumor foci or around the fibrous connective tissue. These results suggest the advisability of combined intra-arterial and intra-portal injection of the carcinostatic agent. At least the carcinostatic agent should be administered intra-portally when intra-arterial injection is technically impracticable. The result of the experiment by MATSUMURA[13] in which he found that the portal dye was predominant in hepatoma type of DAB carcinoma is highly suggestive of the importance of intra-portal administration of carcinostatic agents for hepatoma.

However, when liver cancer is too far advanced, a single injection of a large dose of a carcinostatic agent is not only non-effective but even harmful. Regarding this point the result of the present experiment is in agreement with ITO's[8] clinical experience.

V. SUMMARY

The effects of intra-arterial and intra-portal injection of mitomycin C on transplanted liver cancer were compared, and the blood supply to the liver tumor after injection was studied using dye infusion technique.

Suspension of Brown-Pearce carcinoma cells was inoculated into the rabbit liver by direct puncture of liver parenchyma, and 7 to 9 days later mitomycin C 2 mg/kg was injected into either the proper hepatic artery or the portal vein. Three to six days after the injection, growth of the liver tumor was observed and colored gelatin solutions were infused into the hepatic artery and the portal vein at twice the normal blood pressures in order to clarify the blood supply of liver tumors. The results obtained are summarized as follows:

1) Both the intra-arterial and intra-portal injection were shown to be effective when compared with the control in all criteria of (1) inhibition of gross tumor growth, (2) inhibition of widespread multiple hepatic metastases and (3) degeneration of tumor cells and fibrosis around the tumor foci. The intra-arterial was more effective than the intra-portal injection in inhibiting gross tumor growth and causing degeneration of tumor cells, but there was no recognizable difference in the proliferation of fibrous tissue and the inhibition of widespread multiple hepatic metastases between the two routes of administration.

2) Results of dye infusion were as follows:

i) In the internal area of the large tumor foci only arterial dye was found, but in the marginal area some portal dye was found, although the arterial dye predominated. In the areas of degeneration or necrosis no dye could be found.

ii) In the small metastatic tumor foci both dyes were observed in nearly equal amounts.

iii) In the liver parenchyma the portal dye was predominant, but approaching the large tumor foci the arterial dye was found in increasing amounts so that in the area in contact with tumor tissue the two dyes were found in almost equal amounts. This tendency was not observed around the small metastatic tumor foci.

iv) Around the tumor foci where degeneration or fibrosis occurred following the injection of mitomycin C, only the arterial dye was found in the fibrous connective tissue while in the liver tissue itself arterial dye tended to predominate more so than in the controls.

Relation of anti-tumor effect and the results of dye infusion has been discussed. Based upon the findings of the considerable effectiveness of intra-portal injection, it is suggested that combined intra-arterial and intra-portal injection, or in some cases the latter alone, may be of value.

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(* Written in Japanese)

和文抄録

Mitomycin C による実験的肝癌の治療に関する研究

肝動脈内注入と門脈内注入の比較

京都大学医学部外科学教室第2講座（指導：木村忠司教授）
和歌山赤十字病院外科（外科部長：内藤行雄博士）

加　戸　弘　二

移植肝癌に対する mitomycin C の動脈内注入と門脈内注入の効果を比較し，肝腫瘍の血行を色素液注入法を用いて検索した．Brown-Pearce 癌の細胞浮遊液を肝実質に直接穿刺で移植し，7～9日後 mitomycin C 2 mg/kgを固有肝動脈あるいは門脈内に注入した．更に3～6日後肝腫瘍の発育を観察し，肝腫瘍の血行を明らかにするために肝動脈と門脈内に着色ゼラチン液を健常実兎の動脈圧，門脈圧のそれぞれ2倍の圧で注入し，次のような結果を得た．

1) 動脈内注入，門脈内注入共に対照に比して腫瘍増大抑制，肝内遠隔転移抑制と組織学的な腫瘍細胞の変性および腫瘍周囲の線維化の全ての点で明らかな効果が見られた．動脈内注入は腫瘍増大抑制，腫瘍細胞の変性の点では門脈内注入より優れ，線維化および肝内遠隔転移抑制の点では両者間に差が見られなかつた．

2) 色素注入の結果次のような所見を認めた．

i) 大きな腫瘍巣の内部では動脈性色素のみが見られ，辺縁部では一部に門脈性色素も見られた．変性あるいは壊死に陥つた部では色素は見られなかつた．

ii) 小転移巣では両方の色素がほぼ同程度に見られた．

iii) 肝実質では門脈性色素が優勢であるが大きい腫瘍巣に近づくに従つて動脈性色素が増加し，門脈性色素が減少する傾向があり，腫瘍組織に接する部では両者が同程度となる．このような傾向は小転移巣の周囲では見られなかつた．

iv) Mitomycin C の注入で変性あるいは線維化を来した腫瘍巣の周囲では，線維性結合組織には動脈性色素のみが見られ，肝組織には動脈性色素が対照に比して優勢であつた．

二つの投与経路と色素注入所見との関係について考察を加えた．門脈内注入がかなりの効果を示すことから動脈内と門脈内の併用投与は検討に値すると考えられ，症例によつては門脈内投与が行なわれるべきであろう．

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