

REGIONAL INTRAVASCULAR CHEMOTHERAPY FOR EXPERIMENTAL LIVER METASTASIS

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ABSTRACT

To investigate the effectiveness of intravascular chemotherapy, the rats implanted with two different sizes and ages of Yoshida sarcoma into the liver were treated with Mitomycin-C through either the hepatic artery or the portal vein. The administration of Mitomycin-C was effective by both arterial and portal routes on the rats bearing small young tumor, whereas it was effective only by arterial route on the rats bearing large developed tumor. Tumor regression was also observed on the rats bearing the tumor after ligation of the hepatic artery.

Further, to observe the relationship between tumor growth and its blood supply, microangiography was performed by injection of AgI colloid through the hepatic artery and the portal vein. The branches of the hepatic artery flourished around the tumor at the 4th day after implantation and gradually entered into the tumor area at the 6th day. Thereafter, tumor area was occupied by the arterial branches and later the branches were occluded by the progressive growth of tumor. On the other hand, the branches of the portal vein were obliterated earlier than those of the hepatic artery and at the 5th day no branches were observed in the tumor area.

These vascular changes are closely related to tumor growth, so that special regard should be paid to the change of the blood supply when chemotherapeutic agents are given.

INTRODUCTION

In the use of intravascular chemotherapy, a knowledge of the distribution of the hepatic artery and the portal vein to metastatic cancer in the liver is of great importance. Lodged metastatic tumor cells from circulation are not static; their progressive growth causes invasion and compression of surrounding tissues and obliteration of blood vessels. Accordingly, the vascular pattern may change as time goes by and tumor develops. Experimentally, Ackerman *et al.*¹⁾ reported that the blood supply of small tumors in the liver was different from that of large tumors in which tumors less than 30 mg in weight were fed by both the hepatic artery and portal vein and tumors more than

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30 mg received a predominantly arterial blood supply.

Such knowledge lead us to consider that the route of drug administration should be altered according to the change of blood supply of tumor. A considerable number of reports have appeared but little attention has been paid on this point. The present study was concerned with the intravascular chemotherapy for experimental liver metastasis with relation to blood supply of tumor.

MATERIALS AND METHODS

Tumor implantation:

Male Wistar rats weighing 150~180 g were used in the present experiments. After the animal was anesthetized with intraperitoneal injection of 10~15 mg of Ketamine [2-(*o*-chlorophenyl)-2-methylamino-cyclohexanone], the liver was exposed through a midline abdominal incision. A solid tumor of Yoshida sarcoma was cut into small pieces and implanted into the parenchyma of the left lateral lobe with a trocar. To keep the tumor in place and to stop bleeding a short period of pressure was applied to the implated site. Oxygel (oxidized cellulose) was applied if bleeding was intractable.

Intravascular injection:

For the injection into the hepatic artery, the aorta was clamped by Bulldog forceps above and below the celiac artery take-off. Arterial branches of the celiac artery to the gastrointestinal tract and to the spleen were also clamped. The hepatic artery of rat is too fine to inject, so that hepatic circulation was carried out by injection into the aorta at the site of celiac artery take-off instead. In portal vein study, injection was carried out directly into the portal vein after clamping below the injected point. A drop of Aron Alpha A (alkyl- α -cyanoacrylate monomer, Sankyo, Co., Ltd., Tokyo) was applied on the injected point for hemostasis.

Intravascular chemotherapy and hepatic artery ligation:

1) *Experiment I*; This experiment was aimed to observe the difference in the effectiveness of chemotherapy between a small, young tumor and a large, grown-up tumor. Smaller tumors were produced by the implantation of the pieces of Yoshida sarcoma weighing approximately 30 mg. To produce larger tumors, implants of approximately 60 mg were used. Young tumor in this experiment means the tumor which was present shortly after implantation and its vasculature has not yet been established. On the other hand, grown-up tumor means the tumor whose vasculature has well been established. Therefore, intravascular chemotherapy was performed 2 days later on the rats implanted with a small tumor (2 days old) and 5 days later on the rats with a large tumor (5 days old). The animals received one-shot injection of Mitomycin-C in a dose of 1.46 mg/kg in 0.5 ml solution into the hepatic artery or

portal vein. All control rats were sham-operated on. One week after the injection, all survived rats were sacrificed and the tumors were weighed.

2) *Experiment II*; To observe the blood supply of the metastatic tumor, the regional artery was ligated. Rats implanted with 60 mg of Yoshida sarcoma in the liver were divided into 6 groups: Group 1 received ligation of the hepatic artery on the 4th day after tumor implantation. Group 2 received 1.46 mg/kg of Mitomycin-C into the hepatic artery on the 4th day. Group 3 was sham-operated on the 4th day. Group 4 received ligation of the hepatic artery on the 4th day as did in Group 1 and 1.46 mg/kg of Mitomycin-C was injected into the portal vein on the 7th day. Group 5, sham-operation was performed on the 4th and 1.46 mg/kg of Mitomycin-C was injected into the portal vein on the 7th day. Group 6, sham-operation was done twice; on the 4th and 7th day. The surviving rats were sacrificed on the 10th day and the tumors were weighed.

Microangiography:

To investigate the progressive changes in the blood supply of the tumor whether it is from the hepatic artery or portal vein, microangiography was performed on the rats implanted with 60 mg of Yoshida sarcoma by injection of silver iodide colloid into either route. The rats were sacrificed for microangiography as time went by. Silver iodide colloid was made by mixing 20 g of AgNO_3 and 10 g of Arabic gum in 100 ml distilled water with 30 g of KI and 10 g of Arabic gum in 100 ml of water²⁾. Immediately after completion of the injection, the hilus of the liver was ligated and the lobe bearing metastatic tumor was removed. The lobe was fixed in 10% formaldehyde solution and the paraffin section was cut at a uniform thickness of 3 mm and 5 μ . After the section, 3 mm thick, was made clear by immersing it in dimethylbenzene and the angiograph was taken by Softex IE on No. 80 Fuji industrial X-ray film. The section, 5 μ thick, was counter-stained by Hematoxylin-Eosin.

RESULTS

The results are summarized in Table 1. In the animals bearing small young tumor, the administration of Mitomycin-C was effective either by arterial or by portal route. No difference in curability was observed between the animals given Mitomycin-C into the hepatic artery and those given it into the portal vein ($P > 0.05$). In the animals bearing large grown-up tumor, 6 of 12 rats were free from the tumor after the drug given into the hepatic artery, whereas none of 12 given it into the portal vein.

Further, to observe hepatic artery circulation of grown-up tumor, the hepatic artery was ligated. As shown in Table 2, implanted tumors were marked regressed in Group 1 as compared to those in Group 3. The tumor

TABLE 1. Effect of Mitomycin-C Given into the Hepatic Artery or Portal Vein on the Rats Bearing Small or Large Tumor

Site of injection	No. of rats	Total weight of tumor (mg)	Total weight of tumor/No. of rats (mg)	No. of rats without tumor (%)	X ² -test ^{a)}
Small, young tumor ^{b)}					
Hepatic artery	18	2520	140	12 (≅67)	N.S.
Portal vein	18	1860	103	15 (≅83)	
Untreated	18	21000	1167	0	H.S.
Large, developed tumor ^{b)}					
Hepatic artery	12	2800	233	6	H.S.
Portal vein	12	5650	470	0	
Untreated	12	20400	1700	0	H.S.

a) N.S.: not significant, H.S.: highly significant.

b) See Materials and Methods.

TABLE 2. Effect of Hepatic Artery Ligation on the Growth of Metastatic Tumor in the Liver

Treatment	No. of rats	Total weight of tumor (mg)	Total weight of tumor/No. of rats (mg)
H.A. ^{a)} ligation	9	5380	597
MMC ^{b)} into H.A.	7	4350	621
Laparotomy alone	5	9700	1960
H.A. ligation plus MMC into P.V. ^{c)}	8	4150	518
Laparotomy plus MMC into P.V.	7	7520	1074
Laparotomy plus Laparotomy	4	5870	1467

Abbreviation:

a) H.A.; hepatic artery, b) MMC; Mitomycin-C, c) P.V.; portal vein.

in Group 4 which received ligation of the hepatic artery and later received Mitomycin-C into the portal vein were also smaller than those in Groups 5 and 6, which had no ligation of the hepatic artery but received Mitomycin-C alone into the portal vein and laparotomy alone, respectively. No effect was observed on the tumor growth by the administration of Mitomycin-C into the portal vein after ligation of the hepatic artery, since the tumor weight showed no difference between Group 1 and Group 4. It can be seen again in this experiment that administration of Mitomycin-C was only effective by the arterial route, as compared with between Group 2 and Group 5. In this series of experiment, no cures occurred. Ligation of the hepatic artery showed a high mortality in which all rats in Group 1 died within 10 days.

Microangiographs of the hepatic artery circulation and corresponding Hematoxylin-Eosin sections are shown in Photos. 1a~6b. The site of implanted tumor which can be clearly seen in Hematoxylin-Eosin section (Photo.

1b) can not yet be observed on the microangiograph at the 2nd day after tumor implantation (Photo 1a). At the 4th day, branches of the hepatic artery flourished surrounding the tumor (Photos. 2a and 2b). These branches went into the tumor area at the 6th day (Photos. 3a and 3b). At the 10th day, increased arterial network was observed especially at the tumor area (Photos. 4a and 4b). On the microangiograph at the 11th day, arterial branches are few at the central area of the tumor. They may be fragile, since scattered silver iodide colloid are seen here and there at the tumor area (Photos. 5a and 5b). At the 13th day, arterial branches encased the tumor as can be seen in Photos. 6a and 6b. This findings indicates that arterial branches may become obliterated by the growth of tumors.

On the other hand, microangiographs of the portal vein and corresponding Hematoxylin-Eosin sections are shown in Photos. 7a~9b. As can be seen on the microangiograph at the 2nd day, typical tree-like branches of the portal vein were absent at the tumor implanted site (Photos. 7a and 7b). At the 3rd day, tumor was slightly filled with AgI by portal route (Photos. 8a and 8b). However, at the 5th day, the branches of the portal vein disappeared abruptly so that a clear line of demarcation between liver and tumor was seen in Photos. 9a and 9b.

DISCUSSION

Although the medical literature relating to the blood supply of tumors in the liver is not extensive, the attempts to treat metastatic cancers of the liver through the hepatic artery have been tried elsewhere. The clinical response seems to be effective⁹⁾⁻¹⁰⁾, as the blood supply of metastatic cancers in the liver appeared to be mainly or purely arterial.

In the present experiments, the treatment of a relatively large tumor in the liver was effective only by the arterial route and ligation of the hepatic artery caused tumor to regress indicating that the hepatic artery plays an important role on the blood supply of tumor in the liver. In an early stage of metastases, however, chemotherapy by both arterial and portal routes was effective suggesting that tumor in this stage is supplied from both routes.

Cancer cells from the alimentary tract may reach the liver through the portal vein, lodge on the endothelium of the blood capillary and develop thrombosis. Blood vessels gradually get into the tumor for nourishment. With the development of tumor, branches of the portal veins are gradually invaded, obliterated and pushed aside. Here, newly formed branches of the hepatic artery play a leading role. Later, these branches become obliterated by the progressive growth of tumor and avascular area appears. A shunting of portal to arterial blood in conjunction with the newly formed arterial plexus of the tumor was demonstrated in experimental animals by Lien and Ackerman¹¹⁾ They suggested that if the branches of the hepatic artery become

obliterated following the growth of the tumor or as a result of thrombosis following prolonged arterial drug perfusion, the portal route should be considered¹²⁾.

In the present experiments, no such effect was observed on the tumor bearing rats treated with hepatic artery ligation plus administration of Mitomycin-C into the portal vein compared with the results of hepatic artery ligation alone. The microangiograph showed few or rather absence of silver iodide injected into the portal vein after ligation of the hepatic artery.

Based on these considerations and experimental results, effective routes of chemotherapy for metastatic cancers in the liver may be categorized into the following: In an early stage of metastases when tumor cells are floating into the portal vein system, treatment via portal route would be available; later, when tumor cells begin to grow and until the branches of the portal vein become obliterated, both arterial and portal routes are effective. Still later, when the blood supply of the hepatic artery is dominant, administration of chemotherapeutic agents through the hepatic artery is effective. Concerning the case when the hepatic artery is occluded by the progressive growth of tumor, the portal route would be reconsidered, but no conclusion was established from the results of this experiments.

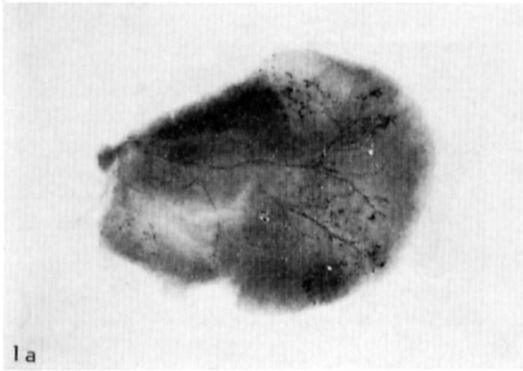
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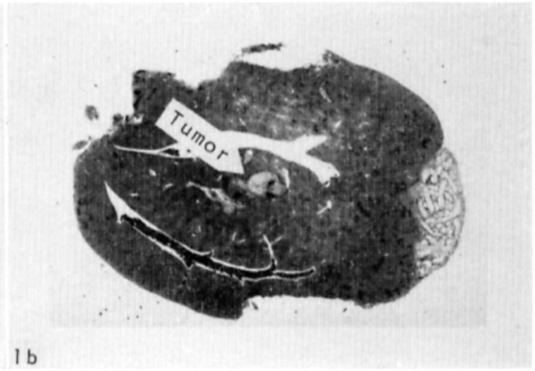
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EXPLANATION OF PLATES

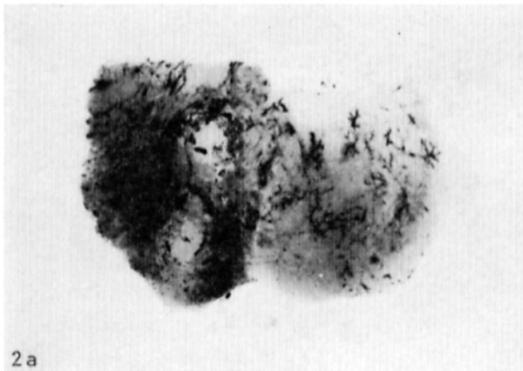
- PHOTO 1 a. Microangiograph of liver injected with AgI colloid into the hepatic artery. Tumor was implanted in the liver 2 days ago but no tumor area was seen.
- PHOTO 1 b. Hematoxylin-Eosin (H-E) section of the same liver of Photo 1 b. Site of implanted tumor was indicated by arrow.
- PHOTO 2 a. Microangiograph of liver implanted with tumor 4 days ago. Arterial branches flourished surrounding the tumor.
- PHOTO 2 b. Corresponding H-E section of the liver of Photo 2 a.
- PHOTO 3 a. Microangiograph of liver implanted with tumor 6 days ago. Many branches were located at the center of the liver.
- PHOTO 3 b. Corresponding H-E section of the liver of Photo 3 a. It was proved that the center of the liver was occupied by tumor.
- PHOTO 4 a. Microangiograph of liver implanted with tumor 10 days ago. Increased arterial plexus was seen at the tumor area.
- PHOTO 4 b. Corresponding H-E section of the liver of Photo 4 a. Tumor was located just at the place angiographed in Photo 4 a.
- PHOTO 5 a. Microangiograph of liver implanted with tumor 11 days ago. Scattered AgI was seen here and there. Center of the tumor became scant.
- PHOTO 5 b. Corresponding H-E section of the liver of Photo 5 a.
- PHOTO 6 a. Microangiograph of liver implanted with tumor 13 days ago. Arterial branches run aside and encased the tumor.
- PHOTO 6 b. Corresponding H-E section of the liver of Photo 6 a. Site of tumor was indicated by arrow.
- PHOTO 7 a. Microangiograph of the liver injected with AgI colloid into the portal vein. Typical tree-like branches of the portal vein were absent at the implanted site.
- PHOTO 7 b. Corresponding H-E section of the liver of Photo 7 a.
- PHOTO 8 a. Microangiograph of liver implanted with tumor 3 days ago. The tumor area was somewhat filled with AgI by portal route.
- PHOTO 8 b. Corresponding H-E section of the liver of Photo 8 a.
- PHOTO 9 a. Microangiograph of liver implanted with tumor 5 days ago showing the absence of the branches of the portal vein in the tumor area. Branches run close to the margin of the tumor and end abruptly.
- PHOTO 9 b. Corresponding H-E section of the liver of Photo 9 a.



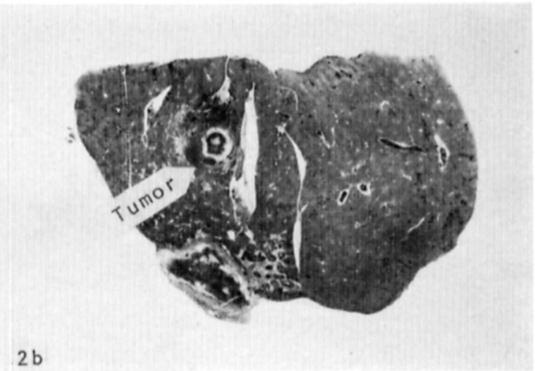
1a



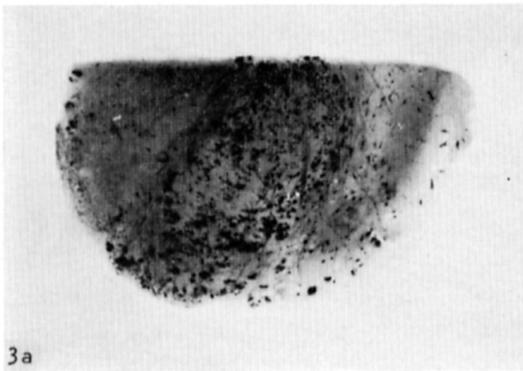
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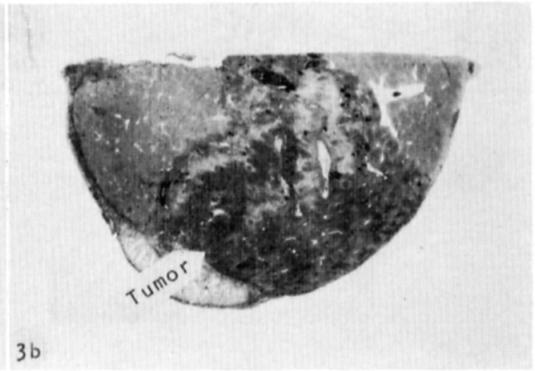
2a



2b



3a



3b

