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Effect of chemoembolization of albumin microspheres containing mitomycin C on AH 272 liver metastasis in rats

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Summary

The utility of synthesized albumin microspheres containing mitomycin C (MMC), as a chemoembolization material against liver tumors in rats was evaluated. By observing angiograms of tumorous and normal rat livers with a soft X-ray apparatus, it was found that the blood flow in target site (tumor site) could be obstructed by infusion of albumin microspheres into the blood vessels of the liver. Furthermore, histological observations demonstrated that the blood vessels of target sites in the liver were embolized by albumin microspheres containing MMC and that parenchymal cells of the liver around the interlobular connective tissue were necrosed by MMC released from the infused albumin microspheres. The antitumor effects of albumin microspheres containing MMC on AH 272 liver metastasis were noticeably increased by intra-arterial administration compared to control (saline infusion) and free MMC. These results suggest that albumin microspheres containing MMC may be very useful as a material for chemoembolization.

Introduction

Techniques to apply locally high concentrations of antitumor drugs specifically to tumor sites were initiated by Klopp et al. (1953) and Seldinger et al. (1953) who developed an intra-arterial infusion therapy which infused antitumor drugs directly to the tumor site using a catheter inserted into the primary nutritional artery of the tumor. Many experiments and therapeutic regimens have been attempted with these techniques of administration. More recently, Yamada et al. (1984) has developed "Balloon Occluded Arterial Infusion Therapy" using a balloon catheter. They reported that satisfactory results were obtained when this technique was applied to several malignant tumors and that it is a useful technique for certain cancer chemotherapies. Also, utilization of microspheres as drug carriers for chemoembolization in these intra-arterial infusion therapies has now become a research focus with the intent of providing better cancer therapy. It was anticipated that drugs which were enclosed in or combined with synthetic microcarriers could be directed to designated sites more selectively with subsequent slow controlled release at these sites. For example a marked antitumor effect has been achieved against cancers of the bladder, kidney and liver with ethylcellulose microcapsules containing mitomycin C by Kato et

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al. (1980, 1981, 1984), and against hepatoma using a lipid lymphographic agent (Lipiodol) containing a hydrophobic antitumor agent, copoly(styrene-maleic acid)-conjugated neocarzinostatin (SMANCS) by Konno et al. (1983). We have also reported in a previous paper (Natsume et al. 1989) the potential of albumin microspheres to be utilized as a material for chemoembolization. In the present paper, we have investigated chemoembolization and antitumor effects of albumin microspheres containing mitomycin C (MMC) using in vivo tumor models.

Materials and Methods

Materials

Human serum albumin (HSA) and Protease were obtained from Sigma Co., Ltd. (St. Louis, MO, U.S.A.). MMC was supplied from Kyowa Hakko Co., Ltd. (Tokyo, Japan). *n*-Octane and 25% glutaraldehyde were obtained from Wako Pure Chemicals (Osaka, Japan). In this experiment 5% glutaraldehyde was used by diluting 25% glutaraldehyde with distilled water. All other reagents used were commercial reagent grade.

Preparation of albumin microspheres

Albumin microspheres containing MMC were prepared as described in a previous paper (Natsume et al., 1989). Briefly, a solution of 30 mg of MMC and 300 mg of HSA in 2.0 ml of distilled water containing 5% glutaraldehyde as a cross-linking agent was emulsified and solidified in 100 ml of *n*-octane containing 1% surfactant (Span 80 and Span 20 mixed 7 : 5) for 3 min. The resulting suspension was heated in an oil bath at 120 °C for 20 min to confirm the solidification. After the suspension was cooled at room temperature, it was mixed with 100 ml of diethylether: petroleum ether (1 : 1) mixture. The solvent was then removed from the albumin microspheres. After drying in a desiccator, the microspheres were sieved to remove smaller particles using a 400 mesh (37 μm) and larger using a 250 mesh (63 μm) sieve. The yield was about 90% and the average diameter of albumin microspheres was approximately 42.3 $\pm$ 6.93 μm. The drug content in the albumin microspheres, determined by digesting them with proteolytic enzyme in pH 7.4 phosphate buffer for 7 days, was 45.6 $\pm$ 1.5 μg/mg.

Animals

Male Wistar rats, weighing 200–250 g, were used in both the soft X-ray observation of embolization and histological observation. Male Donryu rats, weighing 170–200 g, were used to measure the antitumor effects of albumin microspheres containing MMC. Liver metastasis were made by inoculation of 1×10^7 AH 272 tumor cells in the right side of the middle lobe in Donryu rats.

Observation of embolization by soft X-ray angiography

The method for infusing albumin microspheres and contrast media is shown in Fig. 1 (Lindel et al., 1978). Wistar rats were first anesthetized with pentobarbital sodium (50 mg/kg) and the liver artery hidden under the caudate lobe was exposed. The blood flow in the artery through the left lobe and right side of the middle lobe of the liver was

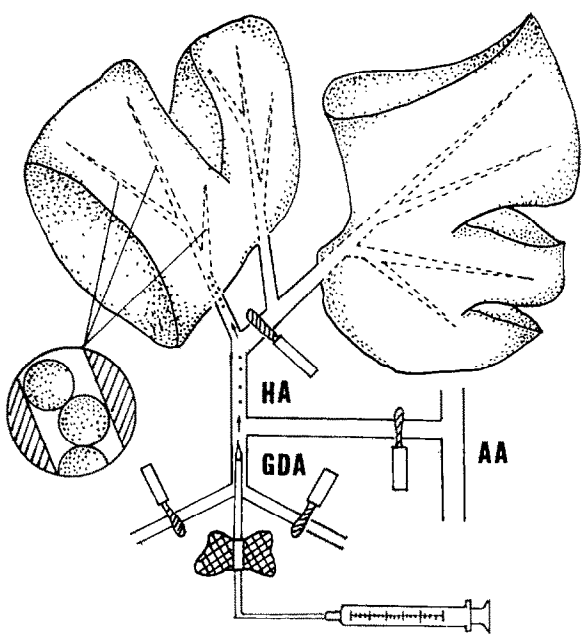

Fig. 1. Position of catheter for injection of albumin microspheres containing MMC and contrast medium into the liver artery. AA, abdominal artery; HA, hepatic artery; GDA, gastroduodenal artery.

cut off with a clamp and 0.4 ml of a suspension of albumin microspheres (20 mg/1.0 ml), containing 0.5% sodium carboxymethylcellulose (CMC-Na) as a suspending agent, was infused with a catheter into the gastroduodenal artery at a speed of 0.01 ml/min. After the infusion, about 0.05 ml of contrast media ($BaSO_4$ 20 g, agar 5 g, distilled water 100 ml) was then infused at 0.1 ml/min and the clamp was removed. The blood flow in the artery which leads to liver artery was interrupted to prevent the reflux of microsphere suspension and contrast media. The time expired during clamping of various arteries was 5–8 min. Microsphere suspension and contrast media were also infused into the liver artery of AH272 tumor-bearing rats. The contrast media alone was infused in a control group. The rat was then sacrificed by decapitation, the entire liver was removed and the angiogram was taken with a soft X-ray angiography apparatus (SOFRON model TRS-630, Soken Co., Tokyo). Photographing conditions were as follows: voltage 28–30 kV, current 5 mA, photographing distance 50 cm and exposure time 30 s (Sasaki et al., 1980; Fujiwara, 1976).

Histological observation of embolization

A suspension of MMC-free albumin microspheres was infused with the same procedure as described above and the liver was removed after 72 h. In addition, a suspension of albumin microspheres containing MMC was infused into the liver artery and the liver was excised after 12 and 24 h. The liver was fixed with 10% formaline, sliced on a microtome and stained with a conventional method (hematoxylin–eosin stain).

Measurement of antitumor effect of albumin microspheres containing MMC against AH 272 liver metastasis in rats

Male Donryu rats were anesthetized with pentobarbital sodium (50 mg/kg). A 1.0 cm abdominal incision was made at the bottom of the right ribs and the middle lobe of the liver was

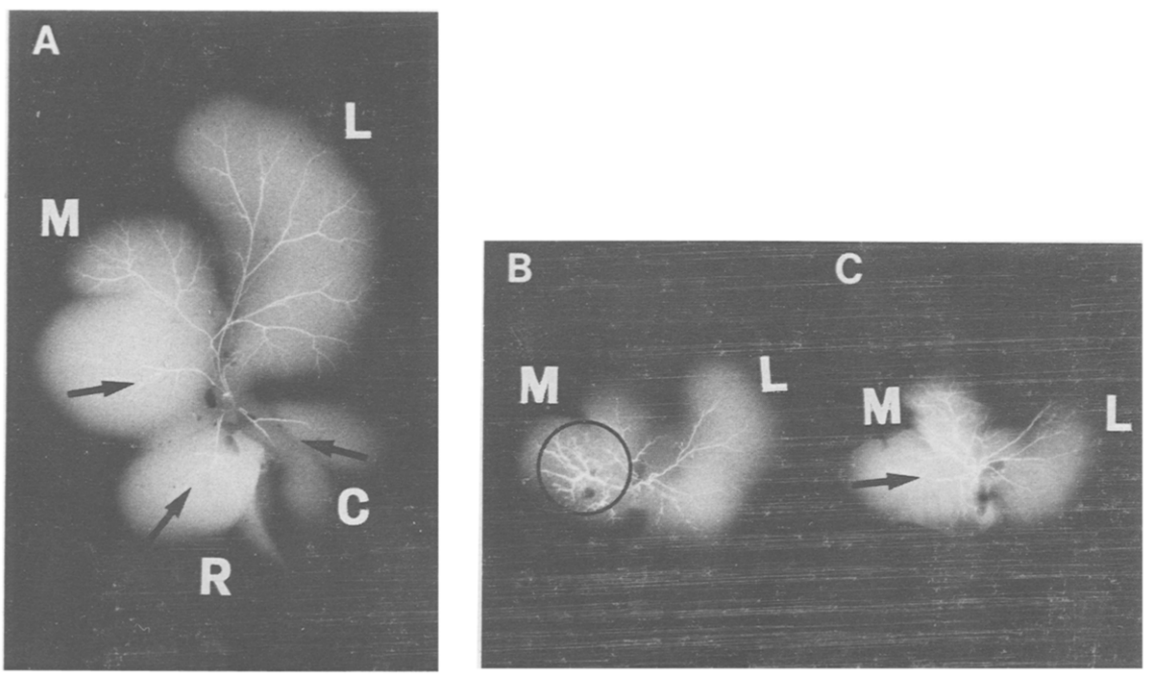

Fig. 2. Angiograms of the liver after embolization with albumin microspheres on rats. A: with injection of albumin microspheres in the normal liver. B: without injection of albumin microspheres in the AH 272 tumor liver. C: with injection of albumin microspheres in the AH 272 tumor liver. L,R,M and C mean the left, right, middle and caudate lobe, respectively. Arrows indicate embolized sites of the vessels. The circle indicates where the tumor developed the neovascularity.

exposed. Three days after implantation of AH 272 tumor cells, the abdomen was cut to open about 3.0 cm. Saline solution (control), MMC-free albumin microspheres, free MMC and albumin microspheres containing MMC were infused into the liver artery as described above. Survival times were measured for each group.

Results

Evidence of embolization of albumin microspheres in the liver artery

Observation of embolization by angiography. Fig. 2 shows angiograms of a normal liver (A) and a tumorous liver (C) after injection of albumin microspheres and contrast media into the liver artery, and of the tumor liver (B) after injection of contrast media only. Normally, arteries of left lobe and right side of middle lobe of the liver were imaged by contrast media in the periphery, because albumin microspheres did not cut off the blood flow of the artery (Fig. 2A). In contrast, the artery of right lobe, caudate lobe and left side of middle lobe of the liver were not imaged because the blood vessels were embolized by the albumin microspheres (arrows in Fig. 2A). Furthermore, we observed that the liver artery of the left side of the middle lobe had developed a complex neovascularization due to the tumorous growth in the liver (circle in Fig. 2B). Only the artery on the left side of the middle lobe which was located near the tumor was embolized by albumin microspheres. Hence, the blood vessels could not be imaged around the tumorous tissues (arrows in Fig. 2C).

Histological observation of chemoembolization. Fig. 3 shows a micrograph taken 72 h (A) after injection of MMC-free albumin microspheres, 12 h (B) and 24 h (C) after injection of albumin microspheres containing MMC into the liver artery. This micrograph indicated that the liver artery was embolized by albumin microspheres. In Figs. 3A and B parenchymal cells of the liver maintained a normal state around the interlobular connective tissue. In contrast, Fig. 3C might indicate that parenchymal cells of the liver were necrosed around the interlobular connective tissue by cytotoxic action of MMC released from al-

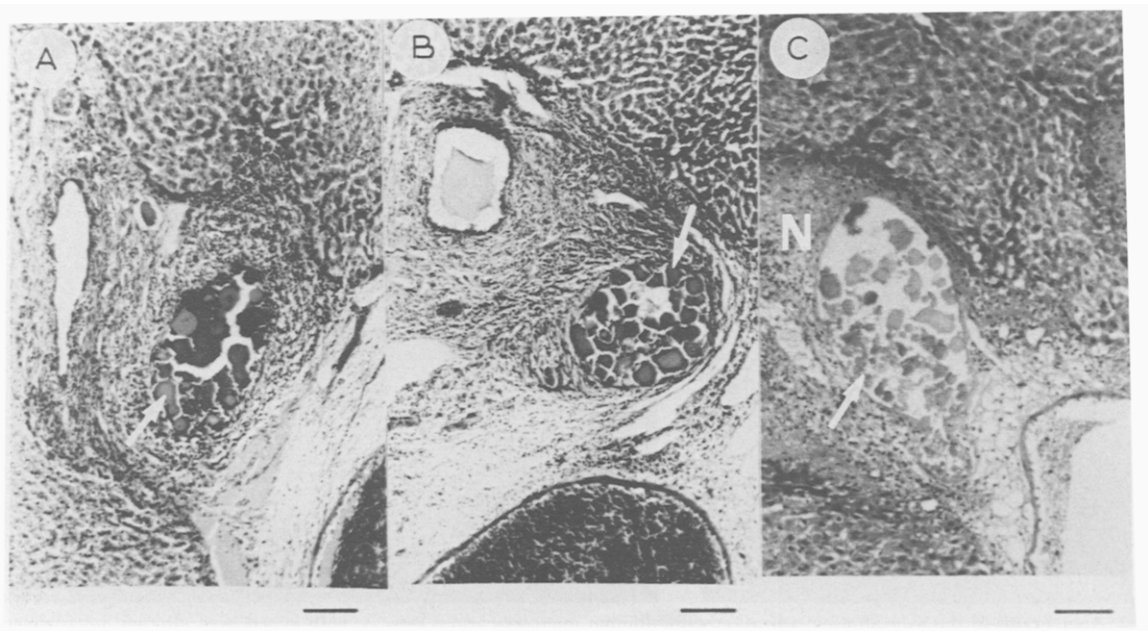

Fig. 3. Histological appearance of the liver after injection of MMC-entrapped and non-entrapped albumin microspheres. N, necrosis area caused by MMC released from albumin microspheres. Arrows indicate an artery embolized with albumin microspheres. Bars = 100 μm.

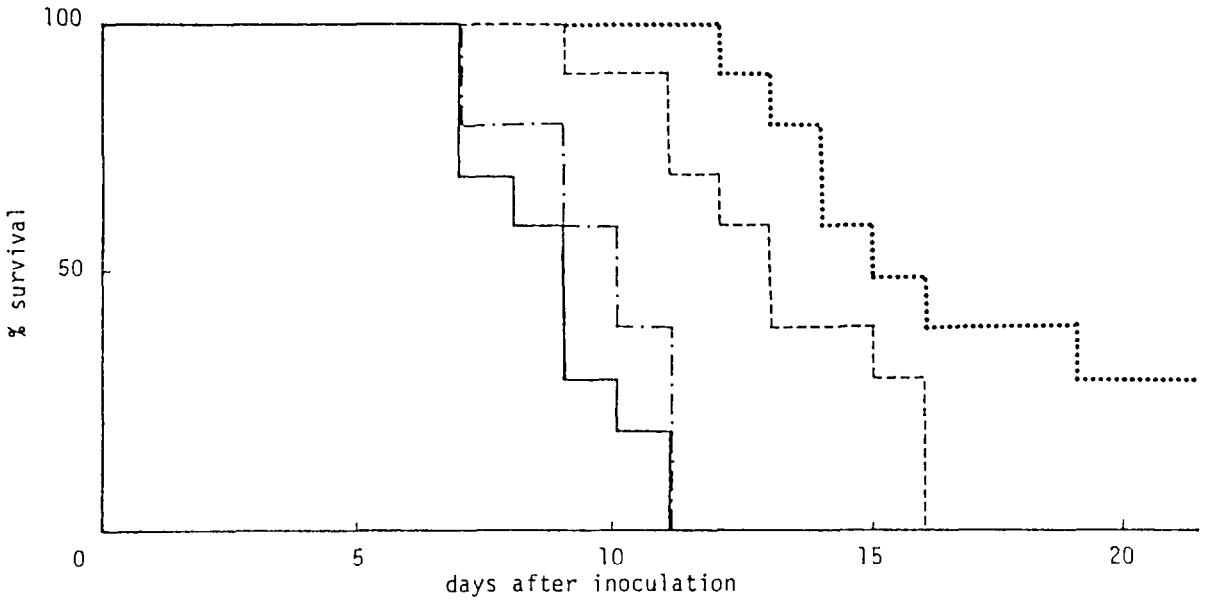

Fig. 4. Effect of albumin microspheres containing MMC on the survival in AH 272 tumor bearing rats. ———, control (saline injection); — · — ·, non-entrapped albumin microspheres; - - - - - - -, free MMC; · · · · · ·, albumin microspheres containing MMC.

bumin microspheres. Necrosis was not found after infusion of free MMC (data not shown).

Antitumor effects of albumin microspheres containing MMC

Fig. 4 shows survival rates after injecting saline (0.9% NaCl solution) (control), MMC-free albumin microspheres, free MMC, and albumin microspheres containing MMC into the liver artery of rats bearing AH 272 liver metastasis. Mean survival times of these groups are 8.8, 9.6, 13.2 and 21.9 days, respectively.

Discussion

Segall (1923) indicated that tumor cells of the liver obviously differ from the normal cells in their localized management of blood vessels. The blood is supplied in the tumor cells from only the liver artery while it is supplied in the normal cells from both the liver artery and the portal vein. Therefore, even if chemoembolizating materials are infused into the liver artery, normal cells may not appear to be influenced by the embolizating liver artery because they receive alternative nutrition from the portal vein. The present study supports this contention (Figs. 2 and 3).

The necrosis observed around the interlobular connective tissue may be produced by cytotoxic action of MMC released from microspheres and not due to obstruction of blood flow. In a previous paper (Natsume et al.), it was demonstrated that MMC release from microspheres continued for 5 days in vitro. It has been reported that the necrotic region in the liver was enlarged 2 weeks after infusion of albumin microspheres containing MMC (Fujimoto et al., 1985). These results indicate that MMC was released slowly at the target sites from the albumin microspheres, and that the in vivo drug release continued for at least 2 weeks with albumin microspheres remaining in those sites.

There was no significant difference in mean survival between the control group and MMC-free albumin microspheres group. Antitumor effect (T/C% which indicated percentage of mean survival of several treated groups to control group) of albumin microspheres containing MMC on AH 272 liver metastasis in rats was about 2.5 times of control and 1.7 times of free MMC. This result indicates that albumin microspheres containing

MMC may be a strong tool with targeting and sustained release in cancer chemotherapy.

Storage therapies of solid tumors, particularly liver tumor, have more recently included new chemoembolization techniques. Several types of percutaneous catheters play critical roles in their effectiveness through artery ligation, embolization and intra-arterial infusion therapy (Kato et al., 1984). Chemoembolization has been carried out in patients with tumors which could not be removed surgically. The resulting therapeutic efficacy was equal to that achieved by surgical therapy. Therefore, the choice of embolizing materials is important. The results described in this report suggest that albumin microspheres are very useful as chemoembolizators.

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