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Advantages and safety of local treatment with MMC/Beriplast P for cancer tumors

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Abstract To target the treatment of small points of cancer, Beriplast P, already used clinically as a physiological tissue adherent drug carrier, was mixed with the anticancer drug, mitomycin C (MMC). In this in vitro study, MMC did not release quickly from the clot of MMC/Beriplast P. The antitumor effect of this mixture was examined for its effect on cancer growth. In one series of experiments, tumor tissues were inoculated with MMC/100 μl Beriplast P and in another series, MMC/100 μl Beriplast P was injected into tumors at a weight of 300 mg. In the first series of experiments, tumor tissue treated with 0.3 mg MMC/100 μl Beriplast P was replaced with plasma cells and lymphocytes, and no viable cancer cells could be found. In the second series, MMC/100 μl Beriplast P delayed tumor growth, and the survival of Balb/c mice injected with 0.08 mg MMC/100 μl Beriplast P was significantly longer than that of mice injected with 0.08 mg MMC/100 μl saline solution ($P = 0.026$). In addition, the abdominal aorta, vena cava, and intestine around the area of treatment with 1.6 mg MMC/100 μl Beriplast P were not damaged. These results indicate that the mixture of Beriplast P and MMC is more effective than MMC solution alone in the local treatment of residual cancer.

Key words Local treatment · Beriplast P · Mitomycin C

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Introduction

Regional/local treatment for cancer has been attempted in various ways, and the clinical effects have been reported [3]. The advantage of these treatments is that a higher dose of administered drug can reach a targeted tumor mass in contrast to systemic therapy. Recurrence after surgical dissection of metastatic lymph nodes and resection of a tumor mass is often found clinically. If local treatment is performed together with surgical dissection or resection, recurrences may be reduced. For local treatment, we mixed an anticancer drug with a fibrinogen preparation.

Beriplast P [4] was used as the fibrinogen preparation producing a slow-release effect on the incorporated drug [9], because it is widely used clinically and its safety for humans has been confirmed. Mitomycin C (MMC), popular as an anticancer drug for gastrointestinal cancer [1] and easily dissolved in water, was mixed with Beriplast P. The advantages of this clotted mixture were then examined in the local treatment of malignant tumor. The effects on the large vessels and intestine around the region treated with this mixture were also examined because skin necrosis around the site of MMC injection has been reported when the MMC has failed to be administered intravenously [5].

Materials and methods

Materials and reagents

Beriplast P was purchased from Hoechest Japan (Japan). Beriplast P comes as a physiological fibrin adhesive set, a combined preparation for tissue adhesion/closure and wound healing. It comprises dry particles containing 80 mg/ml human fibrinogen, 60 times of factor XIII materials, and 300 unit/ml thrombin, and also a solution of aprotinin and calcium chloride. At the apropriate time, fibrinogen and factor XIII were dissolved in the aprotinin solution (solution A), and thrombin was dissolved in the calcium chloride solution (solution B) with an equal volume of aprotinin solution. The mixing of

these drugs rapidly formed a clot. MMC was obtained from Kyowa Hakko Kogyo (Japan). Male Fisher rats and Balb/c mice at 5–6 weeks of age were purchased from Clea Japan. The SCC-131 cell line (squamous cell carcinoma originating from the Fisher rat) was kindly provided by the Japanese Cancer Research Resources Bank. The Meth A cell line (methylcholanthrene-induced sarcoma originating in Balb/c mice) was obtained from Dr. Mashiba.

Time course of MMC release from the Beriplast P mixture

Various amounts of MMC from 3 to 10 mg were mixed with 1 ml Beriplast P. The procedure was as follows. Various amounts of MMC from 6 to 20 mg were dissolved in 0.5 ml solution B. Solutions A and B (50 µl of each) were simultaneously placed in a cryogenic vial (Corning No. 05-664-67). The mixture was allowed to stand for 30 min to coagulate. Fetal bovine serum (1 ml) was inoculated into each vial. About 800 µl serum was aspirated from each vial at the apropriate time. For the assay of MMC concentration, the supernatant was used. The MMC concentration was determined by HPLC (Trirotar, Jasco, Japan) with detection at 350 nm [6]. The stationary phase was a Cosmosil $5C_{18}$ packed colum (4.6 × 150 mm, Nakarai chemicals), and 80% methanol in water was used as the mobile phase at a flow rate of 0.8 ml/min.

Antitumor effects of MMC/Beriplast P injected into animal tumors

Various amounts of MMC powder from 0.2 to 0.8 mg were mixed in 1 ml Beriplast P or 1 ml saline solution. Various amounts of MMC from 0.4 to 1.6 mg were dissolved in 0.5 ml solution B. Solutions A and B (50 µl of each) were injected intratumorally into the same area at the same time. The total volume of the mixture was finally 100 µl. The LD_{50} of MMC for mice is about 8.4 mg/kg (0.25 mg/mouse) [8]. Meth A tumors were inoculated subcutaneously on the back of Balb/c mice. When the weight reached 300 mg (sixth day) after inoculation, the drug was injected intratumorally. Treatment was performed only once. The dosages for the intratumor injections were as follows: 0.08 mg MMC/100 µl Beriplast P, 0.08 mg MMC/100 µl saline solution, 0.02 mg MMC/100 µl Beriplast P, 100 µl Beriplast P, and 100 µl saline solution. In addition, the dosage for intravenous injection was 0.08 mg MMC/100 µl saline solution. A volume of 100 µl can stay inside a 300-mg tumor without any leakage. Mice were divided into five groups of 12 mice each. The tumor size was measured every other day until the seventh day after treatment. The survival period was recorded.

Safety of MMC/Beriplast P for normal tissue of Fisher rats

Various amounts of MMC powder from 3.0 to 16 mg were mixed in 1 ml Beriplast P or 1 ml saline solution. The procedure was as follows: 50 µl solution A and 50 µl solution B at each concentration of MMC were administered simultaneously in an area of about 0.5 cm^2 using a micropipette (Fig. 1). The total volume of the solution administered to the target area was 100 µl. Beriplast P mixed with MMC powder and a fragment of SSC tumor tissue were attached to the abdominal aorta of Fisher rats. The effects of the MMC/Beriplast P adhering to the abdominal aorta, vena cava and intestine were assessed. The rats were divided into three groups. The LD_{50} of MMC for rats is 5 mg/kg (1.3 mg for a Fisher rat). Three rats in each group were treated only once. The dosages were as follows; 0.3–1.6 mg MMC/100 µl Beriplast P, 100 µl Beriplast P, and no treatment. The tissue around the MMC/Beriplast P application site was removed on the 28th day after treatment.

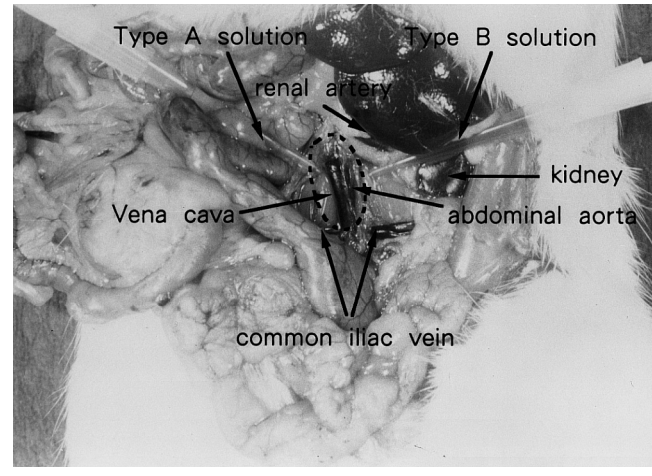

Fig. 1 Technique for administration of A and B solutions. Solution A was fibrinogen and factor XIII dissolved in aprotinin solution. Solution B was thrombin dissolved in calcium chloride solution. MMC was mixed with B solution. A and B solutions were administered simultaneously to an area of about 0.5 cm^2

Growth inhibitory effects of MMC/Beriplast P impasted onto tumor tissue

About a 50-mg fragment of SSC-131 tumor was implanted subcutaneously into the dorsum of Fisher rats, and was transplanted to the abdominal aorta of each Fisher rat using Beriplast P mixed with MMC powder. Rats were divided into four groups. The dosages were as follows: 0.03 mg MMC/100 µl Beriplast P, 0.1 mg MMC/100 µl Beriplast P, 0.3 mg MMC/100 µl Beriplast P, and 100 µl Beriplast P. The drugs were impasted over an area of about 0.5 cm^2. There were five rats in each group. Tumors were weighed every 7 days till the 28th day after treatment.

Statistical analysis

The significance of the differences in tumor weights in each group was determined using Student's *t*-test. The survival rate was calculated by the Kaplan-Meier method, and the significance of differences was analyzed using the Cox-Mantel test.

Results

Time course of MMC release from the Beriplast P mixture

As shown in Fig. 2, we examined the concentration of MMC released from the insoluble mixture of MMC/Beriplast P in serum. An initial burst of MMC occurred within the first 30 min after adding 1 ml serum to the vial containing each mixture. The proportions of MMC released from 0.3, 0.6 and 1.0 mg MMC/100 µl Beriplast P within the first 30 min were only 54%, 18% and 15%, respectively. With 0.3 and 0.6 mg MMC/100 µl Beriplast P, little MMC was released after 30 min. With 1 mg MMC/100 µl Beriplast P,

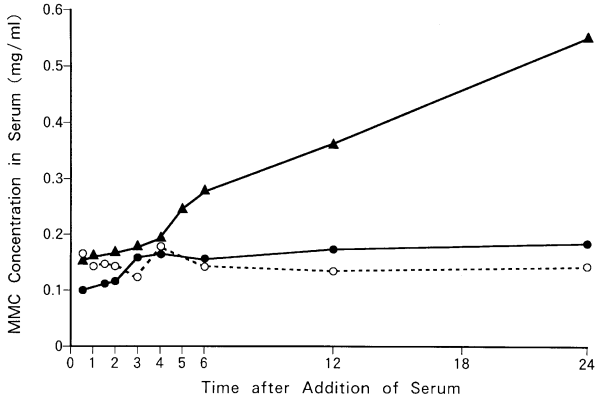

Fig. 2 Time course of MMC release from the Beriplast P mixture (○ 0.3 mg MMC/100 µl Beriplast P, ● 0.6 mg MMC/100 µl Beriplast P, ▲ 1 mg MMC/100 µl Beriplast P)

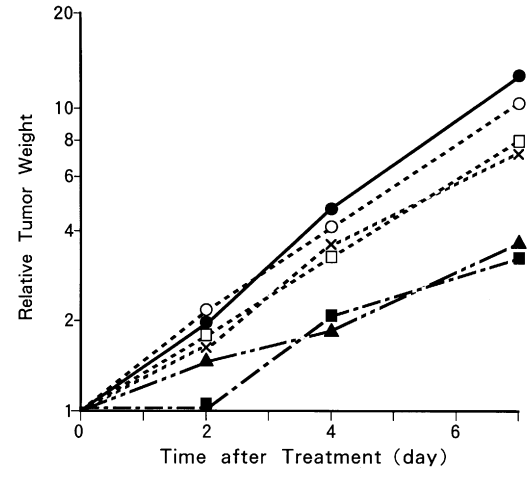

Fig. 3 Antitumor effects of MMC/Beriplast P against Meth A tumors in Balb/c mice. (● 100 µl saline solution, ○ 100 µl Beriplast P, □ 0.08 mg MMC/100 µl saline solution, ▲ 0.02 mg MMC/100 µl Beriplast P, ■ 0.08 mg MMC/100 µl Beriplast P, × 0.08 mg MMC)

sustained release of MMC was seen after 30 min, and 24 h was required to attain a plateau level in the serum. From these results, it appears that the MMC was released gradually from the clot of MMC/Beriplast P under in vivo conditions.

Antitumor effects of MMC/Beriplast P on Meth A tumors in Balb/c mice

This experiment aimed to explore the advantages of local treatment with MMC/Beriplast P for minimum but unresectable tumors. On the seventh day after injection into Meth A tumors of 0.02, 0.08 or 0.2 mg MMC and 100 µl Beriplast P, the tumor weights in the mice were 1.2, 1.1, and 1.0 g, respectively, thus demonstrating that the inhibition of tumor growth had reached a maximum with 0.08 mg MMC/Beriplast P. As shown in Fig. 3, at 7 days after intratumor injection of MMC, the tumor weights of the four groups treated with 100 µl saline solution, 0.08 mg MMC/100 µl saline solution, 0.02 mg MMC/100 µl Beriplast P or 0.08 mg MMC/100 µl Beriplast P were 12.5 ± 3.5, 8.0 ± 3.2, 3.7 ± 2.2 and 3.3 ± 0.77 g, respectively. The tumor weight at 7 days after intravenous injection was 7.8 ± 2.1 g, which was not significantly different from the weight after injection of 0.08 mg MMC/100 µl saline solution. The difference between the groups treated with 100 µl saline solution and 0.08 mg MMC/100 µl saline solution was significant ($P < 0.01$). Tumor growth with 0.08 mg MMC/Beriplast P was inhibited significantly more than that with 0.08 mg MMC/saline solution ($P < 0.001$). As shown in Fig. 4, the survival time of Balb/c mice injected intratumorally with 0.08 mg MMC/Beriplast P was significantly greater than that of mice receiving 0.08 mg MMC/saline solution ($P = 0.026$). MMC/Beriplast P was thus shown to be superior to MMC/solution even at comparatively low doses of MMC.

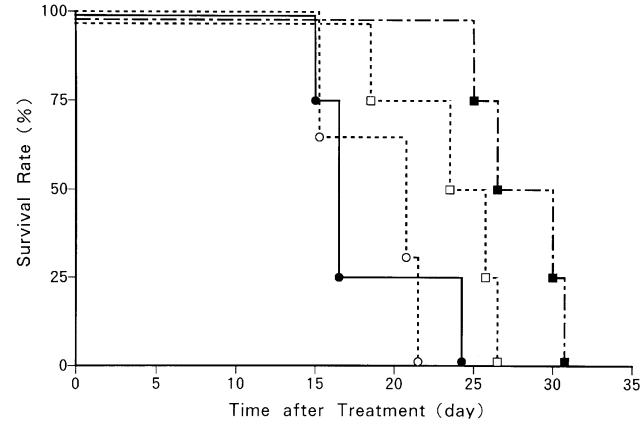

Fig. 4 Survival of Balb/c mice bearing Meth A tumors after intratumoral injection of MMC/Beriplast P. (● 100 µl saline solution, ○ 100 µl Beriplast P, □ 0.08 mg MMC/100 µl saline solution, ■ 0.08 mg MMC/100 µl Beriplast P)

Growth inhibitory effects of MMC/Beriplast P in SCC-131 tumors in Fisher rats

The purpose of this experiment was to explore the advantages of local treatment with MMC/Beriplast P for residual cancer cells after dissection of metastatic lymph nodes or after resection of uncapsulated cancer tissue. As shown in Fig. 5, the weight of SCC-131 tumor tissue without MMC/Beriplast P treatment gradually increased to 6 g by day 28. However, the weight of MMC/Beriplast P-treated tumors hardly increased, and plasma cells and lymphocytes were apparent in the region of the tumor fragment transplanted on the 28th day after treatment with 0.3 mg MMC/Beriplast P. Local treatment with MMC/Beriplast P thus appears useful for minimal and less serious cancer.

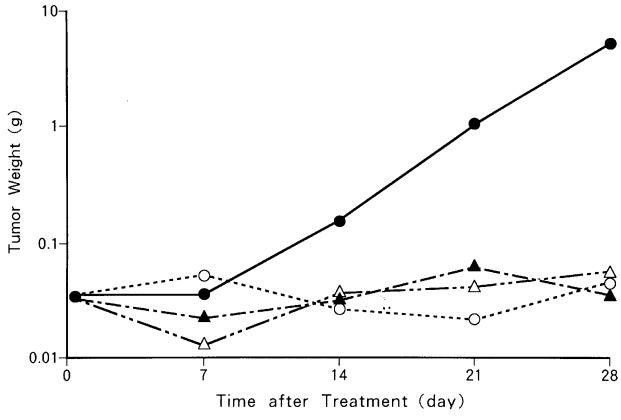

Fig. 5 Growth inhibitory effects of MMC/Beriplast P against SSC-131 tumors in Fisher rats. (● 100 µl Beriplast P, ○ 0.03 mg MMC/100 µl Beriplast P, ▲ 0.1 mg MMC/100 µl Beriplast P, △ 0.3 mg MMC/100 µl Beriplast P)

Effects of MMC/Beriplast P on the aorta, vena cava and intestine

When MMC/Beriplast P was administered to the region after dissection of lymph nodes, the aorta and vena cava were exposed. After dissection of lymph nodes, the intestine often adheres to the region. Therefore, we examined the effects of MMC/Beriplast P on the aorta, the vena cava, and the intestine of rats. The concentrations (0.3–1.6 mg) of MMC used were near the LD_{50} for MMC in animals according to the safety of MMC/Beriplast P. MMC/Beriplast P was the impasted at 0.5 cm^2. As shown in Fig. 6, the aorta, vena cava and intestine were not damaged even by treatment with 1.6 mg MMC/100 µl Beriplast P. The endothelial wall of the aorta on the 28th day was arranged regularly but was slightly swollen. As for endothelial wall of the vena cava, the endothelium was arranged slightly irregularly, and the tunica media and externa vasorum were disordered. However, these changes may have been due to the surgical treatment because they were also seen in rats that received only laparotomy without MMC/Beriplast P treatment. Damage to the serosa and mucosa of the intestine was not observed. It was thus confirmed that MMC/Beriplast P does not seriously damage the abdominal aorta or the vena cava.

Discussion

Activated carbon [2], liposomes [11], and microspheres [10] have been shown to be useful as slow release agents for anticancer drugs. Local treatment with these drugs has various advantages over systemic cancer chemotherapy [3]. In our experiments, the anticancer drug, MMC, mixed with Beriplast P containing fibrinogen and factor XIII, was applied to tumors. This mixture is thought to be useful for targeted treatment of small areas rather then regional areas such as the abdominal cavity [3, 7]. An insoluble fibrin clot is formed from the mixture of fibrinogen and factor XIII, which can be mixed with an anticancer drug. The anticancer drug is gradually released from the clot over a long period [4]. As a fibrinogen preparation, Beriplast P is used clinically as a physiological tissue-adhering drug, and its safety for humans has been confirmed. A mixture of Beriplast P and MMC was thus used in our study as local treatment for residual cancer cells.

Firstly, whether MMC/Beriplast P allows the slow release of MMC was examined in vitro. The MMC was not completely released from the mixture during the first 30 min. For instance, the amounts of MMC released from 0.3, 0.6 and 1.0 mg MMC/100 µl Beriplast P were only 54%, 18%, and 15%, respectively. The proportion released thus depended on the MMC concentration in the Beriplast P mixture. The release of MMC from the 1 mg MMC/Beriplast P mixture was sustained for 24 h. Thus, MMC did not release quickly from the clot of MMC/Beriplast P.

Secondly, we examined the safety of MMC/Beriplast P in the local region where high doses of MMC were to remain for a long time, because MMC injections has been found to induce skin necrosis when the MMC has failed to be administered intravenously. After dissection of lymph nodes, MMC/Beriplast P was applied to the area of the abdominal aorta and vena cava. Most lymph nodes are generally situated along the abdominal aorta, and the vena cava runs beside the abdominal aorta. Therefore, we examined the effects of MMC/Beriplast P on the abdominal aorta, the vena cava and the intestine. The effects on the intestine were examined because the intestine often adheres to the area of dissection of lymph nodes. This study was conducted bearing in mind the limitation that the MMC dose to confirm the safety of MMC/Beriplast P was based on 8.4 and 5.0 mg/kg, the LD_{50} for mice and rats, respectively [8]. The MMC/Beriplast P mixture (100 µl) was applied to an area of about 0.5 cm^2 of the abdominal aorta and vena cava. The tissues examined in this study were not damaged, even by treatment with 1.6 mg MMC/100 µl Beriplast P.

Thirdly, the advantages of MMC/Beriplast P for treating unresectable cancer was examined in preventing reccurrence after dissection of lymph nodes. The antitumour effect was studied. In one series of experiments, tumor tissue was inoculated with MMC/Beriplast P at the same time, and in another series of experiments, MMC/Beriplast P was injected into tumors at a weight of 300 mg. In the first experiments, tumors treated with MMC/Beriplast P hardly grew. No viable cancer cells could be found in the area transplanted with a tumor fragment after treatment with 0.3 mg MMC/Beriplast P. In the second experiments, MMC/Beriplast P delayed tumor growth and prolonged the survival of mice. These results indicate

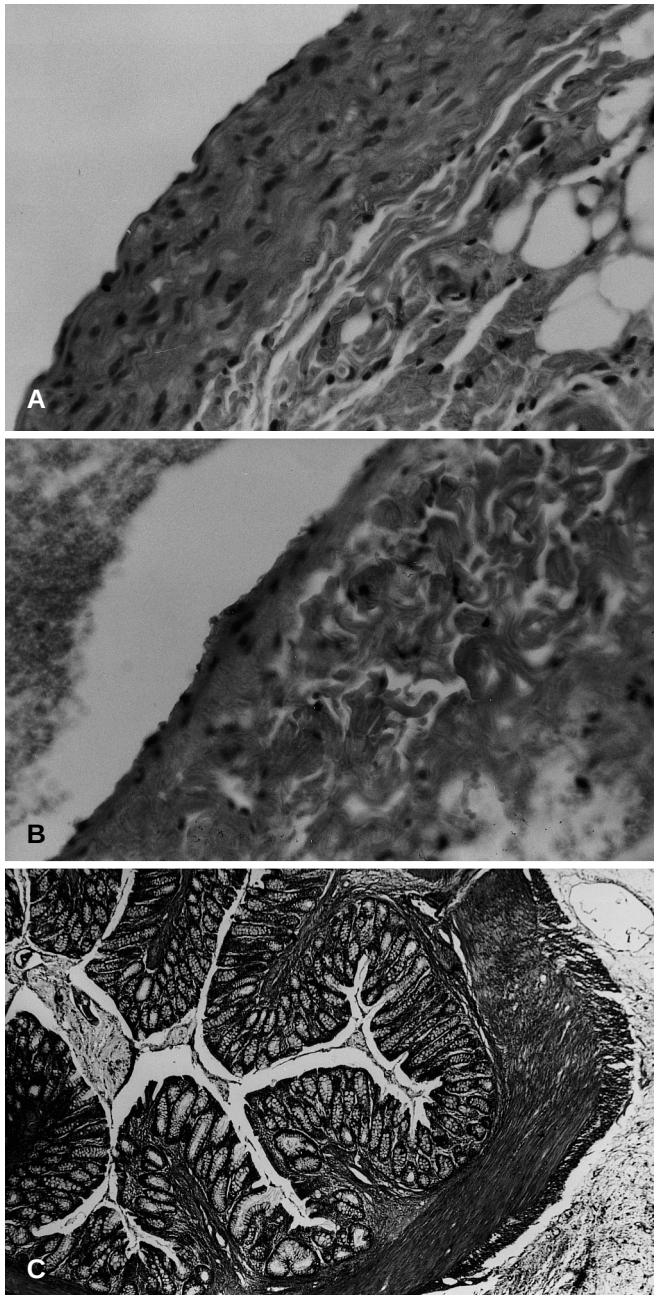

Fig. 6A–C Histopathological features of the abdominal aorta (**A**), vena cava (**B**) and intestine (**C**) at the 4th week following treatment with 1.6 mg MMC/Beriplast P

that MMC/Beriplast P would be most useful for the treatment of imperfectly resectable and invisible cancer, and may be helpful for adjuvant therapy for residual small cancer.

In conclusion, local treatment with MMC/Beriplast P would probably not be harmful to normal tissues, and may be very useful for treating residual cancer cells. MMC/Beriplast P should be used clinically in cancer therapy. For clinical use, 1 mg MMC/100 µl Beriplast P for an area of 1 cm^2 might be an adequate compromise between the releasing effect and safety.

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