Antitumor Effect of Pluronic F-127 Gel Containing Mitomycin C on Sarcoma-180 Ascites Tumor in Mice[1)]

Shozo MIYAZAKI,*[,a] Yutaka OHKAWA,[a] Masahiko TAKADA,[a] and David ATTWOOD[b]

Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University,[a] Ishikari-Tohbetsu, Hokkaido 061-02, Japan and School of Pharmacy, University of Manchester,[b] Manchester, M13 9PL, U.K. *Received March 26, 1992*

Pluronic F-127 (PLF-127) gels were evaluated as a sustained-release vehicle for intraperitoneal administration of mitomycin C (MMC) in order to enhance the therapeutic effects of MMC against a Sarcoma-180 ascites tumor in mice. Tumor cell injections were made on day 0 and injections of MMC in 25% (w/w) PLF-127 on day 1, both intraperitoneally. A prolongation of the life span of tumor-bearing mice following injection of therapeutic PLF-127 was noted, and PLF-127 containing MMC was therapeutically more active than free drug. The high chemotherapeutic efficiency of MMC in PLF-127 was striking at high doses, which would be toxic in the case of the drug alone.

PLF-127 gels exhibit reverse thermal behavior and are fluid at refrigerator temperature, but are soft gels at body temperature. The *in vitro* release experiments indicated that Pluronic gel might serve as a rate-controlling barrier and be useful as a vehicle for sustained-release preparations of MMC to be administered intraperitoneally. These results suggest that sustained-release occurs in the peritoneum and that effective drug concentrations can be maintained by the preparation.

Keywords Pluronic F-127; mitomycin C; gel preparation; reverse thermal gelation; sustained-release; intraperitoneal administration; Sarcoma-180 ascites tumor; mouse

It is well known that free type cancer cells are often observed in the peritoneal cavity of gastric cancer patients with serosal invasion, which may cause carcinomatous peritonitis.[2)] Mitomycin C (MMC) is a clinically significant antineoplastic antibiotic and has been administered intraperitoneally in solution form for carcinomatous peritonitis.[3)] The method is not always successful to control the local lesion, because MMC administered in solution form is rapidly absorbed through the serosal surface into the blood plasma and it is difficult to keep a high concentration of the drug for a long time in the peritoneal cavity.[2)] In order to overcome this difficulty, attempts have been made to achieve sustained intraperitoneal delivery of MMC by means of drug delivery systems such as activated carbon particles[3)] and poly L-lactic acid microcapsules.[4)]

Pluronic F-127 (PLF-127) is a polyoxyethylene–polyoxypropylene block copolymer which consists, by weight, of approximately 70% ethylene oxide and 30% propylene oxide with an average molecular weight of 11500.[5,6)] PLF-127 appears to have good potential for use in topical drug delivery systems since it exhibits reverse thermal gelation behavior[5,7)] and has good drug release characteristics[8,9)] and low toxicity.[5,10)]

PLF-127 has been evaluated as a vehicle for novel dosage forms, either for dermatological use[5,8)] or ophthalmic application.[10)] Morikawa *et al.*[11)] have tested the feasibility of PLF-127 as a sustained-release vehicle for subcutaneous administration of interleukin 2. In our previous papers, it was suggested that indomethacin[12)] and tegafur[13)] preparations based on PLF-127 aqueous gel may be practically useful as a rectal preparation with prolonged action and reduced side effects.

The PLF-127 gel exhibits reverse thermal behavior and is therefore fluid at refrigerator temperature (5 °C), but a soft gel at body temperature.[5)] This suggests that when administered into a peritoneal cavity, the preparation will form a solid artificial barrier and sustained release depot. In this report, we investigated whether the antitumor effect of MMC is augmented by PLF-127 used as a sustained-release vehicle for intraperitoneal administration.

Experimental

Materials MMC was supplied by Kyowa Hakko Kogyo Co., Tokyo. PLF-127 was a gift from Asahi Denka Kogyo Co., Tokyo and used without further purification.

Preparation of PLF-127 Gel PLF-127 gel (25% w/w) was prepared by the cold method described by Schmolka.[5)] A weighed amount of PLF-127 was slowly added to cold phosphate buffer at pH 7.4 in a vial containing a magnetic stirring bar with gentle mixing. The vial was left overnight in a refrigerator to ensure complete dissolution. Eventually, a clear, viscous solution formed. An appropriate amount of MMC was then dissolved in the cold PLF-127 solution.

Measurement of Drug Release from PLF-127 Gel Release rate was measured using a plastic dialysis cell containing a membrane barrier (Visking Co., type 36/32).[9)] The drug concentration of the sample was determined with a spectrophotometer at 363 nm. All experiments were carried out in triplicate and average values were plotted.

Animal Experiment Male ddY mice, 25—35 g, were purchased from Gunma Experimental Animals Co. and used to evaluate the antitumor effect of the drug loaded PLF-127 gel. They were inoculated intraperitoneally with 1×10^6 Sarcoma-180 ascites tumor cells. One day after inoculation of the cells, the mice were injected either with 0.2 ml PLF-127 solution containing MMC or with 0.2 ml MMC solution (free MMC) in pH 7.4 phosphate buffer. Changes in body weight and survival time of the mice were recorded for up to 60 d.

Toxicity Studies The toxicity of the PLF-127 was evaluated on the basis of survivors and changes in body weight on day 14 after the intraperitoneal single administration (25% w/w, 0.2 ml) to normal ddY mice.

Results

***In Vitro* Release of MMC in PLF-127 Gel** The amount of MMC which can be released from PLF-127 gel into the phosphate buffer was determined with a dialysis cell.[9)] Figure 1 shows plots of the data, expressed as the cumulative amount of drug released *versus* time. In contrast with rapid release of free MMC, the release of the drug from PLF-127 gel through the membrane was slow, indicating that sustained-release occurs. Thus, incorporation of MMC into 25% w/w PLF-127 gel resulted in a retarded release of the drug.

Antitumor Effect of MMC in PLF-127 against Sarcoma-180 Ascites Tumor First, the antitumor effect of MMC in

PLF-127 was evaluated by following the changes in body weight of mice. Figure 2 shows the results with free MMC and with MMC in PLF-127, as well as those in normal and tumor-bearing mice. Two dose levels, 3.6 and 5.2 mg/kg, were used in this study. At a dose of 3.6 mg/kg (Fig. 2A), the body weight curve of the mice treated with MMC in PLF-127 was identical with that of the mice receiving free MMC and the increase in body weight was less than that in untreated tumor-bearing mice. At a dose of 5.2 mg/kg (Fig. 2B), loss of body weight was observed after administration of MMC in PLF-127, but the weight of the mice tended to recover to that of the control group. In the groups receiving free MMC, on the other hand, a significant decrease in body weight due to its toxicity was observed at this dose.

Next, the antitumor effect was evaluated in terms of animal survival data. Figure 3 and Table I summarize the antitumor effects of free MMC and MMC in PLF-127. When 1×10^6 cells of Sarcoma-180 ascites carcinoma were inoculated intraperitoneally into mice, the animals died between 7 and 23 d later due to the tumor, and the mean survival time was 15.3 d. At a dose of 3.6 mg/kg, administration of MMC in PLF-127 to tumor-bearing mice cured 3 of the 6 mice examined, whereas administration with free MMC cured 1 of the 6 mice; the survival time (31.0 d) of the remainder after administration of MMC in

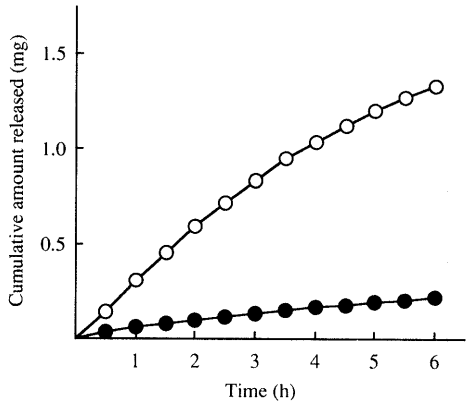

Fig. 1. Release of MMC from Pluronic F-127 Gel at 37 °C

○, free MMC; ●, Pluronic F-127 gel. Concentration of MMC was 0.05% (w/v). Each value represents the mean of 3 experiments.

TABLE I. Effect of Pluronic Gel Containing MMC and Free MMC on Survival Time of Mice Bearing Sarcoma-180 Ascites Tumor

Compound	Dose (mg/kg)	Survival time (d)[a]	T/C[b] (%)	Survivors at 60 d
Control	—	15.3±1.3	100.0	0/16
Free MMC	3.6	30.8±4.4[c]	201.3	1/6
	5.2	26.0±4.6[d]	170.0	0/6
Pluronic gel containing MMC	3.6	31.0±6.1[c]	202.6	3/6
	5.2	37.0±7.6[c]	241.8	2/6

a) Mean±S.E. *b*) Mean survival time of treated mice/mean survival time of control. *c*) Significantly different ($p<0.001$) from the control. *d*) Significantly different ($p<0.005$) from the control.

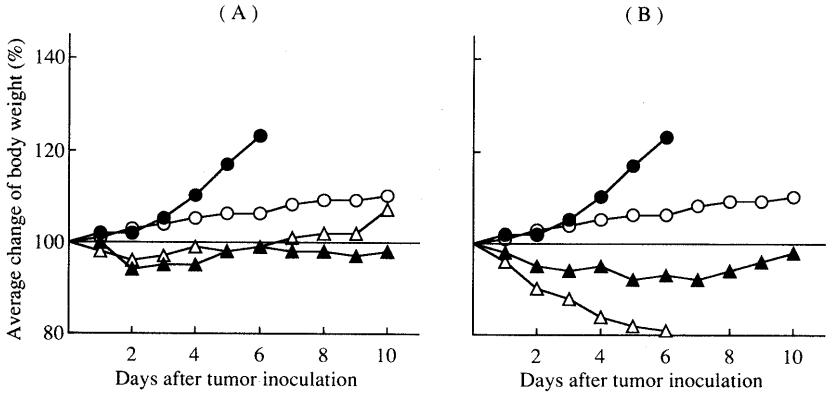

Fig. 2. Changes in Body Weight of Sarcoma-180 Ascites Tumor-Bearing Mice after Injection of Pluronic F-127 Solution Containing 3.6 (A) and 5.2 (B) mg/kg of MMC

○, control normal mice; ●, control tumor-bearing mice; △, treated with free MMC; ▲, treated with MMC in PLF-127.

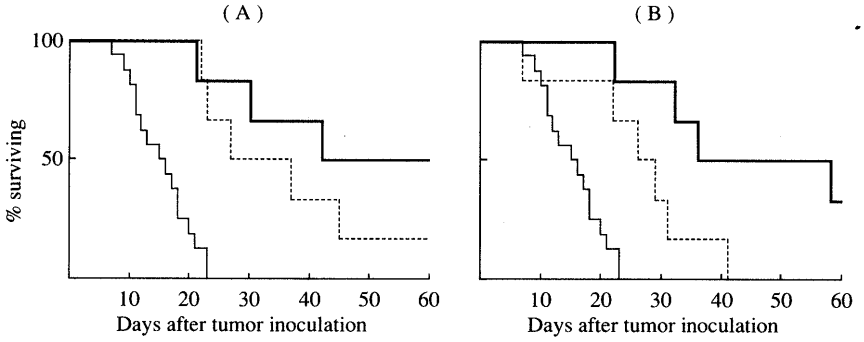

Fig. 3. Effect of Pluronic F-127 Gel Containing 3.6 (A) and 5.2 (B) mg/kg of MMC and of Free MMC on Survival Time of Mice Bearing Sarcoma-180 Ascites Tumor

——, control; – – –, free MMC; ▬, MMC in PLF-127.

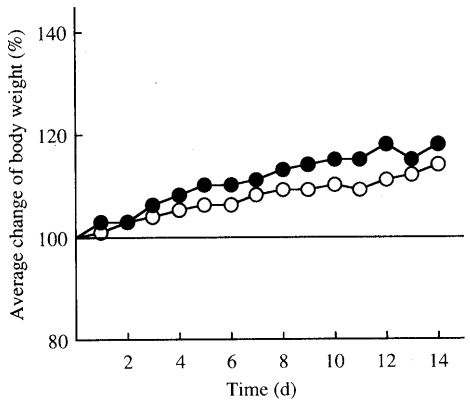

Fig. 4. Changes in Body Weight of Normal Mice after Injection of Pluronic F-127 Solution without Drug

○, normal untreated mice; ●, treated with PLF-127 without drug. Each value represents the mean of 6—8 experiments.

PLF-127 was almost the same as free MMC (30.8 d) and *T/C* values were 202.6 and 201.3%, respectively. At a dose of 5.2 mg/kg, administration of MMC in PLF-127 to tumor bearing mice cured 2 of the 6 mice examined. Moreover, the span of mean survival time of mice treated with MMC in PLF-127 (37.0 d) was much longer than that of mice given MMC alone (26.0 d). Administration with free MMC cured none of the mice. These results suggest that the *in vivo* antitumor effects of MMC are augmented by the use of PLF-127.

Toxicity to Mice The 25% (w/w) Pluronic F-127 solution was prepared without drug and evaluated in terms of the number of survivors and evidence of rejection 14 d after intraperitoneal injection into normal ddY mice. None of the 8 mice receiving 0.2 ml PLF-127 solution per mouse died and no localized inflammation or foreign reaction in the peritoneum was observed. The changes in body weight after injection of the PLF-127 solution were similar to those of normal untreated mice (Fig. 4). Thus, PLF-127 gel showed good biocompatibility.

Discussion

Recently, much effort has been made to enhance the antitumor effect of anticancer agents in cancer chemotherapy. One possible approach is the topical administration of a sustained-release preparation on cancerous lesions. This study was designed to evaluate PLF-127 gel for use as a sustained-release vehicle for intraperitoneal MMC delivery.

The antitumor effect of MMC was augmented by the use of PLF-127 as a sustained-release vehicle for intraperitoneal administration. As the PLF-127 gel containing MMC limited the increase in body weight due to tumor growth and prolonged the life span of the tumor-bearing mice, it may be inferred that sustained-release occurs in the peritoneum and that effective drug concentrations can be maintained by the preparation. The high chemotherapeutic efficiency of MMC in PLF-127 was striking at high doses which would be toxic in the case of the free drug. This increased survival can be interpreted as a sign of reduced toxicity. The MMC in PLF-127 appears to be less toxic than the equivalent dose of free MMC. LD_{50} of free MMC in mice is estimated to be 5.2—7.5 mg/kg,[14] but may be higher when MMC is administered in the form of PLF-127 solution containing the drug.

PLF-127 is of particular interest since concentrated solutions (20—30% w/w) of the copolymer are transformed from low viscosity transparent solutions into solid gels upon warming from 5 °C to body temperature.[5] When a cold liquid form of PLF-127 is injected into the peritoneal cavity, the preparation will form a sustained-release depot immediately. In preliminary experiments (data not presented), we observed that when 25% (w/w) PLF-127 solution was injected intraperitoneally into mice, the PLF-127 gel was present in the cavity for at least 1 h.

It is likely that MMC is released by diffusion through extramicellar aqueous channels of the gel matrix, which is micellar in nature,[8,15] and by decomposition of the PLF-127 gel matrix itself. In addition, since injected PLF-127 has low toxicity[5,10] and is excreted into the urine,[5] PLF-127 is injectable repeatedly and need not be removed from the body at the end of therapy.

The preparation of gels based on PLF-127 is a simple matter as compared with other sustained-release vehicles such as activated carbon particles[3] and poly L-lactic acid microcapsules.[4] One may use either a "cold" or a "hot" technique.[5] Once the Pluronic is completely dissolved, ingredients may be added.

We conclude that MMC in PLF-127 showed superior therapeutic efficacy against intraperitoneally inoculated tumor as compared with MMC aqueous solution. PLF-127 gel might be potentially useful as a sustained intraperitoneal delivery of MMC for carcinomatous peritonitis.

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