Poloxamer 188 and Propylene Glycol-Based Rectal Suppository Enhances Anticancer Effect of 5-Fluorouracil in Mice

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The tumoricidal and apoptosis-inducing activities of 5-fluorouracil (5-FU) have been demonstrated in experimental and clinical investigations. Clinically, the 5-FU suppository form has been widely adopted for its advantages of less systemic toxicity, higher local tissue concentrations, and reduced first-pass effect. In this study, we investigated the feasibility of rectal administration of 5-FU suppository based on poloxamer 188 (P188) and propylene glycol (PG) and its anticancer effect on the murine experimental cancer models. The rectal suppository was made with 70% P188 and 30% PG, which was a solid phase at room temperature and instantly melted at physiological temperature. The treatment with the 5-FU suppository was more effective than the oral route in decreasing the volume of rectal cancer in mice. In addition, the survival rate of the mice with rectal cancer was higher in the group treated with the 5-FU suppository than in the group treated with 5-FU orally. Furthermore, in mice skin cancers induced by inoculation of murine CT-26 colon carcinoma cells, the anticancer effect of 5-FU was significantly enhanced by the rectal administration of the suppository than by oral treatment. Taken together, the results suggest that a poloxamer gel system with 5-FU/P188/PG is an effective rectal dosage form for the treatment of both rectal and non-rectal cancers.

Key words poloxamer 188; propylene glycol; 5-fluorouracil; rectal suppository; cancer treatment

Colorectal cancer is among the most common human malignancies, and remains a leading cause of cancer-related morbidity and mortality. Patients with rectal cancer, despite surgery, still have a significant probability of relapse and cancer-related death. Adjuvant treatment is a rapidly evolving field of rectal cancer treatment. The standard approach is a combination of chemotherapy and radiation therapy (RT). The combined chemotherapy and RT for rectal cancer has shown to increase the proportion of patients cured of their disease.1,2) 5-Fluorouracil (5-FU) has been widely used in the treatment of solid malignancies.3,4) 5-FU administered via suppository has shown less systemic toxicity, while permitting direct topical contact to rectal cancer and higher rectal tissue concentrations than intravenous administration.5,6) 5-FU is also used as an adjuvant chemotherapeutic agent for rectal cancers.7) It has also been reported that 5-FU suppository delivery combined with radiation causes less systemic toxicity and is more effective than intravenous administration.7)

A conventional rectal suppository such as a suppository formulated with a polyethylene glycol base, which may soften or melt slowly in the site of application due to its relatively high melting point, cannot be rapidly absorbed in the mucous membranes of the absorption site.8,9) Furthermore, conventional suppositories, which may reach to the end of the canal of the application site, because of their poor mucoadhesive properties, lose drugs at that level, and may also allow the carried drugs to undergo the first-pass effect.10) To solve these problems associated with the use of conventional solid suppository, it would be desirable to develop a novel solid suppository, which was a solid phase at room temperature and instantly melted at physiological temperature, and had mucoadhesive properties to such level that it would attach to the site of application, i.e. the rectal mucous membranes.11)

Recently, we have established the mixture composed of poloxamer 188 (P188) and propylene glycol (PG) for suppository having the suitable melting point (30—37 °C) and mucoadhesive property.12)

Thus, the purpose of this study is to investigate whether the solid suppository with P188 and PG is a candidate of rectal dosage form for 5-FU in treating not only rectal cancers but skin cancers in mice.

MATERIALS AND METHODS

Materials The murine CT-26 colon carcinoma cell line was purchased from Korean Cell Line Bank (Seoul, Korea). Fetal bovine serum (FBS) was purchased from GIBCO (Grand Island, NY, U.S.A.). 5-FU was provided by Dong-A Chemical (Seoul, South Korea). P188 which is a copolymer of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) with the ratio of POE/POP of 80/20 and the molecular weight of 8350 was obtained from BF Goodrich (Breesville, OH, U.S.A.), and PG was from DC Chemical Co., Ltd. (Seoul, South Korea). Propylene glycol was of USP grade. The semipermeable membrane tube (Spectra membrane tubing No. 1) was from Spectrum Medical Industries Inc. (Los Angeles, California, U.S.A.).

Preparation of P188 and PG-Based Suppository P188 and PG were mixed and heated up to 55 °C. 5-FU was then slowly added to the solution with continuous agitation. The resulting solution was then placed into the suppository mould and cooled down to 25 °C. The melting point of the suppository was determined using DSC (Model 200, Netzsch, U.S.A.) at the raising temperature 10 K/min, as previously described.11)

Dissolution Test Each suppository containing 100 mg 5-FU in different proportions of P188/PG [180/20% (2 g) and (70/30%) (2 g)] was inserted into a semipermeable membrane tube. Both sides of the tube were tied up with a thread
to prevent leakage, and then, placed into the dissolution tester (DST-600, Fine Chemical, Korea). The dissolution test was performed at 36.5 °C and 100 rpm with 400 ml phosphate buffer (pH 4.4) as a dissolution medium using the paddle method as previously described. At 0.25, 0.5, 0.75, 1, 1.5, 2 and 3.5 h, 1 ml aliquotes of the medium were sampled and filtered. After 3 h, the samples were analyzed by UV-visible variable wavelength detector (Philips, Model PU 8730) at 254 nm (Kim et al., 2002). 5-FU was analyzed with a UV–VIS wavelength detector at 265 nm (Philips, Model PU 8730, Germany).

**Cell Culture** CT-26 cells were grown at 37 °C in a humidified incubator with 5% CO2/95% air in Dulbecco’s modified eagle medium supplemented with 10% FBS, 1 mM sodium pyruvate, 200 IU/ml penicillin and 200 µg/ml of streptomycin. Culture medium was replaced every other day. After the confluence, the cells were subcultured following trypsinization with 0.25% trypsin–EDTA solution.

**Establishment of Tumor Models in Mice** For rectal cancer model, ten BALB/C mice weighing 20—22 g supplied by Orient Co., Ltd. (Seoul, Korea) were used for each group in the experiment. The mice were anesthetized with ethyl ether and put on a supine position. Under an operative microscope, a 30-gauge needle was inserted into the submucosa of the posterior wall of the rectum just at the anal verge, then the needle was pierced within the submucosa up to the level about 7—8 mm above the anal verge. The needle can be seen beneath the mucosa of the rectum. CT-26 cells were injected slowly into the submucosa of the right flank. The tumor volume, first measured by using a Serum Transaminase assay kit (Asan Pharm, Seoul, Korea) which is based on the Reitman-Frankel method as previously described. In the case of subcutaneous tumors, mice were first administered with 5-FU either orally or via rectal suppository when tumor volume reached about 200 mm3, and thereafter every 3rd day.

**Treatment of Drugs** For the mice having rectal tumors, 5-FU (120 mg/kg) was given once via oral or rectal route 7 d after the tumor injection. The dose of 5-FU was selected based on the previous studies. In the case of subcutaneous tumors, mice were first administered with 5-FU either orally or via rectal suppository when tumor volume reached about 200 mm3, and thereafter every 3rd day.

**Glutamic Oxaloacetic Transaminase/Glutamic Pyruvic Transaminase (GOT/GPT) Assay** GOT/GPT levels were measured by using a Serum Transaminase assay kit (Asan Pharm, Seoul, Korea) which is based on the Reitman-Frankel method.

Measurement of Lipid Peroxidation Tissue homogenate (0.4 ml) was added to 0.1 M potassium phosphate buffer (0.4 ml). After incubation for 4 h at 37 °C, the mixture was added to 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid solution (pH 3.5), and 1.5 ml of 0.8% thiobarbituric acid. The mixture was heated at 95 °C for 1 h, chilled to room temperature, and extracted with 1 ml of n-butanol-pyridine mixture (15:1 v/v). The upper organic layer containing malondialdehyde produced by lipid peroxidation was measured at 532 nm. Synthetic malondialdehyde was used as an external standard, and the level of lipid peroxides was expressed as nmol of malondialdehyde per mg protein.

**Data Analysis** Data were expressed as mean±standard error of the mean. ANOVA and Student–Newman–Keuls’ test for individual comparisons. p values of less than 0.05 are considered statistically significant.

**RESULTS AND DISCUSSION**

The P188 and PG mixtures were easily prepared by mixing, heating and cooling P188 and PG in a ratio of 80/20% (w/w), 70/30% and 50/50%, respectively. PG and P188 itself showed the peak melting point of at around −10 °C and 55 °C, respectively, whereas increasing concentration of PG shifted the DSC curve towards a lower temperature with the melting point of about 45, 32, 10 °C, respectively in the P188 and PG mixtures as shown in Fig. 1. Furthermore, their DSC curves had no peaks of P188 and PG, indicating that the P188 and PG mixtures were homogeneous phase. The P188 and PG mixture at a ratio of 7/3 was selected as a suppository base, since it was a solid form at room temperature and instantly melted near the physiological temperature.

To test whether the ratio of P188/PG affects the dissolution rates of 5-FU from the suppositories, we performed the dissolution studies on the two formulations composed of 5%...
5-FU and 95% the mixture of P188 and PG with the ratio of either 7/3 or 8/2. The 5-FU release from the suppository containing P188/PG (7/3) was significantly higher than P188/PG (8/2) as depicted in Fig. 2, indicating that PG increases the dissolution rates of the drug from the suppository, which has also been observed in the dissolution rate of other drugs from the solid suppository with P188 and PG. Therefore, not only the melting point but the dissolution profile supported that as a base for 5-FU solid suppository, the ratio of 7/3 for the mixture of P188/PG is the proper one. The higher dissolution rate in the suppository with higher PG content may be due to the lower melting point which facilitates the drug release at the physiological temperature.

To examine the effectiveness of the 5-FU suppository over the oral administration on the rectal cancers, the mice received 120 mg/kg 5-FU via oral and the suppository 7 d after inoculation of CT26 cancer cells within the submucosa of rectum. The 5-FU administered either orally or in the form of the P188/PG-based suppository significantly reduced the volume of rectal cancer. However, the anticancer effect of 5-FU in the suppository form was more prominent, as shown in Fig. 3A. Moreover, in a separate experiment, the 5-FU suppository administration on the 7th day of cancer cell inoculation into the rectum prolonged the survival of rectal cancer mice as shown in Fig. 3B.

In order to evaluate the effectiveness of the 5-FU suppository on the cancers other than rectal tissues, we also established and examined the anticancer effects of the 5-FU suppository on the skin tumor model. The volume of skin cancers treated with 5-FU in a three intermittent administration regimen either orally or via rectal suppository was decreased in a dose-dependent manner (Fig. 4). However, rectal administration of 5-FU through the P188 and PG-based suppository was more effective than 5-FU oral treatment against CT26-derived skin cancers in mice. These results may be explained by the previous studies of other groups that 5-fluorouracil after oral administration undergoes first-pass metabolism by both the liver and intestinal mucosa.

Next, we also investigated any difference in toxicity of oral or the suppository administration. The hepatic damage was assessed by measuring glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) activities in the serum as well as lipid peroxidation of liver tissue. The mice that received 40 or 120 mg/kg 5-FU orally or through rectal suppository showed no statistical difference in the toxicity grading of glutamic oxaloacetic transaminase/glutamic pyruvic transaminase (GOT/GPT) and lipid peroxidation (Fig. 5). Previous other studies have also reported that 5-FU suppository administration is not inducing any side effect and is safer route than i.v. administration since i.v. but not suppository causes side effects of diarrhea, weight loss and...

Fig. 2. The Dissolution of 5-FU from the P188/PG-Based Suppository
P188/PG-based suppository (4 g) containing 200 mg 5-FU was used as dissolution samples. Dissolution tests were performed at 36.5 °C using the paddle method at 100rpm with 400 ml phosphate buffer (pH 4.4) as a dissolution medium. At 0.5, 1, 1.5, 2, 3 and 4 h, 5 ml aliquotes of the medium were sampled and filtered, equal volumes of fresh medium were replaced to maintain the sink condition. The filtrates were analyzed by UV/visible variable wavelength detector (Philips, Model PU8730) at 254 nm (Kim et al., 2002). The data are expressed as the mean ± S.E.M. (n=5).

Fig. 3. Effects of 5-FU in Oral or P188/PG Suppository Form on the Size (A) and Survival Rate (B) of Rectal Cancer Mice
The mice injected with 1×10^6 cells of CT-26 into the submucosa of the posterior wall of the rectum received one dosage of 5-FU either oral or suppository. The tumor size was measured after the rectum was taken out from a decapitated mouse on the 2nd day of 5-FU treatment (n=8), while the measurement of survival rate was kept until the last animal was dead (n=7). SUPP represents suppository. **p<0.01, compared to untreated control. ***p<0.01, compared to the group treated with 5-FU orally.

Fig. 4. The Effect of 5-FU-Loaded P188/PG Suppository on the Mouse Subcutaneous Cancer
BALB/C male mice were inoculated with 1×10^6 CT-26 cells into the subcutaneous tissue of the right flank. The tumor volume was first measured two weeks later and thereafter twice a week. The SUPP represents suppository. *p<0.05, compared to untreated control. #p<0.05, compared to the group treated with 5-FU orally.
myelosuppression in rats. However, more studies are still required to manifest whether 5-FU rectal suppository based on P188 and PG does not induce any toxicity in gastrointestinal tract and hematopoiesis other than liver.

In conclusion, the present study clearly showed that treatment with 5-FU suppository composed of 70% P188 and 30% PG, which was a solid form at room temperature and instantly melted at physiological temperature, decreased the tumor size and increased the survival rate of rectal cancer mice. Moreover, our results further suggest that the 5-FU suppository may be an effective rectal dosage form for the treatment of not only rectal cancers but other cancer types.

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