ENHANCEMENT OF LYMPHATIC TRANSPORT OF 1-(2-TETRAHYDROFURYL)-5-FLUOROURACIL BY POLYACRYLIC ACID AQUEOUS GEL AND EMULSION TYPE SUPPOSITORIES IN RATS

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1-(2-Tetrahydrofuryl)-5-fluorouracil (FT-207) concentrations in lymph and plasma were measured after rectal administration of three types of suppositories containing FT-207. The suppositories used in this study were prepared from polyacrylic acid aqueous gel bases, emulsion type bases and a commercial fat base, witepsol w-35. Both aqueous gel and emulsion type suppositories were superior to the suppositories from witepsol with regard to FT-207 concentration in lymph and plasma. The addition of aqueous gel to the polyacrylic acid aqueous gel was found to be effective for the lymph transport from the aqueous gels. Polyacrylic acid aqueous gel containing 1% glycyrrhizin was better than other suppositories. Solidification of the emulsion type bases are also investigated, and it is suggested that these may be better substitutes of the oily suppository.

Keywords—drug delivery system; 1-(2-tetrahydrofuryl)-5-fluorouracil; lymph; plasma; polyacrylic acid aqueous gel; emulsion type base; *in situ* rectal absorption; gelatin; Tween 80; glycyrrhizin

INTRODUCTION

For the effective cancer chemotherapy, anticancer agents must reach the tumor tissue at a sufficiently high concentration and the concentration must be maintained for a required period. In order to prevent the metastasis along the lymph pathways, the enhancement of lymphatic delivery of anticancer drugs is desired. We have previously reported parenteral emulsions for mitomycin C,¹⁾ bleomycin,²⁾ FT-207,³⁾ and cephalothin.⁴⁾ Hashida *et al.* reported the investigation for drug distribution to lymphatic ducts in the stomach wall and regional lymph nodes.^{5,6)} On the other hand, we have also reported the aqueous gel bases to suppositories for insulin⁷⁾ and ibuprofen.⁸⁾ There are few reports for anticancer drugs up to the present. This paper deals with the formulation of rectal suppositories to increase the lymph and plasma concentrations of an anticancer drug, FT-207. Oil in water emul-

sion and polyacrylic acid aqueous gel as well as a commercially available fat, witepsol, were used as suppository bases. However, instability of emulsion is one of the problems from the viewpoint of administration as suppository. Solidifying emulsion may solve some of the problems in usage and storage. For this reason, we attempted to utilize solidifying emulsion. The effect of addition of some surfactants to the bases was also investigated.

EXPERIMENTAL

1) *Materials* — FT-207 was supplied from Taiho Pharmaceutical Co., Ltd. Witepsol W-35 was obtained from Mitsuba Trading Co. Gelatin, Tween 80, glycyrrhizin and polyacrylic acid (Hiviswako 105) were used without further purification. Other reagents were of reagent grade.

2) *Preparation of Suppositories* — Gel Sup-

positories: The gel was prepared by adding 10% NaOH solution to various concentrations of polyacrylic acid presoaked in distilled water for 15 h. The pH of the gel was adjusted to 5, 7 or 9 and then 100 ml of the gel was mixed with 1 g of FT-207. For gelatin-(or Tween 80-) containing suppositories, 97 ml of the aqueous gel was mixed with 1 g of FT-207 after the addition of 3 ml of 10% gelatin (or 3 ml of Tween 80). For glycyrrhizin containing suppositories, 99.7 ml (or 99.0 ml) of aqueous gel was mixed with 1 g of FT-207 after the addition of 300 mg (or 1 g) of glycyrrhizin.

Witepsol Suppository: Ten g of witepsol w-35 and 100 mg of FT-207 were mixed at 40°C.

O/W Emulsion Suppository: 2.5 ml of distilled water, 2.5 g of witepsol w-35, and gelatin (or Tween 80) at the concentration of 3% were mixed with 50 mg of FT-207 and the mixture was sonicated at 50°C for 1–2 min by using Branson Sonifire B-12 (20 kc).

3) Procedure of Animal Experiments—Male Wistar rats weighting 200–250 g were anesthetized with sodium pentobarbital and the thoracic lymph duct was cannulated according to the method of Bollman *et al.*⁹⁾ A heparin-filled catheter (i.d. 0.5 mm, o.d. 0.8 mm, Dural Plastic and Eng. Pty. Ltd., Australia) was inserted into the thoracic lymph duct and fixed with the aid of a drop of tissue cement, Aron Alpha A (Sankyo Co., Ltd.). One ml of the drug preparation (10 mg of FT-207) was injected into the loop of the rectum. The lymph was collected periodically in the heparinized tube and the volume was calculated from its weight. Two ml of physiologic saline were administered intraperitoneally every hour to obtain a constant flow rate of the lymph. Blood specimen was drawn every hour from the tail vein.

4) Analytical Methods—Two ml of lymph and plasma (each sample consists of samples collected from 4 animals) were extracted with 20 ml of chloroform after the addition of 0.1 ml of 1N HCl. FT-207 was completely transferred to the organic phase. After the centrifugation, the organic phase was evaporated to dryness. The

residue was dissolved in 2 ml of distilled water and the concentration of FT-207 was determined by HPLC (Waters Associates, 6000 A). A stainless steel column of μ Bondapak C 18 was used. The chromatographic conditions were as follows: the mobile phase: methanol-water (20:80, v/v), flow rate: 1.5 ml/min. The detector (Waters Associates, Model 440, 254 nm) was set at a highest sensitivity. FT-207 concentration in lymph and plasma were determined by each calibration curve.

RESULTS

Lymph and Plasma Transport from Emulsion Type Suppositories

Since the nature of the emulsion type suppository is intermediate between aqueous and oil type suppositories, we examined an emulsion type suppository to compare with a witepsol suppositories. The lymph and plasma concentration of FT-207 were measured at given intervals after the administration of emulsion type suppositories containing gelatin or Tween 80. Results are shown in Fig. 1 and 2 with those for witepsol suppositories. It was observed that the lymph concentrations of FT-207 after the administration of emulsion type suppositories were significantly higher than those for witepsol suppositories. Following the administration of witepsol suppositories, FT-207 concentration in lymph reached the maximum value of 35 μ g/ml at 3 h after the administration and it remained at above 20 μ g/ml during the period up to 7 h. For the emulsion type suppositories, however, FT-207 was transported rapidly and the concentration reached maximum at 1 h after the administration and decreased thereafter to the same level as witepsol suppositories. The patterns of concentration curves for plasma were similar to those for lymph. Subsequent to this experiment, lymph flow did not much change at any time by supplying saline into the intraperitoneal (0.5–0.7 ml/h) cavity.

Lymph and Plasma Transport from Polyacrylic Acid Aqueous Gel at Various pH

The lymphatic delivery of FT-207 from

polyacrylic acid aqueous gel at various pH was studied and results were shown in Fig. 3. FT-207 concentrations after the administration of all aqueous gel suppositories were 1.7 times or higher

than that of witexsol suppository, in particular 2.5–3 times at 1 h. The lymphatic transport of the drug did not change with the gel pH. As shown in Fig. 4, the concentration in plasma was

FIG. 1. Lymph Concentration of FT-207 Following Rectal Administration of O/W Emulsion (Gelatin) and O/W Emulsion (Tween 80)

●: witexsol, △: O/W emulsion (gelatin), ▲: O/W emulsion (Tween 80).
Given values are means $\pm$ S.D. ($n=5-7$).

FIG. 2. Plasma Concentration of FT-207 Following Rectal Administration of O/W Emulsion (Gelatin) and O/W Emulsion (Tween 80)

Symbols are the same as in Fig. 1.
Given values are means $\pm$ S.D. ($n=5-7$).

FIG. 3. Lymph Concentration of FT-207 Following Rectal Administration of the Gel Preparations

●: witexsol, ○: 0.5% polyacrylic acid gel (pH 5), △: 0.5% polyacrylic acid gel (pH 7), □: 0.5% polyacrylic acid gel (pH 9).
Given values are means $\pm$ S.D. ($n=5-7$).

FIG. 4. Plasma Concentration of FT-207 Following Rectal Administration of the Gel Preparations

Symbols are the same as Fig. 3.
Given values are means $\pm$ S.D. ($n=5-7$).

about 30 $\mu\text{g/ml}$ in the case of witepsol suppository and 40–50 $\mu\text{g/ml}$ in the case of aqueous gel suppositories. The time courses of the concentra-

FIG. 5. *Effect of Gel Concentration of the Lymph and Plasma Concentrations of FT-207 Following Rectal Administration of the Gel Preparations*
 ○: 0.5% gel, ●: 2% gel, —: lymph, ----: plasma.
 Given values are means $\pm$ S.D. ($n=5-7$).

FIG. 6. *Effect of Tween 80 in 0.5% Gel on the Lymph and Plasma Concentration of FT-207 Following Rectal Administration*
 ●: 0.5% gel + Tween 80, ○: 0.5% gel, —: lymph, ----: plasma.
 Given values are means $\pm$ S.D. ($n=5-7$).

FIG. 7. *Effect of Additives (Gelatin and Tween 80) in 2% Gel on the Lymph and Plasma Concentrations of FT-207 Following Rectal Administration*
 ●: 2% gel + gelatin, ○: 2% gel + Tween 80, —: lymph, ----: plasma.
 Given values are means $\pm$ S.D. ($n=5-7$).

FIG. 8. *Effect of Glycyrrhizin in Aqueous Gel on the Lymph and Plasma Concentrations of FT-207 Rectal Administration Concentration of FT-207 Following Rectal Administration*
 ●, ○: 2% gel, ▲, △: 2% gel + 0.3% glycyrrhizin, ■, □: 2% gel + 1% glycyrrhizin, —: lymph, ----: plasma.
 Given values are means $\pm$ S.D. ($n=5-7$).

tion in plasma are similar in the pattern to those in lymph for every formulation. There is no apparent difference in the concentration of FT-207 among various gel pH.

Effect of Gel Concentration of Lymph and Plasma Transport

Fig. 5 shows the effect of gel concentration of lymph and plasma transport of FT-207 from aqueous gel suppositories. No apparent difference in concentration of the drug between the two formulations was observed between in both lymph and plasma. However, each value of concentration in plasma is lower than that in lymph.

Transport from Aqueous Gel Suppository Containing Tween 80

As shown in Fig. 6, FT-207 concentration in lymph was slightly increased by the addition of Tween 80 to the gel (0.5%), while the concentration in plasma was not altered. However, when gel concentration was increased from 0.5% to 2%, FT-207 concentrations in both lymph and plasma decreased, compared with those for the gel without Tween 80.

Transport from 2% Aqueous Gel Suppositories Containing Tween 80 and Gelatin

As shown in Fig. 7, the transferred amounts of FT-207 from 2% aqueous gel suppositories into both lymph and plasma by the addition of gelatin were greater than those by Tween 80. The mode of the drug transport from gel suppositories into both lymph and plasma seemed to be unchanged by the addition of gelatin to the gels in comparison with 2% aqueous gel (Fig. 5).

Lymph and Plasma Transport from Aqueous Gel Suppositories Containing Glycyrrhizin

Since glycyrrhizin, extracted from a glycyrrhizae radix, has an action as a surfactant,¹⁰⁾ this compound was examined as a natural adjuvant like gelatin. Fig. 8 shows the effect of glycyrrhizin in gel suppositories on lymph and plasma transport of FT-207. When 1% of glycyrrhizin was added to the gel preparation, FT-207 concentration in lymph was the highest, which was increased by 40% in comparison with 2% gel suppository. On the other hand, when 0.3% of glycyrrhizin was added to the gel suppository, FT-207 concentra-

tion in lymph was almost the same as that in the case of 2% gel suppository. Higher concentration (1%) of glycyrrhizin resulted in higher FT-207 concentrations in plasma. The concentration in plasma was remarkably increased compared with that of 2% gel suppository.

DISCUSSION

Witepsol is an oil base commonly used in pharmaceutical industries, because of its many merits in suppository production processes. However, drug absorption from witepsol suppositories is generally slow, since the release of the drug from the base is slow. On the other hand, aqueous and emulsion type suppositories have benefits that a drug in the suppository is easily dissolved, easily released, and easily dispersed into the secreting fluid in the rectal lumen. In spite of many benefits, these suppositories have not been widely used in the chemotherapy. Therefore, in the present study, we have attempted to obtain basic information for aqueous gel or emulsion type suppositories containing gelatin, Tween 80 or glycyrrhizin as surface active agents. In the case of rectal administration of aqueous gel and emulsion type suppositories, the rate of drug release from the suppositories would be rapid and the therapeutic effect in both systemic and local action can be also expected. Although, one must take into consideration the membrane damage due to some surfactants, it is considered that the damage of mucosal membrane by natural surfactants such as gelatin and glycyrrhizin are less than that caused by synthetic ones such as Tween 80.^{11,12)} As shown in Fig. 1 and 4, FT-207 concentrations in lymph following rectal administration of both aqueous gel and emulsion type suppositories are higher than that of witepsol suppository. In other words, it seemed that aqueous gel and emulsion type suppositories were superior to the witepsol suppository. In the present study, it was confirmed with naked eyes that the used emulsion type suppositories were hardened at room temperature and that the suppositories were molten at 37°C in the rectum although it is generally said that emulsion type suppositories are

difficult to form a hard mass. As shown in Fig. 5, it may be considered that the affinity between FT-207 in suppositories and the secreted fluid in the rectal lumen is independent of the increase of the viscosity and the release from suppositories. On the other hand, it may be considered from Fig. 7 that stability and release are increased by addition of gelatin.¹³⁾ Gelatin preparation is excellent in the affinity to the secreting fluid in the rectum and in the release of FT-207 from the suppository. In the case of Tween 80, release of FT-207 from suppositories to the secreting fluid may be inferior. The results shown in Fig. 4, 7 and 8 also suggest that surface active properties of glycyrrhizin are contributed to the suspension and the emulsion of FT-207 in suppositories more than that of Tween 80 and that glycyrrhizin is superior to Tween 80 with regard to the enhancement effect on drug absorption. However, little is known about the mechanism of the enhanced absorption by glycyrrhizin at present. Nevertheless, since glycyrrhizin is a nontoxic material to the organism, it is anticipated that glycyrrhizin as a natural material can be used as a substitute for a synthetic one such as Tween 80. Thus it is concluded that in terms of increase and maintenance of FT-207 concentration in both lymph and plasma after rectal administration of various suppositories, the modified aqueous gel or emulsion type suppositories are superior to the witpsol suppository.

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