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Lymphatic and plasma transport of 1-(2-tetrahydrofuryl)-5-fluorouracil by polyacrylic acid aqueous gel after rectal administration and its antitumor effect on Yoshida sarcoma implanted in rats

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Summary

The inhibition of tumor growth estimated by the size and the weight of the implanted Yoshida sarcoma was investigated in the rat after the administration of suppositories containing 1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207) alone or in combination with adjuvants. The inhibitory effect on the tumor growth was shown to be stronger by rectal administration of FT-207 as a polyacrylic acid aqueous gel suppository containing additive (uracil) than by a Witepsol suppository. FT-207 concentrations in both plasma and the tumor tissue after a single rectal administration on the 10th day after the tumor implantation were higher from the aqueous gel suppository than the Witepsol suppository, and were further increased by the addition of oleic acid into the aqueous gel suppository. While the plasma concentration of FT-207 following repeated administration was almost the same as those after the single administration, the tumor concentration of drug tended to increase with repeated administration, especially in the case of polyacrylic acid aqueous gel suppositories with or without uracil. It can be considered from the result of the present study that the increase in the inhibitory effect on the tumor growth by the coadministration of additive (uracil) with FT-207 are due to the inhibition of 5-fluorouracil degradation by uracil rather than the increased absorption of FT-207 by the adjuvant.

Introduction

With regard to effective cancer chemotherapy, it is important that anticancer agents be selectively delivered and concentrated in tumor tissues and surrounding lymph nodes.

Thomson et al. (1966) used an emulsion type suppository and suggested that anticancer agents can considerably pass into lymphatic systems from the administered site. Ballard (1968) also used polymers as the base and reported that the behavior of drugs administered as such suppositories was similar to that by using an emulsion suppository. We have investigated the lymphatic transport of anticancer agents by using various dosage forms

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(Nakamoto et al., 1975 a and b, 1979, 1981, 1983). The lymphatic transport of 1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207) was increased when the anticancer agent was administered into the loop of the rat small intestine as w/o emulsion and electronmicrograph revealed that oil droplets are mainly transported via lymphatic system (Nakamoto et al., 1979). The enhancement of the lymphatic transport of FT-207 was also demonstrated by using an aqueous gel suppository or an emulsion suppository with or without adjuvants (Nakamoto et al., 1983).

On the other hand, it is reported that uracil inhibited the degradation of 5-fluorouracil in the liver and that the coadministration of uracil with FT-207 increased 5-fluorouracil level in the tumor tissue and the antitumor activity of FT-207 (Fujii et al., 1978, 1979; Kawaguchi et al., 1980; Cohen, 1975; Ikenaka et al., 1979).

In the present study, we prepared polyacrylic acid aqueous gel suppositories containing FT-207 alone or in combination with adjuvants, uracil or oleic acid, and examined the concentrations of FT-207 in lymph and plasma after rectal administration.

Further, Yoshida sarcoma cells were implanted subcutaneously into the scapular region of Donryu rats and FT-207 concentration in the tumor tissue and the inhibitory effect of FT-207 on the tumor growth were evaluated after a single or repeated administration of various FT-207 suppositories to the rectum.

Materials and Methods

Materials

1-(2-Tetrahydrofuryl)-5-fluorouracil (FT-207) was supplied from Taiho Pharmaceutical Co., Japan. Witepsol W-35 (Mitsuba Trading Co., Japan), polyacrylic acid (Hiviswako 105, Wako Pure Chemical Ind., Japan), oleic acid (Kishida Chemical Co., Japan), and uracil (Kyowa Hakko Kogyo Co., Japan) were used without further purification. [5,6-^{3}H]uracil (spec. act. 378 mCi/mg) was purchased from Amersham International plc (England).

Other regents were of reagent grade.

Preparation of suppositories

Witepsol suppositories. Ten grams of Witepsol W-35 and 100, 200 and 400 mg of FT-207 were mixed at 40 °C and the mixture was cooled in the mold.

Aqueous gel suppositories. 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 7.0 and 100 ml of the gel was mixed with 1 g of FT-207. For the suppository formulation containing uracil, 1 g of uracil was added to the above control suppository. For the suppository containing oleic acid, 97 ml of the aqueous gel was mixed with 1 g of FT-207 after the addition of 3 ml of oleic acid.

O/w emulsion suppository. 2.5 ml of 4% polyacrylic acid aqueous gel containing 50 mg (100 mg) of FT-207 and 2.5 g of Witepsol W-35 were mixed and sonicated at 50 °C for 2–3 min by Branson Sonifire (20 kc).

Procedure of animal experiments

Slc-Wistar/ST and Donryu male rats weighing 200 g were housed in a room at 24 °C, 55% relative humidity, 18 changes of air passed HEPA filter per hour, and with a 12 h (06.00–18.00 h) light–dark cycle. Both a standard laboratory diet (NMF, Oriental Yeast Co., Japan) and water were allowed ad libitum before and during the experiment. Male slc-Wistar/ST rats weighing 250–300 g were anesthetized with sodium pentobarbital and the thoracic lymph duct was cannulated according to the method of Bollman et al. (1948). Namely, cannulation of the thoracic lymph duct is accomplished in the incision to the last rib, extending from the midline anteriorly to the medial border of the left quadrarus lumborum muscle posteriorly. The peritoneum and veins are dissected superficially and retracted to the right until the aorta is exposed. The thoracic lymph duct is visible just posterior to the aorta. It is 1–2 mm in diameter. The thoracic lymph duct is exposed for a length 5 mm by gentle blunt dissection. A heparin-filled catheter (i.d. 0.5 mm, o.d. 0.8 mm, Dural Plastic and Eng., Australia) was inserted into the thoracic lymph duct and fixed with the aid of a drop of tissue cement, Aron Alpha A

(Sankyo Co., Japan). 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 specimens were drawn every hour from the tail vein. For the experiments to examine the antitumor effects of FT-207, Yoshida sarcoma were selected. Yoshida sarcoma cannot be adaptive to Wistar rate and not be grown at the scapular region of Wistar rat, because the in vivo culture can only be performed by using Donryu rat. The cells, Yoshida sarcoma (2×10^6), were subcutaneously implanted into the scapular region of Donryu rats weighing 100 g and the tumor growth was observed for 10 days after the tumor implantation. The drug preparation was administered into the rectum on the 10th day (single administration) or every day until sacrifice (repeated administration). The tumor size was measured daily with callipers and the volume of the tumor (cm^3) was calculated by the following equation of Geran et al. (1973): Size $= a \times b^2/2$, where a and b are the largest and the smallest diameters of the tumor in cm. At 3 h after the last administration of suppositories, animals were sacrificed by decapitation and the tumor weight was measured immediately. The tumor tissues were homogenized with physiologic saline and centrifuged at 3000 rpm for 10 min. An aliqout of the supernatant was taken for analysis of FT-207 or [^{3}H]Uracil.

Analytical method

FT-207 in plasma and lymph were determined according to our previous method (Nakamoto et al., 1983). Namely, 2 ml of lymph and plasma (each sample consists of samples collected from 4 animals) were shaken with 20 ml of chloroform after the addition of 0.1 ml of 1 N HCl. After centrifugation, the organic phase was evaporated to dryness in vacuum. The residue was dissolved in 2 ml of distilled water and the concentration of FT-207 was determined by HPLC (Waters Associate Inc., 6000 A). The chromatographic conditions were as follows: Column, a stainless steel column of μBondapak C-18; mobile phase, methanol–water (20 : 80, v/v); flow rate, 1.5 ml/min; sensitivity, 0.02 AUFS at 254 nm. FT-207 concentrations in lymph and plasma were calculated from the calibration curve. [^{3}H]Uracil in blood was determined according to methods of Ikenaka et al. (1979) and Kawaguchi et al. (1980) using a liquid scintillation spectrometer (Tri-Carb 3255, Packard Co., U.S.A.) after combustion with a sample oxidizer (ASC-113, Aloka, Japan) and expressed as dpm.

Statistical Analysis

The values presented in this paper are the mean ± standard error. Statistical significance was calculated using the matched-pair Student's t-test.

Results and Discussion

The inhibitory effect of various FT-207 suppositories on the growth of tumor cells was evaluated by measuring the size and the weight of Yoshida sarcoma implanted subcutaneously into the scapular region of the rat. The results are shown in Figs. 1 and 2. In the control group, the size could be measured on the 4th day after the implantation,

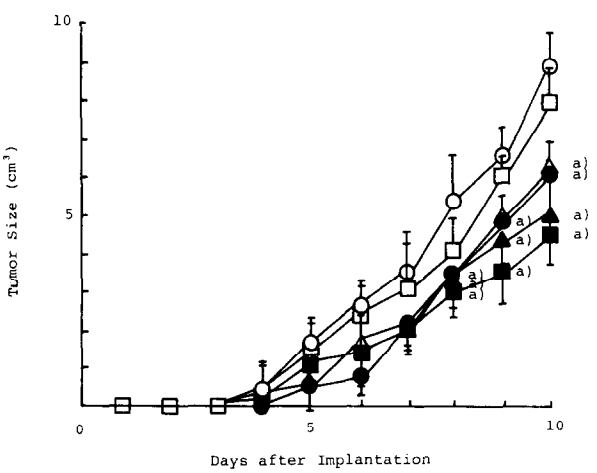

Fig. 1. Effect of various suppositories on the growth of Yoshida sarcoma. ○, control; □, Witepsol (10 mg); ■, Witepsol (40 mg); ●, polyacrylic acid aqueous gel (10 mg); △, polyacrylic acid (10 mg plus oleic acid); ▲, polyacrylic acid (10 mg plus uracil). Suppositories were administered every day just after the implantation. Results are expressed as the ± S.E. of at least 5 animals. a) $P < 0.05$.

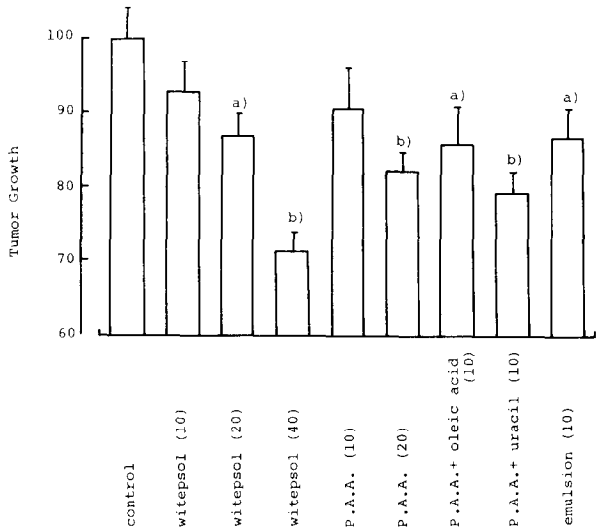

Fig. 2. Effect of FT-207 suppositories administered every day on tumor weight of 10th day implantation of Yoshida sarcoma. Witepsol (10), (20) and (40): Witepsol containing 10, 20 and 40 mg of FT-207, respectively. P.A.A. (10) and (20): polyacrylic acid aqueous gel containing 10 and 20 mg of FT-207, respectively. P.A.A. + oleic acid and P.A.A. + uracil: polyacrylic acid aqueous gel containing 10 mg of FT-207 and 3 ml of oleic acid or 1 g of uracil, respectively. Emulsion (10): Emulsion containing 10 mg of FT-207. Results are expressed as the mean of at least 10 animals. $^{a}P < 0.05$; $^{b}P < 0.01$.

though the formation of tumor cannot be confirmed within 3 days. On the 10th day, the tumor size was 8.9 ± 0.7 cm^3. By the rectal administration of FT-207 at the doses of 10 and 40 mg as Witepsol suppositories, the tumor size reduced to 7.9 ± 0.6 and 4.5 ± 0.7 cm^3, respectively; the inhibitory effects on the tumor growth were 11.2% and 50.0%, respectively (Fig. 1).

On the other hand, in comparison with the group administered a Witepsol suppository containing 10 mg of FT-207, the tumor size became small by the administration of polyacrylic acid aqueous gel suppository containing 10 mg of FT-207 from the 8th day after implantation, suggesting that the latter suppository is superior to the former one in the inhibitory effect on tumor growth. Furthermore, the inhibitory effect was enhanced by the addition of uracil into the aqueous gel suppository and the tumor size was 4.5 ± 0.7 cm^3. The percentage of the inhibition was 19.8.

These findings are similar to the report that oral coadministration of uracil with FT-207 is effective in cancer chemotherapy (Fujii et al., 1978). However, there was no effect of oleic acid added in the aqueous gel suppository (Fig. 2).

Fig. 2 shows the effect of various FT-207 suppositories on the tumor weight on the 10th day after implantation; results are expressed as the percentage of the control. As to Witepsol suppositories, the inhibitory effect on the tumor growth became stronger in proportion to the dose of FT-207. An emulsion suppository containing 10 mg of FT-207 is superior to the Witepsol suppository containing 10 mg of FT-207. As is evident from Figs. 1 and 2, the inhibitory effect of polyacrylic acid aqueous gel suppository containing FT-207 on the tumor growth was enhanced by uracil. This may be due to either the increase in rectal absorption of FT-207 by uracil or the in-

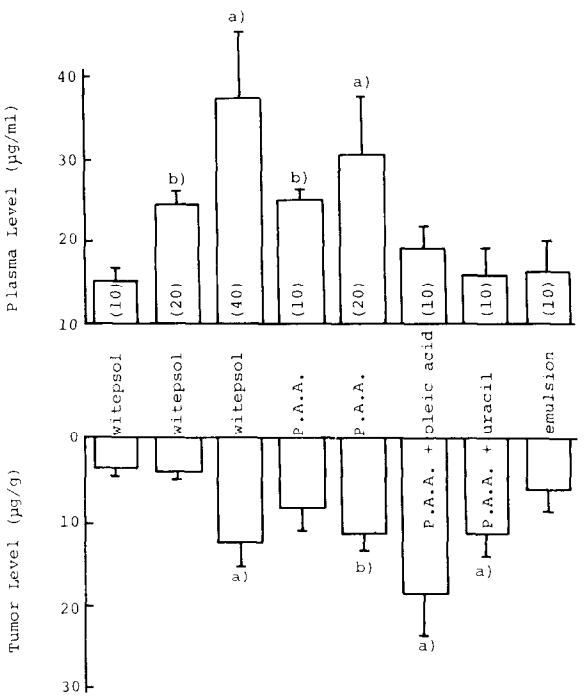

Fig. 3. Effect of various suppositories on the plasma and the tumor tissue levels of FT-207 after rectal single administration. Keys are the same as in Fig. 2. The plasma and the tumor tissue levels were determined 3 h after the administration of a suppository on the 10th day after the implantation. Results are expressed as the mean $\pm$ S.E. of at least 10 animals. $^{a}P < 0.05$; $^{b}P < 0.01$.

hibition of 5-fluorouracil degradation by the absorbed uracil (Kawaguchi et al., 1980).

Fig. 3 shows the plasma and the tumor tissue levels of FT-207 at 3 h following rectal administration of various FT-207 suppositories on the 10th day after the implantation of Yoshida sarcoma (Fig. 3). The plasma concentration of FT-207 was gradually increased with the dose of FT-207. In the suppositories containing 10 mg of FT-207, the plasma level of FT-207 by aqueous gel suppository was 1.7 times higher than by Witepsol suppository and also higher than those by aqueous gel suppositories containing oleic acid or uracil and emulsion-type suppository. As to the concentration of FT-207 in the tumor tissue, aqueous gel suppositories are higher in comparison with Witepsol suppository and with emulsion-type suppositories. FT-207 concentration in the tumor tissue was increased by the addition of oleic acid or uracil into polyacrylic acid aqueous gel suppository. Considering the clinical application, FT-207 suppositories were repeatedly administered once a day for 10 days after the implantation of Yoshida sarcoma and Fig. 4 shows FT-207 concentration in the plasma and the tumor tissue on the 10th day. FT-207 concentrations in both plasma and tumor tissue were increased or tended to increase by the repeated administration in comparison with those after the single administration (Fig. 4). This finding suggested that FT-207 accumulated in the tumor tissue and/or in other organs by the repeated administration. In practice, the accumulation in the tumor tissue was marked in the case of polyacrylic acid aqueous gel suppositories with or without uracil (Fig. 5). Leakage of the administered suppositories from anus stoppered with a pad was not observed at least 4 h after administration. Furthermore, in order to confirm weight of the same volume of administered suppositories, when various suppositories were weighed at 37 °C: Witepsol suppository, polyacrylic acid aqueous gel suppository and emulsion suppository were 0.97 ± 0.03 g, 1.02 ± 0.01 g and 0.98 ± 0.02 g, respectively. After the experiment, we dissected the rectum. The polyacrylic acid aqueous gel suppository was mixed with secretary fluid and seemed to be adsorbed to the membrane, as seen by the naked eye. Then light microscopic preparations,

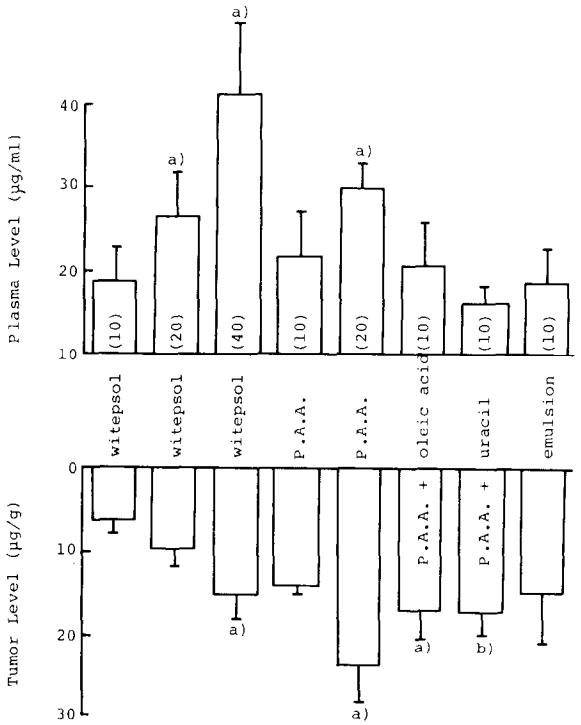

Fig. 4. Effect of various suppositories on the plasma and the tumor tissue level of FT-207 after rectal repeated administration. Keys are the same as in Fig. 2. The plasma and the tumor tissue levels were determined 3 h after the last administration of a suppository on the 10th day after the implantation. Results are expressed as the mean ± S.E. of at least 10 animals. $^{a}P < 0.05$; $^{b}P < 0.01$.

using double staining with both hematoxyline and eosin, of rectum strips were made according to conventional methods. Under light microscopic observation there was no prominent difference in rectum mucosa after administration of various suppositories.

Fig. 5 shows a relationship between the two parameters, namely FT-207 concentration in the plasma and that in the tumor tissue. The broken line in the figure represents the ratio of FT-207 concentration in the plasma to that in the tumor tissue (2 : 1). Moreover, it is quite clear from the figure that FT-207 concentration in the tumor tissue was increased by single or repeated administrations of polyacrylic acid aqueous gel suppositories containing FT-207 with uracil or oleic acid, suggesting that the addition of uracil or oleic acid

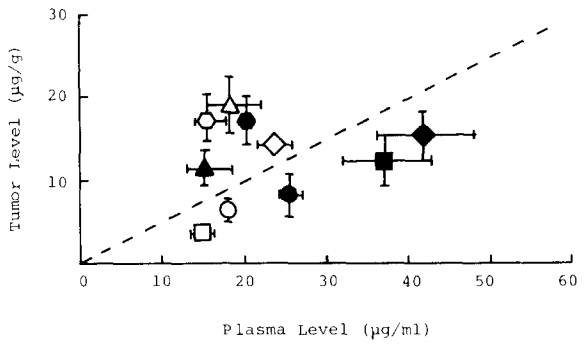

Fig. 5. Relationship between the plasma level and the tumor tissue level. □, Witepsol (10 mg, single); ■, Witepsol (40 mg, single); ○, Witepsol (10 mg, repeated), ◆, Witepsol (40 mg, repeated); ●, P.A.A. aqueous gel (10 mg, single); ◇, P.A.A. aqueous gel (10 mg, repeated); △, P.A.A. aqueous gel (plus oleic acid, single): ○, P.A.A. aqueous gel (plus oleic acid, repeated); ▲, P.A.A. aqueous gel (plus uracil, single); ○, P.A.A. aqueous gel plus uracil, repeated). Results are expressed as the mean ± S.E. of at least 5 animals.

into polyacrylic acid aqueous gel suppositories is effective for increasing FT-207 concentration in the tumor tissue. In the group of single or repeated administrations of Witepsol suppository containing 40 mg of FT-207, the concentrations of the antitumor drug in the tumor tissue were low, while those in the plasma were high in proportion to the doses. The addition of uracil increased FT-207 concentration in the tumor tissue and enhanced the inhibitory effect on the tumor growth. In order to investigate the effect of uracil on the rectal absorption of FT-207, the plasma and the lymph concentrations were monitored following the administration of FT-207 suppositories with or without uracil.

Figs. 6 and 7 show FT-207 concentrations in the plasma and in the lymph, respectively. There was no significant difference in the plasma concentration between polyacrylic acid aqueous gel suppositories containing FT-207 alone and with uracil. But there was a tendency to maintain the high plasma concentration in the case of the coadministration (Fig. 6). As for the lymphatic concentration of FT-207, it was rather lower after the coadministration, but not significantly different. These results indicate that uracil has no effect on the absorption of FT-207 (Fig. 7).

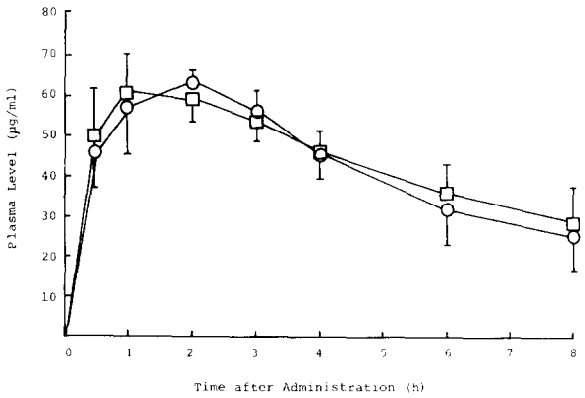

Fig. 6. The plasma levels of FT-207 after rectal administration of the suppositories with or without uracil. ○, polyacrylic acid aqueous gel (FT-207 alone); □, polyacrylic acid aqueous gel (FT-207 plus uracil). Results are expressed as the mean ± S.E. of at least 5 experiments.

Fig. 8 shows the radioactivities in the plasma following the rectal administration of Witepsol suppository or polyacrylic acid aqueous gel suppository containing [^{3}H]uracil. In the case of Witepsol suppositories, the plasma levels of [^{3}H]uracil were low even at 6 h after the administration. On the other hand, [^{3}H]uracil was absorbed more quickly from polyacrylic acid aqueous gel suppository. Kawaguchi et al. (1980) previously reported that the coadministration of uracil with FT-207 orally affected for antitumor effect. Although the route of administration may be dif-

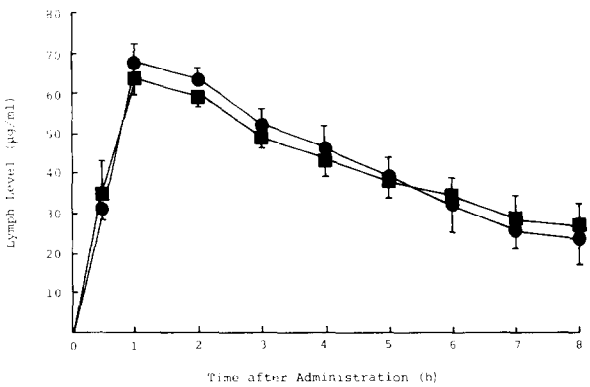

Fig. 7. The lymph levels of FT-207 after rectal administration of the suppositories with or without uracil. ●, Polyacrylic acid aqueous gel (FT-207 alone); ■, polyacrylic acid aqueous gel (FT-207 plus uracil). Results are expressed as the mean ± S.E. of at least 5 experiments.

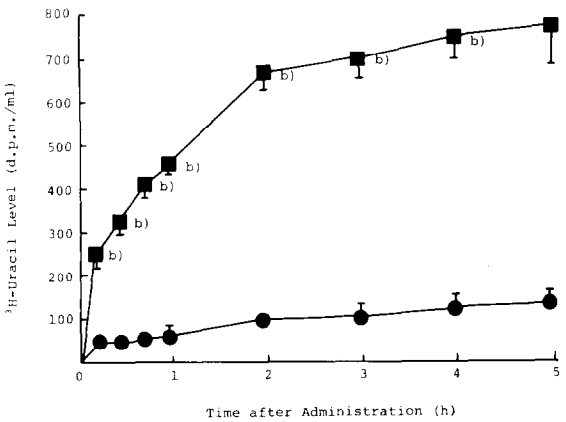

Fig. 8. The plasma levels of [^{3}H]uracil after rectal administration of the suppositories. ●, Witepsol; ■, polyacrylic acid aqueous gel. Results are expressed as the mean ±S.E. of at least 10 animals. ^{b} $P < 0.01$.

ferent between findings reported by Kawaguchi et al. (1980) and ours, from our present results, it is also suggested that absorbed uracil plays an important role in the enhancement of the antitumor effect of FT-207 (Fig. 8).

In conclusion, the tumor growth was inhibited by the administration of FT-207 suppositories, and the inhibitory effect was marked in the polyacrylic acid aqueous gel suppository containing uracil. The enhancement effect of uracil may be due to the inhibition of the metabolic degradation of 5-fluorouracil, an active metabolite of FT-207, after being absorbed.

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