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Selective transfer of 1-hexylcarbamoyl-5-fluorouracil into lymphatics by combination of β-cyclodextrin polymer complexation and absorption promoter in the rat

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Summary

The β-cyclodextrin polymer (polyβCD) was used as a candidate for a macromolecular carrier of a bifunctional delivery system, and the applicability of this new biodegradable polymer was studied using 1-hexylcarbamoyl-5-fluorouracil (HCFU) by administering into the lumen of the large intestine, and into the abdominal cavity. Bifunctional delivery system (mixed micelles + HCFU-polyβCD complex) increased the selective transfer of HCFU into the lymphatics after the large intestinal administration. On the other hand, after the intraperitoneal administration, we found that the administration of HCFU–polyβCD complex without mixed micelles increased the selective transfer of HCFU into the lymphatics. The selective transfer into the lymphatics was found by administration of drug into the abdomen rather than into the large intestine. These results suggest that such a new bifunctional delivery system will be a powerful tool in the field of antitumor chemotherapy.

Introduction

In chemotherapy, it is important to increase the selectivity of a drug to a target site in order to use the drug more effectively and to decrease its toxicity in the other

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(non-target) site. Particularly in cancer chemotherapy, a more effective therapeutic method having lower toxicity that has the selectivity to tumor cells or has the selective transport route into tumor cells has been strongly desired. A review article (Gregoriadis, 1977) has exemplified various drug carriers with effective selectivity to target area in the body such as macromolecules, cells and biodegradable systems. As one of the drug targeting methods, the selective transfer of antitumor drug into lymphatics has been studied by many investigators. For example, when microspheres in oil-type emulsion as a drug carrier was parenterally used, the accumulation of antitumor drugs in the lymphatics was increased (Hashida et al., 1977). However, it was difficult to achieve the lymphotropic selectivity from the enteral route because there are at least three requirements to overcome for driving a drug from the enteral route toward the lymphatic system, which then provides an important route where tumor cells occur. First, drug carriers themselves should penetrate the intestinal barrier such as epithelial cells; it is known that highly water-soluble or large molecular substances cannot be absorbed through them. Second, the size and structure of the lymphotropic carriers must be chosen by considering the anatomical barrier of lymphatic capillary. Third, the highly specific binding of the antitumor drug to the carriers is required so as to achieve the lymphatic delivery. Based on these considerations, we developed a novel device called a bifunctional delivery system which is a combination of macromolecular complex as a lymphotropic carrier, and a lipid-surfactant mixed micelles as an absorption promoter, and have succeeded in the selective transfer of non-absorbable bleomycin (BLM) into the lymphatics from the large intestine (Yoshikawa et al., 1981). In this study, the antitumor drug we used was 1-hexylcarbamoyl-5-fluorouracil (HCFU) (Hoshi et al., 1976), which is the hydrophobic prodrug of 5-fluorouracil (5-FU) and does not have lymphotropic selectivity by itself. Then we gave attention to cyclodextrin which is already known to form non-covalent inclusion complexes with many substances, particularly hydrophobic substances (French, 1957), and is expected to be applied into the field of pharmaceutical sciences (Uekama, 1981a) as a molecular capsule, and further we made the bifunctional delivery system, utilizing the inclusion complex which consisted of HCFU and β-cyclodextrin polymer (polyβCD) together with mixed micelles, and studied the applicability of this drug delivery system using rats.

Materials and Methods

Materials

HCFU and 5-FU were supplied by Mitsuiseiyaku and Kyowa Hakko Kogyo, respectively. The polyβCD was prepared according to the method of Hoffman (1973) and the average molecular weight was estimated to be about 10,000 by gel permeation chromatography using a Shodex B-803 (Tokyo, Japan). The polyoxyethylene lauryl ether (BL9-EX) was supplied by Nikko Chemicals, and linoleic acid was of high purity grade (99.0%, Nippon Oil and Fats). All other chemicals were of reagent grade commercially obtained.

Preparation of inclusion complex

The kneading method (Saenger, 1980) was used for the preparation of HCFU/polyβCD complex. It was as follows: HCFU was added to a slurry of the polyβCD with a small amount of diluted phosphoric acid solution at pH 3 in a mortar. After they were mixed and kneaded for about 20 min, the product was then dried in a vacuum for 24 h at room temperature.

Solubility studies

Solubility measurements were carried out according to Higuchi and Lach (1954). Excess amount of HCFU was added to phosphate buffer of pH 3 containing various concentrations of polyβCD, and were shaken at 25 ± 0.5°C. After equilibrium was attained, an aliquot was centrifuged and pipetted through a cotton filter. The filtrate was then diluted with pH 3 phosphate buffer and analyzed spectrophotometrically at 261 nm.

Preparation of test solutions

Solutions of mixed micelles were prepared by dissolving linoleic acid (2%) and BL-9EX (4%) in distilled water followed by sonication with Ohtake sonicator model 5202 (50 W, 1 min). When we administered HCFU into the large intestine, we used four dosage forms, and dose was equivalent to 20 mg of HCFU/2 ml/kg of rat. They were as follows: first, HCFU was suspended in acetic acid–sodium acetate buffer (pH 4) with 0.5% sodium carboxymethylcellulose (C.M.C.-Na). Second, HCFU was suspended in acetic acid–sodium acetate buffer (pH 4) and then the equivalent of mixed micelles added. Third, HCFU–polyβCD complex was dissolved in acetic acid–sodium acetate buffer (pH 4). The last, HCFU–polyβCD complex was dissolved in acetic acid–sodium acetate buffer (pH 4) and then the equivalent of mixed micelles added. Next, two dosage forms were used by intraperitoneal administration of HCFU, and each dose was equivalent to 10 mg of HCFU/ml/kg of rat. First, HCFU was suspended in acetic acid–sodium acetate buffer (pH 4) with 0.5% C.M.C.-Na, and second, HCFU–polyβCD complex was dissolved in acetic acid–sodium acetate buffer (pH 4).

Absorption experiment

Male Wistar rats weighing 350–370 g were anesthesized intraperitoneally with sodium pentobarbital (32 mg/kg of body weight). In the case of the administration into the large intestine, the intestine was exposed through the midline incision, and a closed loop of the entire large intestine (colon and rectum) was prepared by ligation at the proximal and distal ends. The intestinal contents was cleared, a gentle stream of air was applied to aid in the removal of residual fluids. A polyethylene catheter (i.d. 0.5 mm, o.d. 0.8 mm, Dural Plastics, Australia) was placed into the carotid artery and blood samples were collected periodically. Plasma was separated by a micro-high speed centrifuge, model MR-15A (Tomy Seiko Tokyo, Japan) at 12,000 $\times g$ for 5 min. The procedure employed for the collection of lymph was modified from Bollman's method (Bollman et al., 1948). The thoracic lymph duct was cannulated with a heparin-filled flexible vinyl catheter (i.d. 0.8 mm, o.d. 1.2 mm,

Dural Plastics, Australia) and fixed with a drop of tissue cement (Aron Alpha A, Sankyo). This cannula allowed a continuous drainage of lymph throughout the experiments. In the case of the intraperitoneal administration, a polyethylene catheter and a heparin-filled flexible vinyl catheter were cannulated into the carotid artery and into the thoracic duct lymph, respectively, by the same method described above. After operation, the incised portion was connected with tissue cement. Blood and thoracic duct lymph samples were treated by the same way as described in the large intestinal administration.

Analytical method of HCFU

A 15-ml centrifuge tube containing 50 μl of rat lymph or plasma, 1 ml of 0.1 N hydrochloric acid, and 8 ml of ethyl acetate was placed on a reciprocating shaker for 15 min. The aqueous and organic phases were separated by centrifugation (3000 rpm, 10 min). Then, 7 ml of the organic phase was removed with a Pasteur pipette and placed in a 15-ml centrifuge tube. The organic phase was evaporated at 35°C under vacuum. The residue was dissolved in 200 μl of the mobile phase (methanol–acetic acid–distilled water, 720 : 1 : 280), and 50 μl was injected into a chromatograph. Analysis was performed using a Shimadzu Model LC-3A pump (Kyoto, Japan), and Model SPD-2A ultraviolet (UV) absorbance detector. The column was packed with 5 μm ODS-silicagel (YMC GEL ODS, Yamamura Chemical, Kyoto, Japan). The mobile phase was prepared fresh daily. The flow rate was 1.0 ml/min and pressure was approximately 120 kg/cm^2. Detection was achieved by UV absorption measurement at 261 nm. The detector signal was processed and recorded using a Shimadzu Model C-R1A reporting integrator.

Analytical method of 5-FU

To a 15-ml centrifuge tube containing 50 μl of lymph or plasma, 100 μl of phosphate buffer (pH 7.4) containing 5-bromouracil (0.8 μg/ml) as an internal standard, 1 ml of 0.1 N hydrochloric acid, and 8 ml of ethylacetate was added. After shaking for 15 min, the aqueous and organic phases were separated by centrifugation (3000 rpm, 10 min). Then, 7 ml of the organic phase was removed with a Pasteur pipette and placed in a 15-ml centrifuge tube. The organic phase was evaporated at 35°C under vacuum. The residue was dissolved in 200 μl of the mobile phase (acetonitrile–acetic acid–distilled water, 20 : 1 : 980), and 50 μl was injected into a chromatograph. Analysis was performed using a Shimadzu Model LC-3A pump (Kyoto, Japan) and Model SPD-2A UV absorbance detector. The column was packed with 5 μm ODS-silicagel (Zorbax-ODS), and the mobile phase was prepared fresh daily. The flow rate was 1.0 ml/min and pressure was approximately 40 kg/cm^2. Detection was achieved by UV absorption measurement at 270 nm. The detector signal was processed and recorded using a Shimadzu Model C-R1A reporting integrator.

Results

Absorption of HCFU into blood and lymph from the lumen of the large intestine

When HCFU was administered in C.M.C.-Na suspension as a control experiment, both lymph and plasma HCFU concentrations showed low values as seen in Fig. 1a, and lymph HCFU concentration was not higher than plasma HCFU concentration except at 1.5 h after administration. We calculated the ratios of lymph HCFU concentration relative to plasma HCFU concentration (L/P ratios of HCFU), and then almost unit values (about 1.0) were obtained. With respect to 5-FU concentration (Fig. 1b), both lymph and plasma concentrations gradually increased after administration. However, lymph and plasma concentrations were not higher than 10 μM, and lymph 5-FU concentration also was not higher than plasma 5-FU concentration except at 1.5 h after administration. The ratios of lymph 5-FU concentration relative to plasma 5-FU concentration (L/P ratios of 5-FU) showed low values (about 1.0) at all sampling times. From these results, we may mention that selective transfer of HCFU and 5-FU into lymphatics was not obtained by administering HCFU as a suspension into the large intestine.

Fig. 2 shows the results when HCFU was administered with mixed micelles into the rat large intestine. Plasma HCFU concentration considerably increased at 0.5 h after administration, but lymph HCFU concentration did not go up at this time. After 1.5 h, both plasma and lymph HCFU concentrations showed the same patterns as that in control. L/P ratio of HCFU at 0.5 h was 0.5, and were about 1.0 at the other times. With respect to 5-FU (Fig. 2 b), lymph concentration was almost equal to or lower than plasma concentration, and L/P ratios of 5-FU were also about 1.0. Then, we may mention that the selective transfer of HCFU and 5-FU into

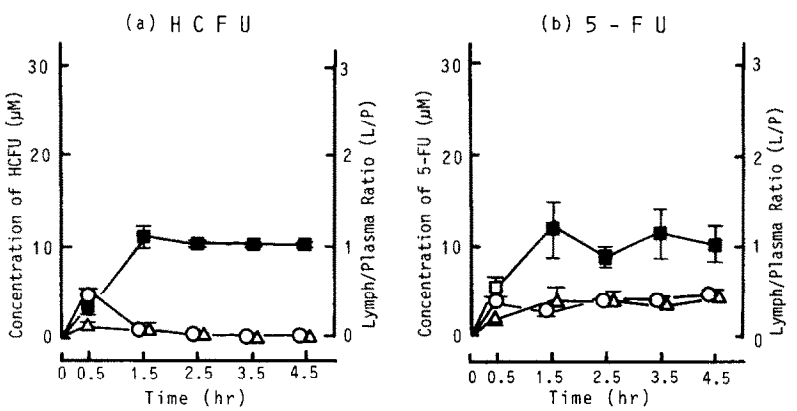

Fig. 1. Concentration of HCFU or 5-FU in the plasma and the lymph of the thoracic duct, and ratio of HCFU or 5-FU concentration in the lymph relative to the plasma (L/P) after administration HCFU as suspension into the large intestine. (a) HCFU; (b) 5-FU. ○, plasma concentration; △, lymph concentration; ■, ratio of concentration in the lymph relative to the plasma. Each value represents the mean ± S.E. for 6 experiments.

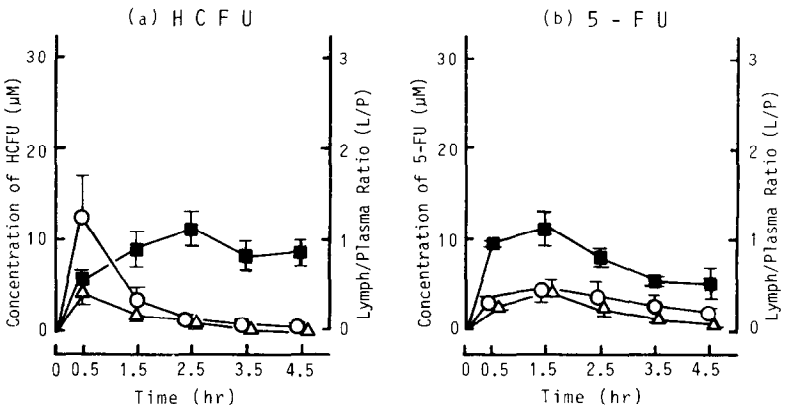

Fig. 2. Concentration of HCFU or 5-FU in the plasma and the lymph of the thoracic duct, and ratio of HCFU or 5-FU concentration in the lymph relative to the plasma (L/P) after administration HCFU with mixed micelles into the large intestine. (a) HCFU; (b) 5-FU. ○, plasma concentration; △, lymph concentration; ■, ratio of concentration in the lymph relative to the plasma. Each value represents the mean ± S.E. for 4 experiments.

the lymphatics was not achieved by administering HCFU with mixed micelles.

HCFU polyβCD was administered to rats and the results are shown in Fig. 3. As shown in Fig. 3a, both lymph and plasma HCFU concentrations showed considerably low values, and L/P ratios of HCFU were still low values (about 1.0). With respect to 5-FU (Fig. 3b), plasma concentration showed a somewhat higher value than that of control experiment (Fig. 1b). In addition, L/P ratios of 5-FU were not higher than 1.0. These results suggest that lymphotropic selectivity of HCFU and

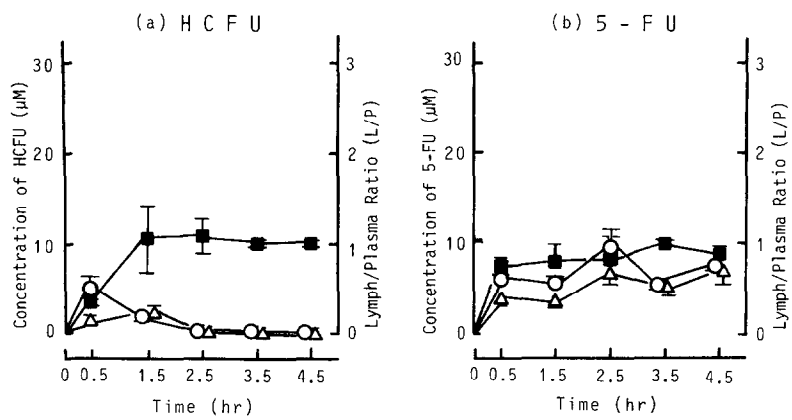

Fig. 3. Concentration of HCFU or 5-FU in the plasma and the lymph of the thoracic duct, and ratio of HCFU or 5-FU concentration in the lymph relative to the plasma (L/P) after administration HCFU as a complex with polyβCD into the large intestine. (a) HCFU; (b) 5-FU. ○, plasma concentration; △, lymph concentration; ■, ratio of concentration in the lymph relative to the plasma. Each value represents the mean ± S.E. for 4 experiments.

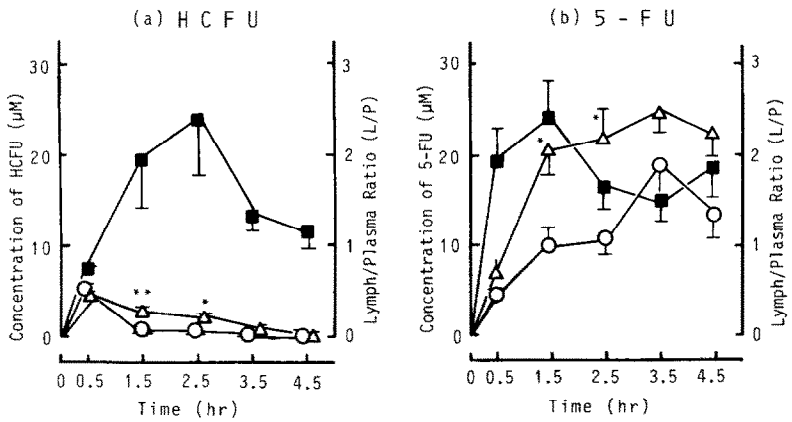

Fig. 4. Concentration of HCFU or 5-FU in the plasma and the lymph of the thoracic duct, and ratio of HCFU or 5-FU concentration in the lymph relative to the plasma (L/P) after administration HCFU–polyβCD complex with mixed micelles into the large intestine. (a) HCFU; (b) 5-FU. ○, plasma concentration; △, lymph concentration; ■, ratio of concentration in the lymph relative to plasma. Each value represents the mean ± S.E. for 6 experiments. Statistical comparison of lymph concentration versus plasma concentration was done by a two-tailed Student's *t*-test: * $P < 0.05$, ** $P < 0.01$.

5-FU was not obtained by administering HCFU as a complex with polyβCD.

To examine the lymphotropic selectivity of HCFU from the enteral route, HCFU–polyβCD complex was administered with mixed micelles into the large intestine, and both plasma and lymph concentrations were measured. As shown in Fig. 4, lymph concentrations of both HCFU and 5-FU were higher than those obtained in the previous experiments (Figs. 1, 2 and 3), and plasma concentration also showed a somewhat higher concentration, but there is no significant difference as compared to lymph concentration. As shown in Fig. 4a, lymph HCFU concentrations were higher than plasma concentrations except at 0.5 h after administration, and L/P ratios of HCFU after 1.5 h were higher than 1.0 (1.2–2.5). Regarding 5-FU (Fig. 4b), lymph 5-FU concentrations were higher than plasma concentrations at all sampling times, which were 2 or 3 times higher than the values obained in previous experiments (Figs. 1b, 2b and 3b). From the above striking results, we may mention that the combination of HCFU–polyβCD complex and mixed micelles could enable the selective transfer of HCFU into the lymphatics from the large intestine.

Absorption of HCFU into blood and lymph after intraperitoneal administration

As a control experiment, HCFU was administered in C.M.C.-Na suspension, and the results are shown in Fig. 5. Fig. 5a demonstrates that only a small amount of HCFU was detected in both lymph and plasma at each time. When L/P ratios of HCFU were calculated, the maximum value of the L/P ratio of HCFU was 1.1 and others were lower than 1.0. On the other hand, both lymph and plasma 5-FU concentrations were lower than 10 μM and lymph concentration was not higher than plasma concentration at all sampling times for the same administration of HCFU suspension into the large intestine, and then L/P ratios of 5-FU were lower than 1.0

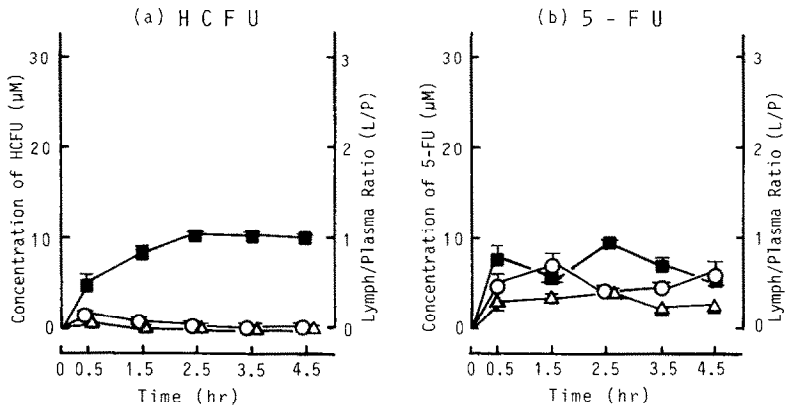

Fig. 5. Concentration of HCFU or 5-FU in the plasma and the lymph of the thoracic duct, and ratio of HCFU or 5-FU concentration in the lymph relative to the plasma (L/P) after intraperitoneal administration of HCFU as suspension. (a) HCFU; (b) 5-FU. ○, plasma concentration; △, lymph concentration; ■, ratio of concentration in the lymph relative to the plasma. Each value represents the mean ± S.E. for 4 experiments.

at all sampling times. Considering those results, it may be mentioned that the selective transfer of HCFU and 5-FU into the lymphatics was not obtained by the intraperitoneal administration of HCFU as suspension. When we administered HCFU–polyβCD complex into abdominal cavity, the lymph HCFU concentration was higher than plasma HCFU concentration, and L/P ratios of HCFU were higher than 1.0 (1.9–2.8) at all sampling times as seen in Fig. 6a. Moreover, lymph 5-FU

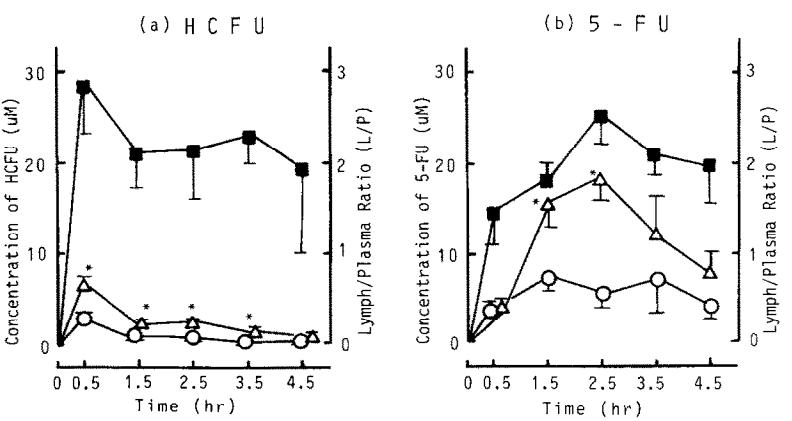

Fig. 6. Concentration of HCFU or 5-FU in the plasma and the lymph of the thoracic duct, and ratio of HCFU or 5-FU concentration in the lymph relative to the plasma (L/P) after intraperitoneal administration of HCFU–polyβCD complex. (a) HCFU; (b) 5-FU. ○, plasma concentration; △, lymph concentration; ■, ratio of concentration in the lymph relative to the plasma. Each value represents the mean ± S.E. for 4 experiments. Statistical comparison of lymph concentration versus plasma concentration was done by a two-tailed Student's *t*-test: * $P < 0.05$.

concentration was about 2- or 3-fold higher than plasma concentration at 1.5 h after administration, and L/P ratios of 5-FU were higher than 1.0 (1.5–2.6) at all sampling times. Furthermore, L/P ratios of HCFU and 5-FU were almost 2–5-fold higher than that of the control experiment (Fig. 5). These results show that the intraperitoneal administration of HCFU–polyβCD complex permitted the selective transfer of HCFU into lymphatics.

Discussion

Blood is known to be the main transport route for absorbed substances from the intestine, because the first-flowing portal venous drainage of the intestine is estimated to be 500 times greater than the flow of the intestinal lymph (Bollman et al., 1948). However, the selective transfer of drugs into the lymphatics is important to avoid the first-pass effect of the liver and to prevent or treat the lymphatic metastasis of cancer (Haagensen et al., 1972). According to the histological differences between the blood vessel and the lymph vessel, the macromolecular substance is considered to transfer into the lymph vessel more selectively than into the blood vessel (Leak, 1970; Garlick and Renkin, 1970). Therefore, we developed a bifunctional delivery system for selective lymphatic transfer of non-absorbable BLM from the enteral route (Yoshikawa et al., 1981). This system in a combination of lymphotropic macromolecular complex (BLM–dextran sulfate), and absorption promoter (lipid–surfactant mixed micelles). BLM–dextran sulfate complex is an ionic complex and the average molecular weight of dextran sulfate is about 500,000. However, BLM–dextran sulfate complex is an ionic complex and such ionic binding cannot be used for uncharged lipid soluble drugs. In order to selectively increase the lymphatic transfer of HCFU (prodrug of 5-FU), we chose, in this study, the β-cyclodextrin polymer (average molecular weight, 10,000) as a candidate of new lymphatic carrier in which CD cavity can include the lipid-soluble drugs.

In the administration to the large intestine, we found that HCFU did not have the lymphotropic selectivity by itself (Fig. 1), and that absorption of HCFU from the large intestine increased to some extent by mixed micelles; however, mixed micelles did not affect the lymphotropic selectivity of HCFU (Fig. 2). After administration of HCFU–polyβCD complex into the large intestine, HCFU in lymph and plasma were detected in spite of the administration without absorption promoter (mixed micelles) because the inclusion complex reached dissociation equilibrium in aqueous solution, and HCFU dissociated from the inclusion complex might have been absorbed from the lumen of the large intestine. In addition, administration of HCFU–polyβCD complex showed the higher plasma concentration of 5-FU compared to the plasma level by HCFU suspension administration (Figs. 1b and 3b). The reason for this result is due to a high dissolution rate of HCFU in aqueous solution by complexation with polyβCD (Uekama et al., 1981b), which was shown in the phase solubility diagram of HCFU–polyβCD (Fig. 7) pointing out Ap type (Higuchi et al., 1965). However, the selective transfer of HCFU into lymphatics was not obtained by the administration of HCFU–polyβCD complex without mixed

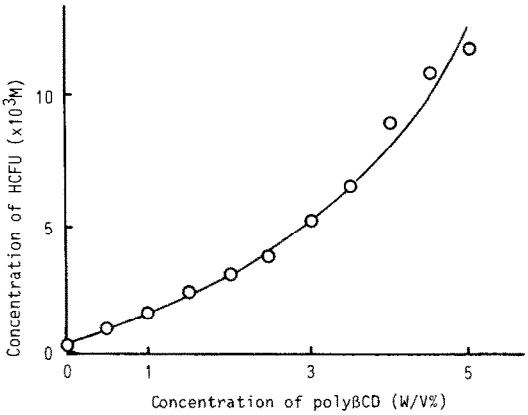

Fig. 7. Phase solubility diagram of HCFU–polyβCD system in phosphate buffer (pH 3) at 25°C.

micelles into the large intestine. We succeeded in the selective transfer of HCFU into the lymphatics from the lumen of the large intestine by the new bifunctional delivery system composed of macromolecular HCFU–polyβCD inclusion complex and mixed micelles (Fig. 4). The detailed mechanism of that bifunctional delivery system is now under study, but considering the mechanism for selective transfer of BLM into the lymphatics by the bifunctional delivery system (Yoshikawa et al., 1983), we may suppose that the macromolecular inclusion complex is absorbed from the lumen of the large intestine by mixed micelles as an absorption promoter, and then the absorbed complex transferred into the lymph vessel selectively due to its large molecular size. In the abdominal cavity, there is no mucosal barrier separating the lumen from the large intestine, so that the absorption promoter is useless to transport of drugs into the blood or the lymph vessels, and the good absorption of HCFU and the lymphotropic selectivity of HCFU was obtained by the intraperitoneal administration of HCFU–polyβCD alone. The lymphotropic selectivity obtained by the intraperitoneal administration was better than that by the large intestinal administration of the inclusion complex with mixed micelles. The reason for this result may be attributed to the fact that the inclusion complex might move more freely to lymphatic system in abdominal cavity than in other sites (Hisaoka et al., 1982), or that permselectivity of the blood–lymph barrier in the abdomen is different from that in the large intestine. In this study, we obtained the high concentration of 5-FU in the lymph in the large intestinal administration of inclusion complex with mixed micelles and the intraperitoneal administration of inclusion complex, and then we can expect the antitumor effect. When we considered the relationship between the lymphotropic property of substances and the molecular weight of that substance (Yoshikawa et al., 1984), we may get more selective lymphatic transfer of HCFU using the larger molecular weight of polyβCD.

Finally, we expect that this new bifunctional delivery system can be used for effective prevention or treatment of tumor metastasis along lymphatic pathway from rectum, colon or abdominal cancer.

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