Molecular Weight-Dependent Lymphatic Transfer of Exogenous Macromolecules from Large Intestine of Renal Insufficiency Rats

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Received October 16, 1991; accepted March 5, 1992

To study the lymphatic delivery of exogenous macromolecules via the enteral route in renal insufficiency, we determined the transfer selectivity to the systemic blood and lymph of fluorescein isothiocyanate-labeled dextrans of different average molecular weight (10, 18, 39, and 69kD). The compounds were administered into the large intestinal lumen of rats with occluded renal circulation, with the aid of lipid–surfactant mixed micelles as an absorption prometer. Whereas concentrations of the smaller dextrans (molecular weight under 18 kD) in the lymph of the thoracic duct and in the peripheral plasma were similar, levels of dextrans over 39 kD were significantly higher in the lymph than in the plasma. Further, dextran plasma concentrations decreased in inverse proportion to increasing molecular weight. The molecular weight threshold for a high lymph-to-blood level ratio (18–39 kD) was higher than that previously found in rats with normal renal function (10–18 kD). This difference was accounted for by reduced renal clearance of the low molecular weight dextrans in renal failure. These results are useful in the design of lymphotropic drug delivery in disease states.

KEY WORDS: lymphatic transfer; macromolecules; rat large intestine; renal insufficiency, blood–lymph barrier.

INTRODUCTION

The permeability of the enteral blood–lymph barrier for various substances has been investigated using the capillary perfusion method, in the small intestine (1–3) and the colon (4). However, owing to the poor absorption, the transfer selectivity from the gastrointestinal tract to the blood and the lymph of exogenous macromolecules was previously not well studied. We have found that the luminal mucosa of the gastrointestinal tract, especially of the colorectum, can be made permeable to these molecules with the aid of lipid–surfactant mixed micelles, as a harmless adjuvant (5–7).

Using these mixed micelles we investigated the molecular weight-dependent lymph/plasma level ratio of exogenous macromolecular dextrans absorbed from the small (8) and the large (9) intestinal lumen in intact rats. We established the existence of a molecular weight threshold of dextran transfer to the blood and lymph, above which significantly higher dextran lymph levels than plasma levels are observed. The lymphatic delivery of drugs in disease states is also of interest. In this paper, using the renal circulation-occluded rat, we evaluated the effect of the molecular weight of dextrans on blood–lymph transfer selectivity, with the aid of mixed micelles from the large intestinal lymph in the state of renal insufficiency.

MATERIALS AND METHODS

Materials. Fluorescein isothiocyanate-labeled dextrans (FDs) were purchased from Sigma Chemicals Co. (St. Louis, MO). Their approximately average molecular weights were 10, 18, 39, and 69kD (abbreviated FD10, FD20, FD40, and FD70, respectively). Polyoxyethylated (60 mol) hydrogenated castor oil (HCO60) was supplied by Nikko Chemicals, Co., Ltd. (Tokyo). The linoleic acid used was of high-purity grade (over 99.0%; Nippon Oil & Fats Co., Ltd., Tokyo). All other chemicals were of analytical grade and obtained commercially. The solution of lipid–surfactant mixed micelles was prepared by dispersing linoleic acid (0.56%, w/v) and HCO60 (0.4%, w/v) in distilled water followed by sonication at 37°C with a 20-kHz sonicator (Model 5202, Ohtake Works, Co., Ltd., Tokyo) at 100 W for 4 min. The test solution of FD for administration was prepared by dissolving each FD (3 mg/ml) in the mixed micellar solution with vigorous shaking, respectively.

Absorption Experiments. Male Wistar rats (Japan S.L.C., Co., Ltd., Shizuoka, Japan) were given a commercial pellet diet and water ad libitum. The rats (200–250 g) were anesthetized intraperitoneally with sodium pentobarbital, and following a midline laparotomy, a closed loop of the entire large intestine (colon and rectum) was prepared by ligation with silicone tubes into the proximal and distal ends of the large intestine. The intestinal contents were removed by slow infusion of a 37°C saline solution into the loop. Once the intestinal content was cleared, a gentle stream of air was applied to aid in the removal of residual fluids. Immediately before administration into the large intestine, the renal circulation was entirely occluded by ligating both arteries and veins of kidneys. Two milliliters of test solution containing each FD (dose of FD, 6 mg/rat) was introduced in the loop of the large intestine. Body temperature was maintained at 37°C by the use of a overhead lamp. A modification of the method of Bollman *et al.* (10) was used for the collection of lymph from the thoracic duct. Only the rats producing lymph continuously during the 5-hr experiment were used for the data. A polyethylene cathether tube (i.d., 0.5 mm; o.d., 0.8 mm) was placed in the carotid artery and a blood sample (50 μl) was collected periodically at the midpoint of lymph collection. Plasma was separated by a centrifuge (Model MA-15, Tomy Seiko, Co., Ltd., Tokyo) at 12,000*g* for 2 min. Plasma and lymph samples were immediately immersed in an ice bath and kept from light after collection.

Gel Filtration. At 5 hr after the administration of test solution, the remaining test solution in the lumen of the large intestine was removed. The test solution from the lumen and the lymph sample during 4–5 hr were filtered through a cel-

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0724-8741/92/0900-1195$06.50/0

lulose membrane (pore size, 0.45 μm; Nihon Millipore Kogyo Co., Ltd., Yonezawa, Japan). These filtered samples were fractionated by gel filtration on a 2.3 × 70-cm glass column of Sephacryl S-200 (Pharmacia Fine Chemicals, Uppsala, Sweden). Fractions (3 ml each) were collected automatically and FD was determined.

Analytical. FD levels in the lymph, plasma, and fraction samples were measured by fluorescence spectrophotometry (8) with a fluorescence spectrophotometer (Model 650-10s, Hitachi, Ltd., Tokyo) at 536 nm using an excitation wavelength of 486 nm.

Statistics. All data are presented as mean ± SE; data comparison was performed using Student's t test and P values under 0.05 are regarded as statistically significant.

RESULTS AND DISCUSSION

Whereas administration without mixed micelles showed no dextran in lymph and plasma, coadministration with mixed micelles allowed absorption of all FDs into both body fluids (Figs. 1 and 2). Figure 1 presents the lymph concentration of FDs 5 hr after administration of linoleic acid–HCO60 mixed micelles into the lumen of the large intestine. The levels of FD10, FD20, FD40, and FD70 in the lymph reached a maximum of 27.6, 25.2, 12.9, and 16.8 μg/ml, respectively, at 2.5 hr and thereafter decreased slowly. A gap in the lymph levels of FDs was detected between those under FD20 and those over FD40.

The concentrations of FDs in the plasma are shown in Fig. 2. Time courses of plasma FDs levels were distinct from those of lymph levels as shown in Fig. 1; plasma levels of FDs rose and reached maximum levels more rapidly compared to lymph concentrations. The peak plasma levels of FDs were 32.2 μg/ml (FD10), 24.3 μg/ml (FD20), 5.1 μg/ml (FD40), and 2.3 μg/ml (FD70), respectively, at 1.5 hr. The effect of the molecular weights of FDs on their plasma concentrations was detected at any sampling time; plasma levels of FDs decreased with increases in molecular weight. Lymph levels of FD40 and FD70 were significantly higher

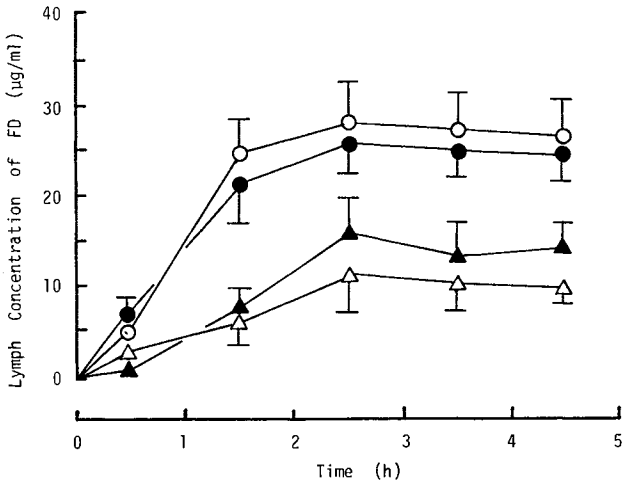

Fig. 1. Lymph concentrations of FDs: FD10 (○), FD20 (●), FD40 (△), and FD70 (▲). Each point represents the mean ± SE of four experiments. SE is indicated unless smaller than the point as plotted.

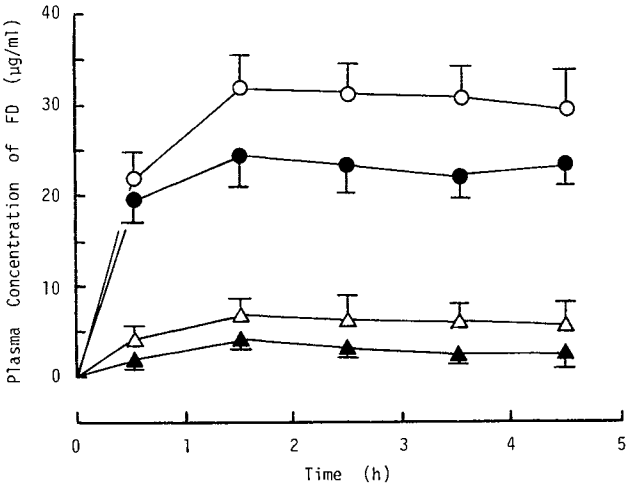

Fig. 2. Plasma concentrations of FDs: FD10 (○), FD20 (●), FD40 (△), and FD70 (▲). Each point represents the mean ± SE of four experiments. SE is indicated unless smaller than the point as plotted.

than plasma levels of each FD at every sampling time except 0.5 hr.

The lymph-to-plasma level ratios (C_L/C_P) of FDs calculated from the data of individual rats in Figs. 1 and 2 rose steadily with increasing molecular weight of FDs. The ranges of C_L/C_P during 5 hr were 0.3–0.9 (FD10), 0.6–1.3 (FD20), 1.3–4.1 (FD40), and 1.7–7.8 (FD70) (not shown).

The molecular weight distribution of FD10 and FD70 was analyzed by gel filtration (Fig. 3). Although in the lumen of the large intestine (at 5 hr after administration), both FD10

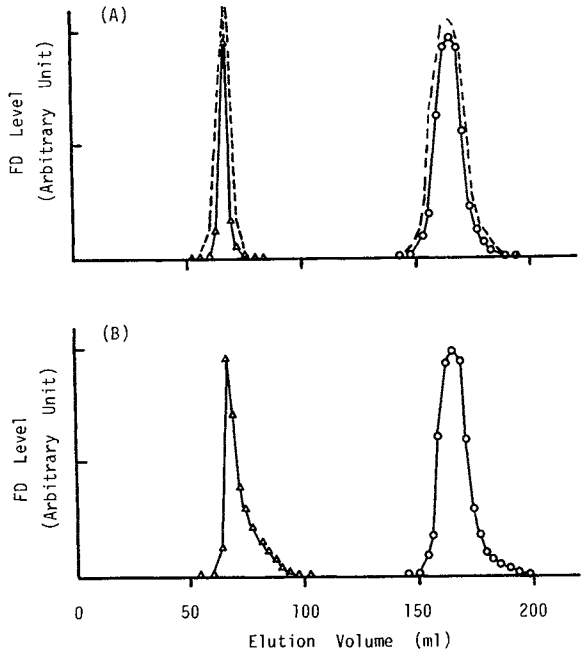

Fig. 3. Gel filtration chromatogram (by Sephacryl S-200) of FD10 (○) and FD70 (△). (A) Residual solution in the lumen of the rat large intestine at 5 hr after administration. Standard of each FD (----). (B) Lymph sample 4–5 hr after administration. Details of the procedure are given under Materials and Methods.

and FD70 showed that they were unaltered dextrans, in the lymph (4–5 hr) a slight degradation was observed by the tailing of the elution curves toward a low molecular weight, especially for FD70.

The same phenomenon was observed in plasma samples at 4.5 hr (not shown). These results suggest that FDs were absorbed intact from the large intestinal lumen by mixed micelles and underwent slight degradation in the tissue or in the body fluids.

Lipid–surfactant mixed micelles drug dosage forms serve as gastrointestinal absorption enhancers (5–7). Using these mixed micelles, we found that the lymph levels of FDs were significantly higher than their plasma levels at average molecular weights over 39 kD when absorbed via the small intestinal lumen (8), and over 18 kD via the large intestinal lumen (9), of intact rats.

In rats with renal insufficiency, the molecular weight of FDs affected their lymph levels (Fig. 1); however, plasma levels of FDs were more distinctly inversely dependent on their molecular weight (Fig. 2). Therefore, the rise in C_L/C_P of FDs with increasing molecular weight results mainly from the difficulty of the larger FDs (FD40 and FD70) to transfer into the blood circulation. The lymph levels of FDs absorbed from the large intestinal lumen of rats with renal insufficiency were significantly higher than the plasma levels (except at 0.5 hr) at an average FD molecular weight over 39 kD (Figs. 1 and 2). The molecular weight threshold of transfer selectivity to the blood and the lymph in the rat with renal failure is therefore considered to be between 18k and 39k, greater than that observed in the normal rat [between 10k and 18k (9)].

Figure 4 depicts the average C_L/C_P of each FD level during 1.5–4.5 hr calculated from the ratio of individual rats. The reduction in C_L/C_P on occluding the renal circulation was larger for FD10 and FD20 than for FD40 and FD70. Further, plasma levels of all FDs in the present study were higher than those in renal-intact rats (9), although their lymph levels were similar. The increase in plasma FD levels during 1.5–4.5 hr after administration was 58–72% (FD10), 54–68% (FD20), 27–42% (FD40), and 20–37% (FD70). These findings suggest that the effect of reduced renal clearance is greater for the smaller FDs.

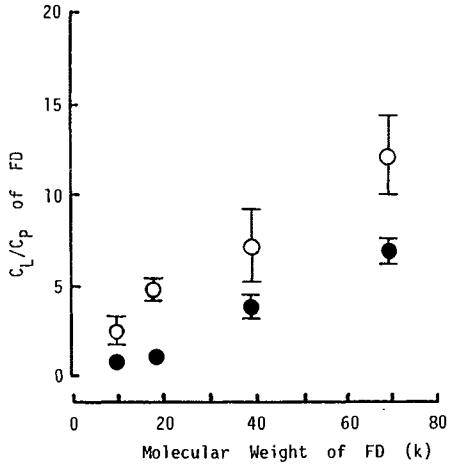

Fig. 4. Lymph-to-plasma level ratio (C_L/C_P) of FDs of each molecular weight. Each point indicates the average $C_L/C_P \pm$ SE of four values 1.5–4.5 hr after administration in the large intestinal lumen of the renal circulation-occluded rat (●), from the data on individual rats in Figs. 1 and 2, and of the intact rat (○), from the data in our previous work (9). SE is indicated unless smaller than the point as plotted.

Renal failure might also affect lymph and plasma FD levels or their ratios. Therefore, we examined the effect of occluding the renal circulation on the lymph flow rate and intestinal membrane permeability. The average lymph flow rates of the thoracic duct lymph (ml/hr · 100 g body weight) during the 5-hr absorption experiment were as follows: FD10 (0.16 ± 0.04, 0.14 ± 0.04), FD20 (0.13 ± 0.04, 0.12 ± 0.05), FD40 (0.10 ± 0.04, 0.13 ± 0.03), and FD70 (0.14 ± 0.06, 0.15 ± 0.05). The data in parentheses are, first, with occlusion and, next, from intact rats (sham operated). There was no statistical difference in lymph flow rate in any pair of the same FD.

Absorption of FDs with the same mixed micelles via the rat large intestine (decreased percentage from the lumen) was as follows: FD10 (24.6 ± 5.8, 27.8 ± 6.4), FD20 (21.5 ± 4.1, 25.2 ± 3.2), FD40 (14.1 ± 2.9, 17.7 ± 3.3), and FD70 (10.2 ± 2.8, 13.5 ± 3.8). Although the intact rats tended to show higher absorption, the difference was not significant. Therefore, blocking the renal blood flow did not significantly affect lymph flow and large intestinal mucosal permeability of FDs in the presence of lipid–surfactant mixed micelles.

Although blood flow rate was not measured, its fluctuation was considered to be small, because lymph flow rate (11) and intestinal permeability (12), both of which are greatly dependent on blood flow rate, were not significantly biased by occluding the renal circulation.

We have reported enhanced lymphotropic delivery of poorly absorbable bleomycins, anticancer drugs, via the rat enteral route using noncovalent linkage to macromolecules and mixed micelles as an absorption promoter (13–15). The present results will be useful in the development and clinical applications of the lymphotropic drug delivery system.

ACKNOWLEDGMENT

This work was supported in part by a Grant from the Ministry of Education, Science and Culture, Japan.

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