Transfer of Exogenous Macromolecules from Rat Stomach Wall to Blood and Lymph is Dependent on Molecular Weight

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Transfer selectivity to the blood and the lymph of exogenous dextrans of various average molecular weight (approximate 4, 10, 18, 39 and 70 kilodaltons (kDa)) after dosing to the subserosal layer of the rat stomach wall was investigated by measuring the fluorescein isothiocyanate labelled dextran concentrations in the lymph of the thoracic duct and the peripheral plasma. The lymph/plasma level ratio of the dextrans rose greatly with the increase in molecular weight (over 10 kDa) owing largely to the lower plasma concentration, although the lymph levels of small dextran (4 kDa) were not significantly higher than the plasma levels. These results indicate that there is an inverse relation between plasma levels of dextrans and their molecular weight, and also suggest that the molecular weight threshold of transfer selectivity of dextran to the blood and the lymph administered in the rat stomach wall is between 4—10 kDa.

Keywords transfer selectivity; dextran; macromolecule; blood capillary; lymph vessel; rat stomach; molecular weight dependency

The blood-lymph barrier concerned with the transfer of substances from blood to lymph has been well studied, and various end- and exogenous macromolecules are known to be able to traverse the blood circulation and enter the tissue space. It also seems important, however, to know the function of this barrier into the blood and lymph from the interstitial spaces, when drugs are administered to these sites. There have been reports on the lymphatic transfer of macromolecules by intramuscular[1,2] subcutaneous injection[3] and on intestinal capillary permeability,[4-6] but no systemic study on the transfer of exogenous macromolecules from the wall of the gastrointestinal tract. The lymphatic transfer of drugs from the stomach wall is considered interesting and important in parenteral cancer chemotherapy, especially in treating and even preventing the lymphatic metastases of gastric tumor cells.

In this paper, we report the transfer of fluorescein isothiocyanate labelled macromolecular dextrans of various molecular sizes into the blood and lymph circulation from the interstitial space of rat stomach wall, and describe the effect of molecular weight on their transfer selectivity to the blood and lymph.

Experimental

Materials Fluorescein isothiocyanate-labelled dextrans (FDs) were purchased from Sigma Chemicals Co., MO., U.S.A. Their mean molecular weights were 4, 10, 18, 39 and 70 kilodaltons (kDa) (abbreviated: FD4, −10, −20, −40 and −70). All other chemicals were of analytical grade and commercially obtained.

Animals Male Wistar rats (300—350 g, Shizuoka Laboratory Animal Center, Shizuoka, Japan) were fed on a commercial diet (CE2 Clea Japan Co., Ltd., Tokyo, Japan). Water was allowed *ad libitum* and the animals were not fasted before use.

Procedure Animals were anesthesized intraperitoneally with sodium pentobarbital. After opening the abdominal cavity by a flank incision on the left, saline solution of each FD (dose, 1 mg/100 μl/kg) was injected into the subserosal layer in the area of the fundus gland of the stomach wall by microliter syringe. The thoracic duct was cannulated with a vinyl catheter tube (i.d., 0.6 mm, o.d., 0.9 mm) according to the modified method of Bollman *et al.*[7] The lymph fluid was collected continuously throughout the experiment for 5 h after the injection. Blood samples (100 μl) were collected *via* a polyethylene (i.d., 0.5 mm, o.d., 0.8 mm) tube cannulated in the carotid artery at the midpoint of the period of lymph collection, and the plasma was separated by centrifugation (at 15000 *g* for 2 min).

Gel filtration was done as follows: each plasma and lymph sample at final sampling time was filtered through a cellulose membrane (pore size: 0.45 μm, Nihon Millipore Kogyo Co., Ltd., Yonezawa, Japan). The filtered sample was fractionated by gel filtration on 2.3 × 70 cm glass column of Sephacryl S-200 (Pharmacia Fine Chemicals, Uppsala, Sweden). Fractions (3 ml each) were automatically collected and FD was determined.

FD levels in the lymph and the plasma were measured by fluorescence spectrophotometric method as previously reported[8] at 536 nm using an excitation wavelength of 486 nm with a Hitachi fluorescence spectrophotometer (650-10S, Tokyo, Japan).

All data are indicated as the mean ±S.D.; data comparison is done using Student's *t*-test and *p* values under 0.05 are regarded as statistically significant.

Results and Discussion

Figure 1 presents the lymph concentrations of FDs in the thoracic duct during the 5 h after injection in the rat stomach tissue. Levels of FD4, −10 and −20 in the lymph reached

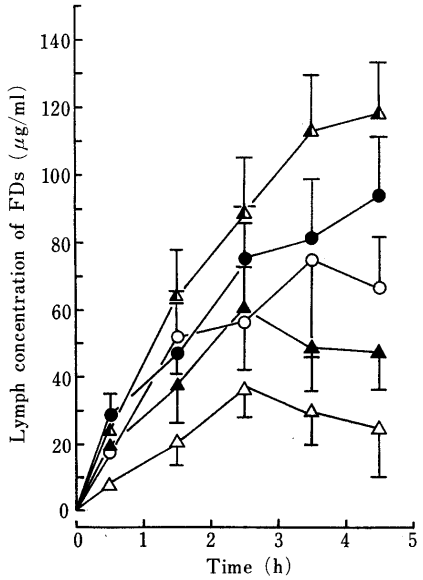

Fig. 1. Lymph Concentrations of FDs after Administration in the Rat Stomach Wall

△, FD4; ▲, FD10; ○, FD20; ●, FD40; ◭, FD70. Each point represents the mean ±S.E. of 6 experiments.

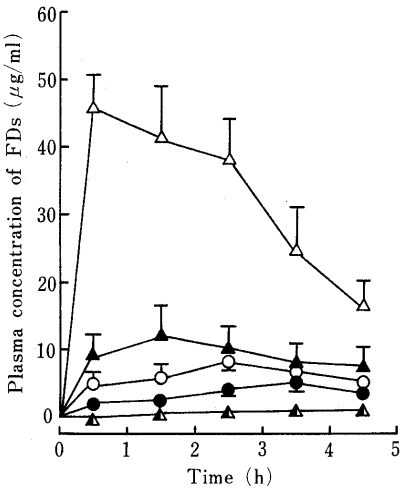

Fig. 2. Plasma Concentrations of FDs after Administration in the Rat Stomach Wall

△, FD4; ▲, FD10; ○, FD20; ●, FD40; ◭, FD70. Each point represents the mean±S.E. of 6 experiments. S.E. is indicated unless smaller than the point as plotted.

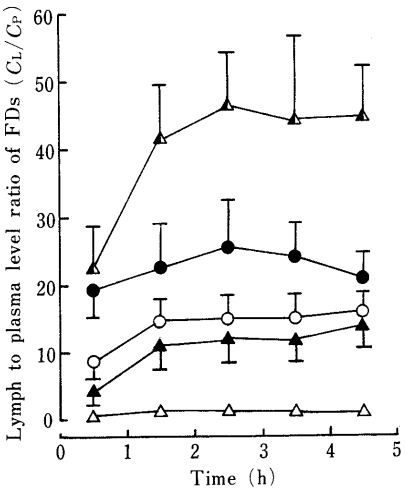

Fig. 3. Lymph to Plasma Level Ratio (C_L/C_P) of FDs after Administration in the Rat Stomach Wall

△, FD4; ▲, FD10; ○, FD20; ●, FD40; ◭, FD70. Each point represents the mean±S.E. of 6 experiments. S.E. is indicated unless smaller than the point as plotted.

a maximum of 37 μg/ml at 2.5 h, 62 μg/ml at 2.5 h and 75 μg/ml at 3.5 h, respectively, the peak time thus tending to be later as molecular weight increased. No obvious peaks were observed for FD40 and 70 during the 5 h, although the lymph levels increased without interuption up to the final sampling time. The lymph levels of FD40 and 70 at 4.5 h were 97 and 118 μg/ml, respectively. The lymph flow rate (ml/h mean±S.D.) in each FD experiment was 0.25±0.13 (FD4), 0.28±0.10 (FD10), 0.26±0.12 (FD20), 0.23±0.09 (FD40) and 0.22±0.13 (FD70), and significant difference of the lymph flow rate between any two of them was not detected. The concentrations of FDs in the plasma are shown in Fig. 2. Time course patterns of plasma FDs levels were almost the same to those of lymph levels as shown in Fig. 1. The peak plasma levels of FDs were 46 μg/ml at 0.5 h (FD4), 12 μg/ml at 1.5 h (FD10), 9 μg/ml at 2.5 h, (FD20) and 5 μg/ml at 3.5 h (FD40). No peak

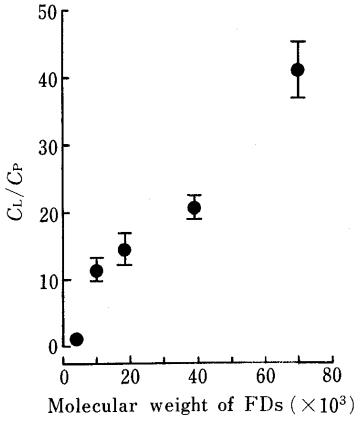

Fig. 4. Effect of Molecular Weight of FDs on C_L/C_P Ratios

Each point is calculated from the data of C_L/C_P ratios in Fig. 3 and represents the mean±S.E. of data at 5 sampling times. S.E. is indicated unless smaller than the point as plotted.

plasma concentration of FD70 was detected and the effect of molecular weight of FDs on plasma levels was more obvious at any sampling time than that on lymph levels; plasma levels clearly decreased in inverse proportion to the increase of their molecular weight.

The molecular weight distribution of FDs was tested by gel filtration chromatography (not shown). Although in the lymph fluid both FD4 and FD70 demonstrated that they were almost unaltered dextrans, in the plasma samples a very small extent of tailing of the elution curves towards low molecular weight was observed, especially for larger FDs. This suggests that FDs were absorbed almost intact and transferred from the rat stomach wall into the circulating lymph and blood without or with only little degradation.

Figure 3 shows the lymph to plasma level ratio (C_L/C_P) of FDs after administration to the rat stomach. These ratios rose steadily with the increase of FD molecular weight. The lymph level of each larger FD (FD10—70) was significantly ($p<0.05$ by Student's t-test) higher than the plasma level of the same FD at any sampling time. The range of C_L/C_P ratios during 5 h was 0.8—1.6 (FD4), 4.8—13.6 (FD10), 8.6—15.5 (FD20), 19.0—26.3 (FD40) and 22.8—47.1 (FD70).

Figure 4 depicts the average C_L/C_P ratios of FDs levels during the 5 h period: 1.4 (FD4), 10.6 (FD10), 13.8 (FD20), 21.4 (FD40) and 42.8 (FD70). Significant difference of mean C_L/C_P ratios by Student's t-test was observed between FD4 and FD10 ($p<0.05$), FD20 and FD40 ($p<0.05$) and FD40 and FD70 ($p<0.01$). While the molecular weight of FDs affected their lymph levels (Fig. 1), plasma levels were more clearly reversely dependent on their molecular weight (Fig. 2). Therefore, the great rise in average C_L/C_P ratios of FDs with increase of molecular weight (Fig. 4) could be largely responsible for the difficulty in FD transfer into the blood capillaries from the interstitial space of the stomach wall.

We have been interested in the promotion of absorption of exogenous macromolecules from the lumen of the gastrointestinal tract[9-11] with the aid of lipid-surfactant mixed micelles, as absorption potentiators, and their subsequent lymphatic transfer.[8,12-16] We earlier reported that lymph levels of FDs in the thoracic duct absorbed by mixed micelles from the small[8] and the large intestine[14]

of rats were significantly higher than their plasma levels at average molecular weight above 39 and 18 kDa, respectively. Using the same method as used in this study, we also found that administration of FDs by injection to the rat small and large intestinal subserosal layers gained almost the same results (unpublished data). Namely, the molecular weight threshold of transfer selectivity of these macromolecules to blood and lymph in the rat small intestine is approximately between 18—39 kDa and 10—18 kDa for the rat large intestine. These findings and the results in this study (the molecular weight threshold of FD for the stomach: 4—10 kDa) may suggest that the transfer selectivity to blood and lymph of exogenous macromolecules is specific to the site of the gastrointestinal tract wall. Regarding this point, other investigators suggested a regional difference of blood capillary permeability among sites of the gastrointestinal tract.[17]

Although blood circulation is, of course, the main means of moving almost all drugs, the lymphatics also play an important role in the transport of certain chemicals, especially macromolecules.[18] Quantitatively, in fact, the calculated cumulative amount of FD70 (% of dose) in this study reached approximately 19%. In qualitative importance, this lymphatic route is valuable because of the necessity of delivering anticancer drugs to treat or even prevent the lymphatic metastases of malignant tumor. This route also avoids metabolism by first-pass in the liver for drugs administered in the gastrointestinal tract.

We believe that the results of this work offer valuable information for development and clinical application of a lymphotropic drug delivery system from the stomach.

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