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EFFECT OF ADJUVANTS ON THE RECTAL ABSORPTION AND LYMPHATIC UPTAKE OF PEPLEOMYCIN IN RATS

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The rectal absorption of pepleomycin sulfate (PEPS) in rats was increased significantly by the coadministration with each of diclofenac (DC), sodium 5-methoxysalicylate (5-MSA) and phenylalanine enamine of ethylacetoacetate (Enamine). 5-MSA increased the lymphatic uptake of PEPS after rectal administration while DC and Enamine did not. The mechanism behind the enhancing action of 5-MSA on the lymphatic uptake of PEPS may be due to the suppressing action of 5-MSA on the vascular permeability to PEPS. DC increased the vascular permeability to PEPS but Enamine did not affect it. Findings obtained in this study may indicate that adjuvant used acts independently at the rectal mucosal membrane and at the vascular membrane for membrane for membrane permeability to PEPS.

Keywords—adjuvant; diclofenac; sodium 5-methoxysalicylate; enamine; rectal absorption; lymphatic uptake; bradykinin

INTRODUCTION

Pepleomycine, a potent antineoplastic drugs, is widely used as intravenous and intramuscular injection since it is weakly absorbed from gastrointestinal tract because of its relative large molecular weight and low lipophilicity.

Nonsurfactant adjuvants such as salicylate,[1-3] enamine derivatives[4,5] and others,[6,7] markedly enhance the rectal absorption of drugs which are weakly absorbed from the gastrointestinal tract.

Furthermore, it has recently been reported[8] that 5-methoxysalicylate significantly enhanced the rectal absorption and lymphatic uptake of water soluble compounds such as sodium cefoxitin and phenol red. Such results suggest that adjuvants will play important roles for the rectal absorption and lymphatic uptake of anti-cancer drugs such as pepleomycin.

In the present study, the adjuvant effect of sodium 5-methoxysalicylate, enamine and diclofenac on the rectal absorption and lymphatic uptake of pepleomycine were investigated. The results suggest that lymphatic uptake of pepleomycin depend on a coadministered adjuvant in spite of each adjuvant used enhancing the rectal pepleomycin absorption.

MATERIALS AND METHODS

Materials—Pepleomycin sulfate was supplied from Nippon Kayaku Co., Ltd. (Tokyo, Japan), diclofenac from Nihon Bulk Yakuhin Co., Ltd. (Tokyo, Japan) and sodium 5-methoxysalicylate from Interx Research Corporation (Kansas, USA). Sodium salt of phenylalanine enamine of ethylacetoacetate was synthesized according to the method described in a previous paper.[9] Bradykinin and ϵ-amino caproic acid were purchased from Sigma Chemical Co., Ltd. (Missouri, USA). Other reagents used were of analytical grade.

Animals—Wistar male rats, 225 to 250 g, were fasted for 16 h prior to the experiments but water was given freely.

Drug Solution Administered—Aqueous solutions containing pepleomycin sulfate were prepared with distilled water with adjuvant when

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needed for the rectal administration. For the intravenous injection, pepleomycin was dissolved in a saline solution. For the study of rectal connective tissue injection, drug solution was prepared with distilled water when additive was added, and control drug solution without additive was prepared with saline solution.

Administration Method—Three administration routes were studied; intravenous injection, rectal administration and intrarectal-tissue injection to the rectal connective tissue. Rectal administration was carried out with polyethylene tubing (PE 50) inserted into the rectum at 1 cm depth from the anus at a dosage volume of 500 μl/kg in microenema. Intrarectal-tissue injection was carried out by injecting into the connective tissue with a microsyringe at a dosage volume of 100 μl/kg. Intravenous administration was carried out by the injection into jugular vein at a dosage volume of 500 μl/kg.

Measurement of Concentrations of Pepleomycine in Plasma and Lymph Fluid—During the experiments, rats were anesthetized with sodium pentobarbital (40 mg/kg, *i.p.*) and kept on the hot plate surface at 38°C. After the administration of drugs, blood was sampled at intervals from jugular vein. The blood sample was centrifuged at 3000 rpm for 10 min. To measure the concentration of pepleomycin in lymph fluid, the thoracic duct was canulated with a polyethylene tubing (PE 50) after the abdominal incision according to the method described previously.[8] Lymph fluid was sampled at 30 min intervals after administration of drugs. Concentrations of pepleomycin in plasma and lymph fluid were measured with the microbiological agar-cup method using bacillus subtillis ATCC 6633 as a test organism according to the method reported previously.[10]

RESULTS AND DISCUSSION

For 3 h after the rectal administration of pepleomycin sulfate (PEPS, 10 mg/kg, alone), the concentration of PEPS in plasma was less than 0.1 μg/ml. Diclofenac (DC, at a dose of 20 mg/kg), sodium 5-methoxysalicylate (5-MSA, at a dose of 40 mg/kg) and sodium salts of phenylalanine enamine of ethylacetoacetate (enamine, at a dose of 40 mg/kg) markedly enhanced the rectal absorption of PEPS in rats (Fig. 1). The concentration of PEPS in plasma significantly increased and reached to maximum at about 20—30 min after administration and then subsided. The rank order of potencies of these adjuvants to the rectal absorption of PEPS is DC > 5-MSA > enamine. This order depended on their potencies to the bioavailabilities of PEPS (Table I). The results suggest DC, 5-MSA and enamine greatly potentiated the rectal absorption of relatively large molecular drugs such as PEPS.

It has recently been reported that 5-MSA also stimulates the transport of low lipophylic com-

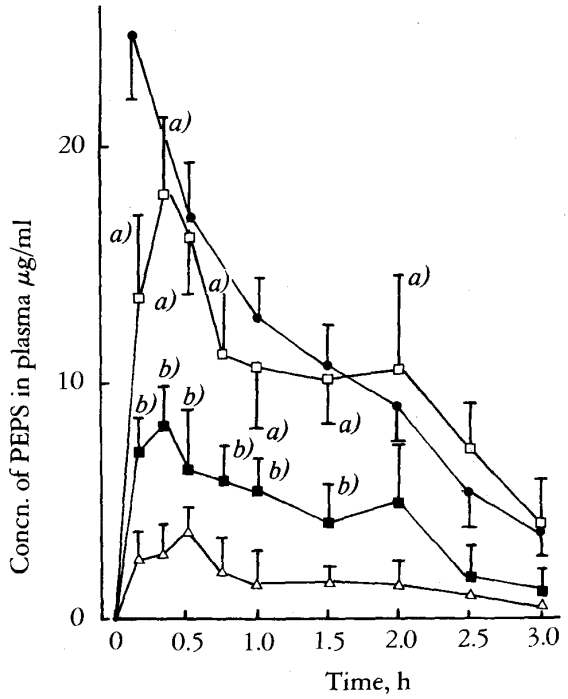

FIG. 1. *Plasma Concentration of Pepleomycin Following i.v. Administration (●) at a Dose of 10 mg/kg in Saline or Rectal Administration at a Dose of 10 mg/kg in Microenema with Each Following Adjuvant; Diclofenac (at a Dose of 20 mg/kg, □) Sodium 5-Methoxysalicylate (40 mg/kg, ■) and Enamine (40 mg/kg, △)*

Each value represents mean± S.D. ($n \geq 4$). For a) $p < 0.001$ against ■ and △, b) $p < 0.001$ against △.

pounds such as cefoxitin and phenol red to lymph fluid *via* rectum.[8] The effects of adjuvants on lymphatic uptake of PEPS in rats were also investigated.

To study the transport of PEPS, a macromolecular weight drug, to lymphatic fluid after rectal administration of microenema containing PEPS and either adjuvant, plasma and lymphatic levels of PEPS were measured as a function of time after the administration (Fig. 2). As a control experiment, the PEPS levels in plasma and lymph after an intravenous administration at the same dose of 10 mg/kg were measured (Fig. 2A). The lymphatic levels of PEPS after intravenous administration were lower than those of plasma levels at the early stages after the administration and approached to the same levels in the plasma at 1.5 h after the administration. From this result, it is confirmed that the appearance of PEPS in the lymphatic system occurs *via* the blood flow after the intravenous administration but it takes 1.5 h to equilibrate the distribution between two body fluids; blood and lymph. Similar results were obtained when coadministered rectally with DC (Fig. 2B). This results may suggest that PEPS after uptaken into the rectal tissue by the presence of DC is mainly transported to the blood stream and then transported to lymphatic flow as well as after the intravenous administration of PEPS. As shown in Fig. 2C, plasma and lymph levels of PEPS showed the very similar pattern even at the early stage after coadministration recrtally with enamine. However, the results obtained when coadministered rectally with 5-MSA was remarkable. During the experimental periods for 3 h, the lymphatic levels of PEPS were maintained 5 times higher than the plasma levels by the rectal administration with 5-MSA (Fig. 2D).

From these results, it may be concluded that the transport of PEPS into lymphatic system after rectal administration is significantly modified by 5-MSA coadministered, and 5-MSA is rather weak in its adjuvant action than that of DC, but is appropriate to enhance the transport of drugs into lymphatic system.

Fig. 3 shows the accumulated amounts of PEPS in lymph fluid collected from thoracic duct as a function of time after administration. The rectal administration of PEPS with 5-MSA showed greatest cumulative amounts of PEPS in lymph in this study and the rectal administration with

TABLE I. *Bioavailability*[a] *of Pepleomycin Sulfate (PEPS) after Rectal Administration, Calculated from the Result Shown in Fig. 1, and Accumulative Amounts of PEPS in Thoracic Lymphatic Duct during 3 h after Administration, Calculated from the Result Shown in Fig. 2*

Administration route	Adjuvant (dose, mg/kg)	[*AUC*] 0 to 3 h μg·h/ml	Bioavailability % (A)	accumulative amount, μg (B)	(B) ×100² / Dose × (A) %
i.v. administration	—	30.48±5.23	100	15.02±2.46	0.15
Rectal administration					
Diclofenac	(20)	30.11±5.34	98.8	14.84±2.16	0.15
Sodium 5-methoxysalicylate	(40)	10.73±3.53	35.2	35.96±4.62	1.02
Enamine		3.57±1.03	11.7	3.96±1.04	0.34

Dose of PEPS was 10 mg/kg for all experiments.
a) Bioavailability = $[AUC]_{rec,\ 0\ to\ 3\ h} \times (Dose)_{i.v.} / [AUC]_{i.v.,\ 0\ to\ 3\ h} \times (Dose)_{rec}$, where $[AUC]_{rec}$ and $[AUC]_{i.v.}$ represent the area under the curve of PEPS during 3 h after rectal and i.v. administration, respectively.

enamine showed smallest results. However, comparison of accumulation of PEPS against the bioavailability after administration should be required to determine the lymphatic uptake of PEPS absorbed. The ratio of lymphatic uptake of PEPS against the total uptake of PEPS in the rat body showed the highest value when coadministered rectally with 5-MSA, with 8 times higher than after an intravenous administration (Table I). The ratio after rectal administration with DC was the same with that after *i.v.* administration and that with enamine was 2 times higher than *i.v.* administration.

The above findings suggest that these adjuvants behave in different way on the transport of PEPS to lymphatic system in spite of all these adjuvants enhancing the transport of PEPS through the rectal mucosal membrane.

Although it is not yet clear what kind of mechanism is involved in the action of adjuvant enchancing the rectal PEPS absorption, another program which concerns with the mechanism of adjuvant influencing the lymphatic uptake of PEPS after taken up into rectal tissue was studied in this report.

An aqueous solution of PEPS containing one of adjuvants was injected into the rectal connective tissue, and the plasma and the lymphatic levels of PEPS were measured, as shown in Figs. 4 and 5. To start the discussion of adjuvant effects

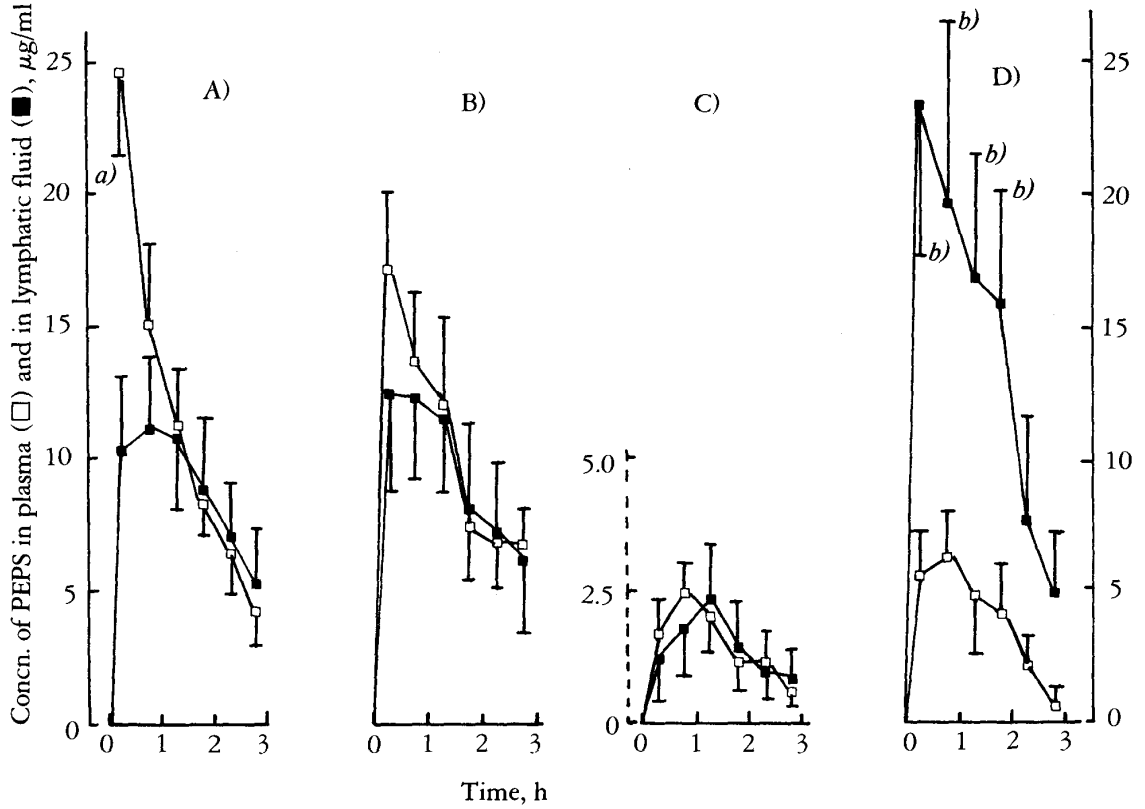

FIG. 2. *Concentration of Pepleomycine in Plasma (□) and in Lymph (■) Following i.v. Administration (A) at a Dose of 10 mg/kg in Saline or Rectal Administration at a Dose of 10 mg/kg in Microenema Following Each Adjuvant; Diclofenac (at a Dose of 20 mg/kg, B), Enamine (40 mg/kg, C) and Sodium 5-Methoxysalicylate (40 mg/kg, D)*

Each value represents mean ± S.D. ($n \geq 4$). For a) $p < 0.001$ against ■, b) $p < 0.001$ against □.

on the lymphatic transport of PEPS, it is necessary to confirm the effect of adjuvant on the transport of PEPS into the blood flow through blood vessel membrane under the condition in which the thoracic duct is comulated to avoid the transport of PEPS from the lymph to the blood. As control experiments, saline solution of PEPS was injected into the rectal connective tissue at a dose of 7.5 mg/kg. In comparison to this results without adjuvants, the coadministration of DC and 5-MSA significantly influenced on the transport of PEPS to the blood flow while enamine did not so much (Fig. 4). A significant enhanced transport of PEPS to the blood flow was observed at the early stage after injection with DC, but the plasma PEPS level after injection with 5-MSA were significantly lower than those in control study.

These results indicate that 5-MSA suppressed the transport of PEPS to the blood stream after injection, while DC enhanced the transport of PEPS to blood flow especially at the early stage after injection. Although it was not shown, the intravenous injection of PEPS with one of DC, 5-MSA and enamine at a same dose used for the rectal connective tissue injection did not influence the profile of plasma levels and lymphatic transport of PEPS compared to those obtained after an intravenous injection of PEPS without any adjuvants. This result suggests that the different transport of PEPS into the blood stream under the influence of each adjuvant originates at the rectal compartment where each adjuvant must be present in high concentration after intrarectal connective tissue injection and rectal administration.

For the purpose to elucidate a possible mechanism involved in adjuvant action at rectal connective tissue influencing the transport of PEPS into the blood flow, the following study was carried out.

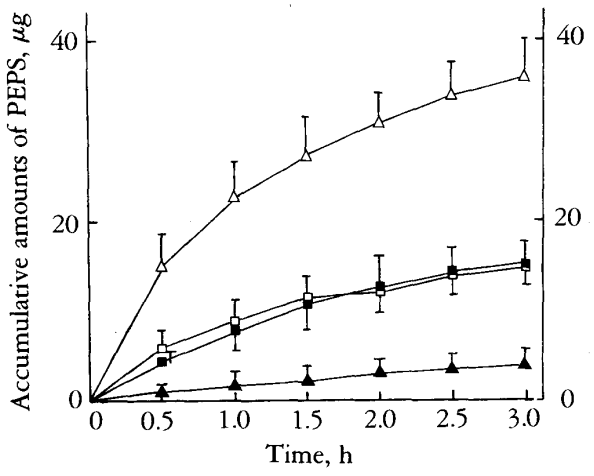

FIG. 3. *Cumulative Amounts of Pepleomycine in Lymph as a Function of Time in the Experiments Shown in Fig. 2*

The results obtained after i.v. administration were symbolized with □. *The results obtained after rectal administration with diclofenac, enamine and sodium 5-methoxysalicylate were symbolized with* ■, ▲, *and* △, *respectively. Each value represents mean* ± *S.D.* ($n \geq 4$).

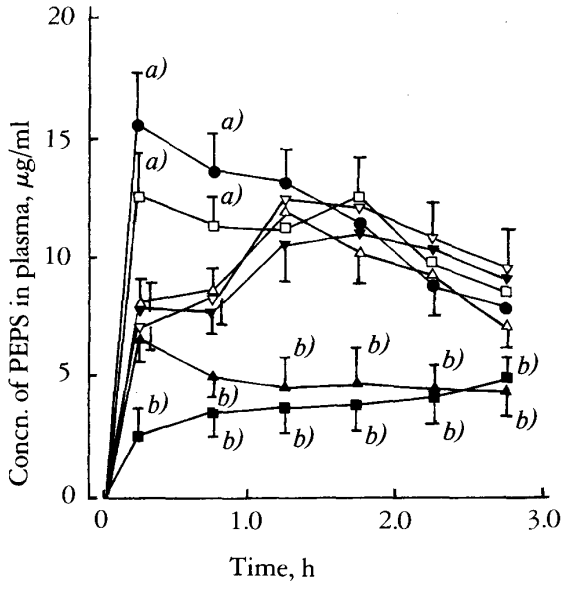

FIG. 4. *Plasma Concentration of Pepleomycine Following Injection into Rectal Connective Tissue at a Dose of 7.5 mg/kg in Saline* (▼), *or the Aqueous Solution* (△) *Following Each Additive; Diclofenac (at a Dose of 2 mg/kg,* ●), *Enamine (10 mg/kg,* △), *Sodium 5-Methoxysalicylate (10 mg/kg,* ▲), *Bradykinin (10 μ g/kg,* □) *and* ε*-Amino Caproic Acid (10 mg/kg,* ■)

Each value represents mean ± *S.D.* ($n \geq 4$). *For a, b)* $p < 0.001$ *against control study.*

Since bradykinin is known to increase the permeability of blood vessel membrane, its effect on the movement of PEPS into the blood flow was studied by the injection of PEPS and bradykinin into the rectal connective tissue (Fig. 4). Similar to the results of DC, bradykinin significantly increased the uptake of PEPS into the initial stage after injection. Similarly, ϵ-amino caproic acid is known to suppress the vascular permeability, thus its effect on the transport of PEPS into the blood flow was also studied (Fig. 4). ϵ-Amino caproic acid suppressed the uptake of PEPS into the blood flow during the experimental periods of 3 h and were similar to those of 5-MSA. On the other hand, the rectal coadministration of each of bradykinin and ϵ-amino caproic acid at a dose of 50 and 40 mg/kg, respectively, did not result in the appearance of PEPS in plasma (less than 0.1 μg/ml) when administered rectally at a dose of 10 mg of PEPS/kg.

Above findings may indicate that the action of DC and 5-MSA at the rectal mucosal membrane where both adjuvant enhance the uptake of PEPS occurs in the separate process from the action of them at the rectal connective tissue where DC increase the uptake of PEPS into blood flow but 5-MSA suppresses the uptake of PEPS into the blood flow. That is, action manner of adjuvants is independent at the rectal mucosal membrane and the rectal connective tissue.

Lymphatic transport of PEPS after the administration into the rectal connective tissue was studied. Lymphatic PEPS levels and the accumulation of PEPS in lymph during 3 h after administration with various additive was summarized in Fig. 5 and Table II. Administration of PEPS with 5-MSA into the rectal connective tissue results in significant high lymphatic PEPS levels, with more than 5 times higher than those after administration of PEPS in a saline solution. And the lymphatic levels of PEPS after injection with 5-MSA also showed 10 times higher than the plasma PEPS levels as shown in Fig. 4. The lymphatic levels of PEPS after administration with each DC, enamine, bradykinin and ϵ-amino caproic acid into the rectal connective tissue showed the very similar pattern with the plasma levels of PEPS except for lower lymphatic PEPS levels at early stages after administration (compared Fig. 5 to 4). It should be noted here that, as unexpected result, coinjection with ϵ-amino caproic acid did not show higher lymphatic PEPS levels compared to the plasma PEPS levels (compare Fig. 4 to 5). For the accumulation of PEPS in lymphatic fluid during 2.5 h after administration into the rectal connective tissue, coadministration of 5-MSA showed significantly greater value compared to other additive (Table II).

The above findings indicate that, since it is widely suggested that there is no barrier function at lymph terminal for drug uptake, significant effect of 5-MSA enhancing lymphatic transport of PEPS at the rectal connective tissue may be due to the suppression of the uptake of PEPS by 5-MSA into the blood flow. And weak effect of ϵ-amino caproic acid on the uptake of PEPS into

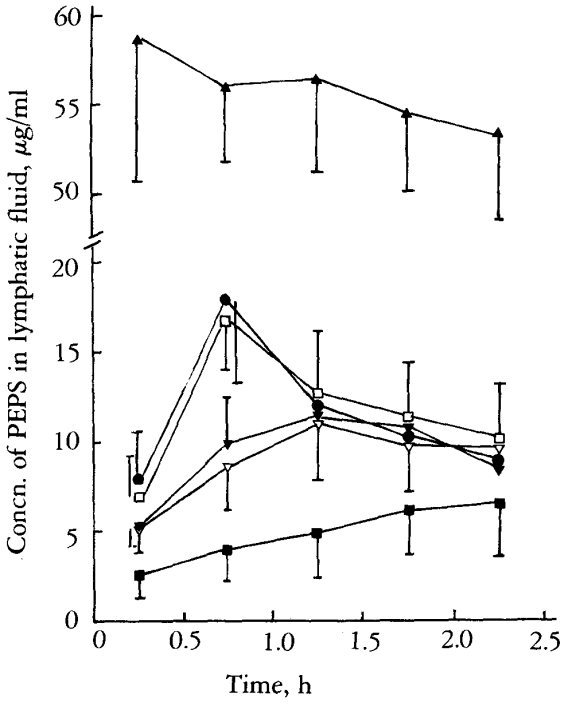

FIG. 5. *Concentration of Pepleomycine in Lymph in the Experiments Shown in Fig. 4*
Each symbol is described in Fig. 4. Each value represents mean ± S.D. ($n \geq 4$).

TABLE II. *Cumulative Amounts and Recovery Percent of Pepleomycine Sulfate (PEPS) in Lymph against the Dose in Experiments Shown in Fig. 5*

Administered solution and additive	Cumulative amounts in lymph during 3 h, μg	$\frac{\text{Cumulative amounts}}{\text{dose}} \times 100$
Saline	13.65±8.13	0.183
Aqueous solution		
Diclofenac	18.15±4.05	0.243
Enamine	12.13±3.62	0.161
Sodium 5-methoxy Salicylate	128.25±26.83[a)]	1.709
Bradykinin	20.23±5.18	0.269
ϵ-Amino caproic acid	6.15±2.43[b)]	0.083

Dose of PEPS was 7.5 mg/kg. Each value represents the mean± S.D. ($n \geq 4$).
a) $p < 0.001$ against the result for saline study (Student's t-test).
b) $p < 0.1$ against the results for saline study.

both lymph and blood flow would be explained by the possible suppressing action of ϵ-amino caproic acid on the diffusion of PEPS from the injection site. The slow uptake of PEPS into lymphatic flow after injection with saline. DC, enamine and bradykinin may be due to the slow flow rate of lymphatic fluid compared to blood flow.

In the present study, we tried to elucidate the possible mechanism involved in the enhancing action of 5-MSA on the lymphatic uptake of PEPS after rectal administration, though it is not clear yet what kind of mechanism is involved in the enhancing action of DC, 5-MSA and enamine on the rectal mucosal transport of PEPS. The enhanced uptake of PEPS by 5-MSA into lymph system is considered to be important for the delivery of neoplastic drugs such as PEPS. The enhancement of the rectal absorption of PEPS by the adjuvant action of 5-MSA and the remarkable increase in the transport of PEPS into the lymphatic system may be prospective for the clinical purpose and worthwhile to perform further studies for cancer treatment.

REFERENCES

1) T. Nishihata, J. H. Rytting, and T. Higuchi: Enhancement of rectal absorption of drugs by adjuvant, *J. Pharm. Sci.*, **69**, 744–745 (1980).
2) T. Nishihata, J. H. Rytting, and T. Higuchi: Enhanced rectal absorption of theophyllin, lidocaine, cefmetazole and levodopa by several adjuvants, *J. Pharm. Sci.*, **71**, 865–868 (1982).
3) T. Nishihata, J. H. Rytting, A. Kamada, T. Higuchi, M. Routh, and L. Caldwell: Enhancement of rectal absorption of insulin in dogs, *J. Pharm. Pharmacol.*, **35**, 148–151 (1983).
4) T. Murakami, N. Yata, H. Tamauchi, I. Nakai, M. Yamazaki, and A. Kamada: Study on the absorption promoters for rectal delivery preparation I, enamine of amino acid for the rectal absorption, *Chem. Pharm. Bull.*, **29**, 1998–2004 (1981).
5) A. Kamada, T. Nishihata, S. Kim, M. Yamamoto, and N. Yata: Study of enamine derivatives of phenylglycine as adjuvant for the rectal absorption of insulin, *Chem. Pharm. Bull.*, **29**, 2012–2019 (1981).
6) H. Yaginuma, T. Nakata, H. Toya, T. Murakami, M. Yamazaki, and A. Kamada: Rectal delivery of anti-inflammatory drugs I, the influence of antiinflammatory drugs on rectal absorption of -lactum antibiotics, *Chem. Pharm. Bull.*, **29**, 2974–2982 (1981).
7) T. Nishihata, S. Kim, S. Morishita, A. Kamada, N. Yata, and T. Higuchi: Adjuvant effect of glyceryl esters of acetoacetic acid on rectal absorption of insulin and inulin in rabbits, *J. Pharm. Sci.*, **72**, 280–285 (1983).
8) L. Caldwell, T. Nishihata, J. H. Rytting, and T. Higuchi: Lymphatic uptake of water soluble drugs after rectal administration, *J. Pharm. Pharmacol.*, **34**, 520–522 (1982).
9) T. Murakami, H. Tamauchi, M. Yamazaki, K. Kubo,

A. Kamada, and N. Yata: Biopharmaceutical study on oral and rectal administrations of enamine prodrug of amino acid-like antibiotics in rabbits, *Chem. Pharm. Bull.*, **29**, 1991–1997 (1981).

10) H. Fujita, T. Shinoda, K. Ogata, and K. Kimura: *In vivo* distribution and indication of pepleomycine, *Jpn. J. Antibiot.*, **31**, 664–671 (1978).