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Effect of Nonsteroidal Anti-inflammatory Drugs on the Absorption of Macromolecular Drugs in Rat Rectum

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The effect of three nonsteroidal anti-inflammatory drugs (NSAID), indomethacin, phenylbutazone and diclofenac sodium, on the absorption of water-soluble drugs with various molecular weights through the rectal membrane was evaluated by using an *in situ* perfusion technique. ^{14}C-Inulin, ^{125}I-insulin, ^{125}I-polyvinyl pyrrolidone (PVP) and ^{125}I-albumin were selected as marker drugs. NSAID increased the absorptions of inulin, insulin and PVP, but not that of albumin. It is suggested that macromolecular drugs with a molecular weight of less than 35000 are able to penetrate through the rectal membrane in the presence of NSAID.

Keywords——nonsteroidal anti-inflammatory drug; water-soluble drug; inulin; insulin; polyvinyl pyrrolidone; albumin; *in situ* perfusion technique; rectal absorption; macromolecular drug; enhancing effect

Introduction

In our previous papers,[1-3] we demonstrated that the permeability of the rectal membrane to drugs was increased by inclusion of some pharmaceutical adjuvants in the formulations, and that the permeability-enhancing effects were accompanied by histological changes and release of protein components from the rectal mucosa. Also, we reported that nonsteroidal anti-inflammatory drugs (NSAID), indomethacin (IM), phenylbutazone (PB), diclofenac sodium (DF) and aspirin (ASA), enhanced rectal membrane permeability to nonabsorbable drugs, sulfanilic acid (SA) and creatinine,[4] and that the interaction of NSAID with membrane components (protein and lipid) played an important role in the permeation process of low-molecular-weight drugs. Further, NSAID induced a solvent-drag effect in the permeation of drugs through the rectal membrane.[5] Recently, we showed by an electrophysiological technique[6] that DF increased the mucosal-to-serosal flux of SA in isolated rat jejunum by enhancing both transcellular and paracellular permeabilities.

In this study, the effect of NSAID on the absorption of macromolecular drugs in the rat rectum was investigated and the mechanism of the enhancing effect was considered.

Experimental

Materials——IM (Medicel Research Laboratory, Japan), PB (Sigma, U.S.A.), and DF (Kodama Pharmaceutical Co., Ltd., Japan) were used as supplied. ^{14}C-Inulin (M_r=5500), ^{125}I-insulin (M_r=6000), ^{125}I-polyvinyl pyrrolidone (PVP, M_r=35000) and ^{125}I-albumin (M_r=69000) were obtained from the Radiochemical Centre, Amersham. They were used after further purification through a Sephadex G-25 column. SA and other chemicals used were reagent grade commercial products.

Absorption Experiments——Male Wistar rats (200—250 g) were used in all experiments. The absorption of marker drugs from the rectum was measured by the *in situ* single perfusion technique as described previously.[1,7] The drug solution (0.3—0.5 μCi/ml) was perfused through the whole rectum at a rate of 20 ml/15 min. Blood samples (0.3 ml) were taken just before the start of the perfusion and every 15 min thereafter. The ratios of the concentration

of the labeled marker drug in the blood to the initial concentration in the perfusate were calculated.[7] As a control run, isotonic phosphate buffer (pH 7.4) containing the marker drug alone was perfused similarly.

The absorption of SA and IM from the rectum was measured by the *in situ* single perfusion technique as described previously.[1] Isotonic phosphate buffer solution containing SA and IM at concentrations of 3 mg/ml and 5 mM, respectively, was perfused. The effect of albumin on the absorptions of SA and IM was examined by the addition of albumin to the above solution.

Analytical Methods——Blood glucose level was determined by measuring the absorbance at 635 nm using the *o*-toluidine–boric acid method (Glucose-Test Kit, Wako Pure Chemical Industries, Ltd.). The analytical methods for SA,[2] IM,[4] ^{14}C-inulin, ^{125}I-labeled insulin, PVP and albumin[7] were described in our previous paper.

Results and Discussion

Figure 1 shows the blood/perfusate concentration ratio–time curve for ^{125}I-insulin and the blood glucose level–time curve, illustrating the effect of NSAID (IM, PB and DF) on the absorption of ^{125}I-insulin in the rat rectum. The blood glucose level was recorded as the relative value with respect to the initial level, which was considered as 100%. As is evident from Fig. 1, the absorption of ^{125}I-insulin was significantly increased by the presence of NSAID. The enhancing effect increased in the following order: PB < DF < IM. Also, it was shown that the blood glucose levels after the perfusion of insulin were decreased about 20—40% at 90 min in the presence of NSAID in comparison with the control value. The degree of the blood glucose-lowering effect was dependent on the enhancing effect of NSAID.[4] To confirm that ^{125}I-insulin was actually absorbed, plasma was treated with trichloroacetic acid. Most of the radioactivity was found in the precipitated fraction, indicating that NSAID enhanced rectal absorption of native insulin without loss of the biological activity.

The absorptions of PVP and albumin, which are known to be poorly absorbable due to their large molecular weights, were examined in the presence of NSAID and the results are shown in Fig. 2. Although the absorption of PVP was increased by NSAID, the extent of PVP absorption in the presence of NSAID was significantly smaller than that of insulin. On the other hand, albumin was not absorbed from the rectal lumen, regardless of the presence or absence of NSAID.

For each marker drug, the area under the blood/perfusate concentration ratio–time curve for 90 min (*AUCR*) was determined. The ratios of these values to those obtained from the control experiments were calculated and the changes in the permeability of the rectal membrane were evaluated. The results are summarized in Table I. The values obtained for adjuvants,[7] such as sodium deoxycholate (SDC), sodium lauryl sulfate (SLS) and disodium ethylenediaminetetraacetate (EDTA), are also listed in Table I. The absorption of macromolecular drugs from the rectal lumen was significantly increased by NSAID. The *AUCR* of inulin (M_r = 5500) in the presence of NSAID was similar to that obtained for insulin (M_r =

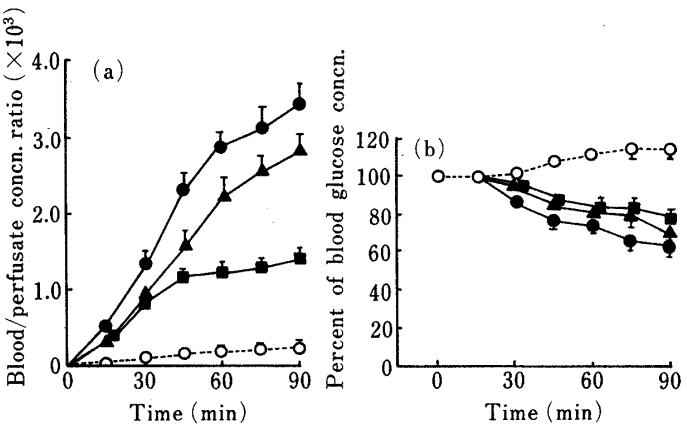

Fig. 1. Effect of Nonsteroidal Anti-inflammatory Drugs on the Absorption of ^{125}I-Insulin (a) and on Blood Glucose Concentrations (b)

---○---, control; —●—, 5 mM indomethacin; —■—, 10 mM phenylbutazone; —▲—, 10 mM diclofenac sodium.

Each point represents the mean ± S.E. of five experiments.

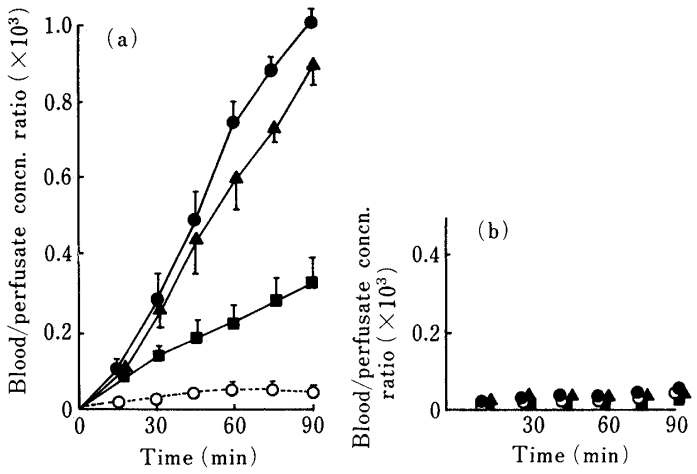

Fig. 2. Effect of Nonsteroidal Anti-inflammatory Drugs on the Absorptions of ^{125}I-PVP (a) and ^{125}I-Albumin (b)

---○---, control; —●—, 5 mM indomethacin; —■—, 10 mM phenylbutazone; —▲—, 10 mM diclofenac sodium.

Each point represents the mean±S.E. of five experiments.

TABLE I. Effect of Nonsteroidal Anti-inflammatory Drugs and Some Other Adjuvants on *AUCR* of Marker Drugs

Adjuvant	Conc. (mM)	Sulfanilic acid		Inulin		Insulin		PVP	
		AUC	Ratio	*AUCR*	Ratio[a)]	*AUCR*	Ratio[a)]	*AUCR*	Ratio[a)]
Control	—	61	1.0	10.1	1.0	13.3	1.0	3.6	1.0
Indomethacin	5	1844	30.2	153.8	15.2	180.0	13.5	45.9	12.6
Phenylbutazone	10	506	8.3	39.9	4.0	86.3	6.5	16.9	4.6
Diclofenac sodium	10	1270	20.9	116.9	11.6	140.4	10.6	40.6	11.2
Sodium deoxycholate	5	4160	68.5	382.5	37.9	384.0	28.9	120.2	33.4
Sodium lauryl sulfate	5	4055	66.7	289.5	28.7	438.0	32.9	76.5	21.3
EDTA	25	422	6.9	84.8	8.4	136.5	10.3	44.6	12.4

AUC, area under the blood concentration–time curve, $\mu g \cdot 90$ min/ml. *AUCR*, area under the blood/perfusate concentration–time curve, $\times 10^{-3} \cdot 90$ min. *a*) The ratio was calculated as *AUCR* of the marker drug with NSAID/*AUCR* of the marker drug without NSAID.

6000). The enhancement ratios for SA by IM and DF were 30.2 and 20.9, respectively. The enhancement ratios for macromolecular drugs (inulin, insulin and PVP) by IM and DF were 10—15 and became larger as the molecular weight of the marker drug decreased. The enhancing effects on the absorption of marker drugs by NSAID were about one-half of those obtained with SDC and SLS (5 mM).

Albumin penetration through the rectal membrane was induced by SDC and SLS, but not by NSAID. It is well known that NSAID binds strongly to serum albumin in the circulation.[8)] Further, there was a good correlation between the amount of NSAID accumulated in the rectal tissue and the enhancing effect of NSAID.[4)] Thus, in order to clarify whether NSAID can enhance rectal permeability to drugs in the presence of albumin or not, we investigated the absorptions of SA and IM in the presence of various concentrations of albumin concentration in the perfused solution was 1—10 μg/ml. These results show that solution, the absorptions of SA and IM were similar to the values obtained in the absence of albumin. In the experiment showing that albumin absorption did not occur (Fig. 2), ^{125}I-albumin concentration in the perfused solution was 1—10 μgml. These results show that NSAID exhibits the absorption-enhancing effect on marker drugs in the presence of albumin at 1 mg/ml, but albumin itself could not penetrate through the rectal membrane even in the presence of NSAID. It appears that macromolecular drugs, having a molecular weight of less than 35000 are able to penetrate through the rectal membrane in the presence of NSAID.

On the other hand, the absorptions of SA and IM were markedly decreased by the

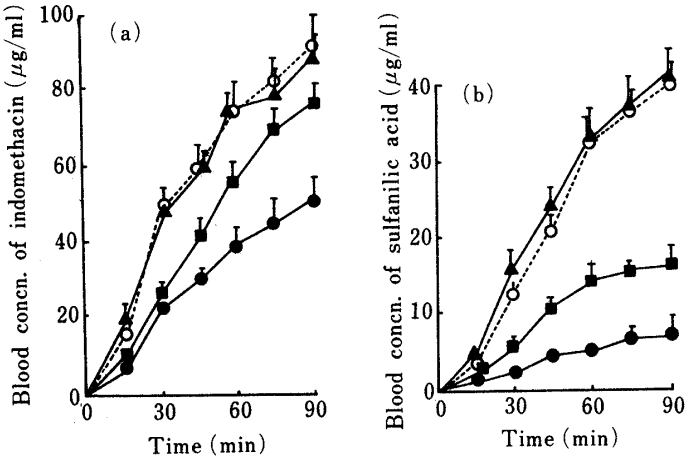

Fig. 3. Effect of Albumin on the Absorptions of Indomethacin (a) and Sulfanilic Acid (b)

---○---, control (in the absence of albumin); —▲—, albumin (1 mg/ml); —■—, albumin (5 mg/ml); —●—, albumin (10 mg/ml).
Each point represents the mean ± S.E. of five experiments.

addition of albumin at 5 or 10 mg/ml in comparison with the values in the absence of albumin. The reduction of the enhancing effect of IM might be due to the decrease of free IM in the perfusate. The ratio of free IM and SA in the presence of albumin was examined by using the equilibrium dialysis method described in our previous paper.[5] Free IM was reduced by about 50% by the addition of albumin (10 mg/ml), but SA did not bind to albumin. Similarly, the accumulation of IM in the rectal tissue in the presence of albumin (10 mg/ml) was one-half of the value in the absence of albumin. Consequently, it is suggested that the decrease in enhancing effect of NSAID in the presence of higher concentration of albumin may be due to the decrease in the accumulation of NSAID in the rectal tissue.

Although it has been reported that rectal absorption of insulin or heparin was enhanced by various adjuvants,[9,10] it was found that NSAID also enhanced the absorptions of inulin, insulin and PVP. It was shown that interaction of NSAID with membrane components (protein and lipid), as well as the induction of solvent drag, were responsible for the enhanced permeability to low-molecular-weight drugs.[5] Further, NSAID enhanced permeation through two pathways (transcellular and paracellular routes) in the case of the low-molecular-weight drug, sulfanilic acid.[6] However, we cannot conclude at present whether or not both pathways contribute to the enhancement of the permeation of macromolecular drugs through the rectal membrane by NSAID.

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