

Influence of Pharmaceutical Excipients on Membrane Transport via the Transcellular Route in the Rat Small Intestine

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Abstract

Original Research Article

Pharmaceutical excipients improve the safety and homogeneity of pharmaceutical drugs, and, thus, increase their usefulness. By definition, pharmaceutical excipients must not disturb the influence of medical therapy with drugs; however, some have been shown to affect epithelial membrane proteins and induce changes in the structure of tight junctions and function of drug transporters, which may alter the disposition of drugs. To clarify alterations in the absorption behavior of drugs by pharmaceutical excipients, the present study examined the effects of pharmaceutical excipients on membrane transport via the transcellular route, which is the main membrane permeation route for lipophilic drugs. The effects of twenty common pharmaceutical excipients from different classes on the mucosal membrane were also investigated, and variations in these effects between different regions of the small intestine were assessed. The *in vitro* sac method was employed to examine the effects of pharmaceutical excipients on the membrane permeation of β -naphthol in the rat jejunum and ileum. β -Naphthol is a drug that permeates through the transcellular route. The membrane permeability of β -naphthol was significantly altered under the dosage conditions of pharmaceutical excipients. Furthermore, the effects of pharmaceutical excipients were site-specific (jejunum and ileum) in the small intestine. The present results demonstrated that some pharmaceutical excipients altered membrane permeability via the transcellular route in the rat small intestine.

Keywords: Pharmaceutical excipient, membrane transport, transcellular route, passive transport, small intestine.

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INTRODUCTION

The Ministry of Health, Labour and Welfare in Japan is promoting the administration of generic drugs that will reduce medical expenses in order to effectively utilize limited medical cost resources. Although generic drugs have been shown to be therapeutically equivalent to brand drugs in biological equivalency tests, issues continue to be reported on their quality, supply system, and information provision system in Japan. Therefore, there is still insufficient trust from medical staff and patients. The low reliability of generic drugs has impeded the promotion of their use. For example, the type and amount of the main drug is equivalent in brand and generic tablets; however, the type and amount of the pharmaceutical excipient differs. Therefore, although these products are not strictly the same, they are regarded as being equivalent under the law.

Pharmaceutical excipients improve the safety and homogeneity of pharmaceutical drugs, and, thus, increase their usefulness. By definition, pharmaceutical excipients must not disturb the influence of medical therapy with drugs; however, some have been shown to affect epithelial membrane proteins and induce changes in the structure of tight junctions (TJ) and function of P-glycoprotein (P-gp), which may alter the disposition of drugs [1-6].

Interactions have been reported between drugs and pharmaceutical excipients, which has led to the development of the concepts of pro-drug formulations and drug delivery systems (DDS) [7-9]. However, few studies have focused on changes in the gastrointestinal mucosal membrane by pharmaceutical excipients [10-12]. Since the influence of pharmaceutical excipients on the gastrointestinal mucosal membrane has not yet been

examined in detail [12, 13], limited information is currently available on interactions between the gastrointestinal mucosal membrane and pharmaceutical excipients of each category.

The membrane permeation routes of drugs are divided into the transcellular and paracellular routes. Drugs are passed between cell-cell junctions (tight junction; TJ) for paracellular permeation, but need to dissolve in cells for transcellular permeation. Many drugs are lipophilic, and, thus, the changes induced in membrane permeability via the transcellular route by pharmaceutical excipients are of importance.

As described above, pharmaceutical excipients differ between brand and generic drugs. Therefore, to clarify the alterations induced in the absorption behavior of generic drugs by pharmaceutical excipients, the present study examined the effects of pharmaceutical excipients on membrane transport via the transcellular route, which is the main membrane permeation route for lipophilic drugs.

MATERIALS AND METHODS

MATERIALS

Naphthol was purchased from Sigma Aldrich Co. Ltd. (Tokyo, Japan). All other reagents were of analytical grade or higher.

Animals

Male Wistar rats (8 weeks old) were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). Animals were housed in a clean room maintained at $23\pm 2^{\circ}\text{C}$ with a relative humidity of $55\pm 10\%$ and a 12-hr light-dark cycle. Animals were fasted for 16-18 hr before the initiation of experiments. Water was freely available during fasting. All animal experiments were performed according to the guidelines of the Tokyo University of Pharmacy and Life Sciences.

Membrane Permeation Experiments Using the *In Vitro* Sac Method

The *in vitro* sac method was performed as previously described [6]. In brief, rats were fasted overnight for 16-18 hr, but allowed free access to water before the experiment. After anesthetizing rats with Somnopentyl[®] (pentobarbital sodium, 50 mg/kg, i.p.), the entire small intestine segment was removed by cutting across the upper end of the duodenum and the lower end of the colon and manually stripping the mesentery. The isolated intestine was carefully washed with normal saline (0.9% w/v NaCl), and 6-cm jejunal (5 cm below the Treitz ligament) and ileal (5 cm above the cecum) segments were removed and cannulated at both ends with plastic tubing. The experimental system was assembled with a silicone stopper and beaker before the intestinal tract dried, Krebs-Henseleit bicarbonate buffer (KHBB: NaCl, 126 mM, KCl, 5

mM, NaHCO_3 1.4 mM, Na_2HPO_4 0.95 mM, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 4.85 mM, CaCl_2 2 g/L, and glucose 3.5 mM; pH 6.5) was added to the receiver side (serosal side, 40 mL) and donor side (mucosal side, 5 mL), and a pre-incubation was started for 10 min. After the pre-incubation, 5 mL of KHBB solution containing β -naphthol with or without each pharmaceutical excipient was added to the donor side. Sampling from the receiver side was performed at the following time points: 0, 20, 40, 60, 80, 100, and 120 min after the administration of β -naphthol.

After the membrane permeation experiment, the intestinal tract was removed from the experimental system, and its effective surface area was measured. Drug solutions and buffer warmed to 37°C were used in this experiment, which was performed at 37°C .

Samples of β -naphthol were rapidly cooled and stored at -80°C for later analyses. β -Naphthol was measured by reverse-phase HPLC using a COSMOSIL 5C18-AR-II column (150×4.6 mm) at 50°C . An analysis of β -naphthol was performed at a wavelength of 246 nm. The mobile phase consisted of a mixture of methanol and water (50:50 v/v) containing 0.05 mol/L KH_2PO_4 and its flow rate was 1.0 mL/min.

The area under the receiver concentration curve (AUC) of β -naphthol was calculated by the trapezoidal method.

STATISTICAL ANALYSIS

All results are expressed as the mean \pm standard error (mean \pm S.E.). The significance of differences between groups was analyzed using the Student's *t*-test and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Influence of diluents on the transcellular permeation of β -naphthol in the rat small intestine

Twenty types of pharmaceutical excipients, which are major pharmaceutical excipients and widely used in the pharmaceutical industry, were selected in the present study and their effects on membrane permeation via the transcellular route were examined. We also investigated the effects of each pharmaceutical excipient at two different concentrations. (Table-1) The concentrations of pharmaceutical excipients were selected based on the clinical dosage. Each category of pharmaceutical excipient was included in a tablet (200 mg) as follows: diluents: 20 - 30%, disintegrants: 5 - 10%, binders: 5 - 10%, lubricants: 1%, and sustained release substrate: 20 - 30%. Since each tablet was taken with 100 - 200 mL of water, we set the dose (concentration) of each pharmaceutical excipient based on the volume of water consumed and the amount of pharmaceutical excipient included in the tablet.

Table-1: Abbreviations and concentrations of pharmaceutical excipients

Application	Compound Name	Abbreviation	Concentration (w/v %)	
Diluents	methyl- β -cyclodextrin	M-beta-CD	0.08	0.8
	lactose hydrate	LH		
	corn starch	CS		
	microcrystalline cellulose	MCC		
Disintegrants	sodium carboxymethyl starch	SCMS	0.02	0.2
	low substituted hydroxypropyl cellulose	LHPC		
	croscarmellose sodium	CCS		
Binders	hydroxypropyl cellulose	HPC	0.02	0.2
	hydroxypropylmethyl cellulose	HPMC		
	povidone (K29/32)	PVP (K29/32)		
	povidone (K90)	PVP (K90)		
	pullulan	-		
Lubricants	talc	-	0.004	0.04
	stearic acid	SA		
	calcium stearate	CaS		
	magnesium stearate	MgS		
	glyceryl monostearate	GMS		
	soft anhydrous silicic acid	SAAS		
Sustained release substrate	ethyl cellulose	EC	0.08	0.8
	methyl cellulose	MC		

In the present study, physical changes due to the co-existence of the substrate drug (β -naphthol) and pharmaceutical excipients (such as changes in substrate degradability and solubility as well as the binding of pharmaceutical excipients to the substrate drug) were not examined. Therefore, changes in the amount of the substrate drug absorbed were evaluated by calculating AUC_{0-120} from the amount of the substrate drug transferred to the serosal side. We also examined regional differences between the jejunum and ileum in terms of the influences of pharmaceutical excipients.

The effects of diluents on transcellular permeation were examined using 0.08 and 0.8% (w/v) of the diluent. Previous studies described the influence of methyl- β -cyclodextrin (M-beta-CD) on membrane transport via the paracellular route [6, 14, 15, 16]. Under the dosage conditions of M-beta-CD, the membrane permeation of β -naphthol was not significantly affected in the jejunum or ileum (Fig. 1A, B). This result showed that β -naphthol did not pass through the paracellular route, and also demonstrated

that M-beta-CD did not affect the membrane permeation of β -naphthol in the jejunum or ileum. Since very limited information is currently available on the impact of lactose hydrate (LH), corn starch (CS), and microcrystalline cellulose (MCC) on transcellular permeability, it is important to clarify the effects of these diluents on the gastrointestinal mucosal membrane. LH did not affect the membrane permeation of β -naphthol in the jejunum or ileum, similar to M-beta-CD (Fig-1A, B). On the other hand, under the dosage conditions of high concentrations of CS and MCC (0.8 w/v %), the AUC_{0-120} of β -naphthol in the jejunum was significantly lower than that in their absence. In contrast, under the dosage conditions of high concentrations of CS and MCC (0.8 W/V %), the AUC_{0-120} of β -naphthol in the ileum was significantly increased (Fig-1A, B). Regional differences were observed in the effects of CS and MCC; however, the underlying mechanisms currently remain unknown. Based on these results, diluents may have influenced transcellular permeability in the rat small intestine.

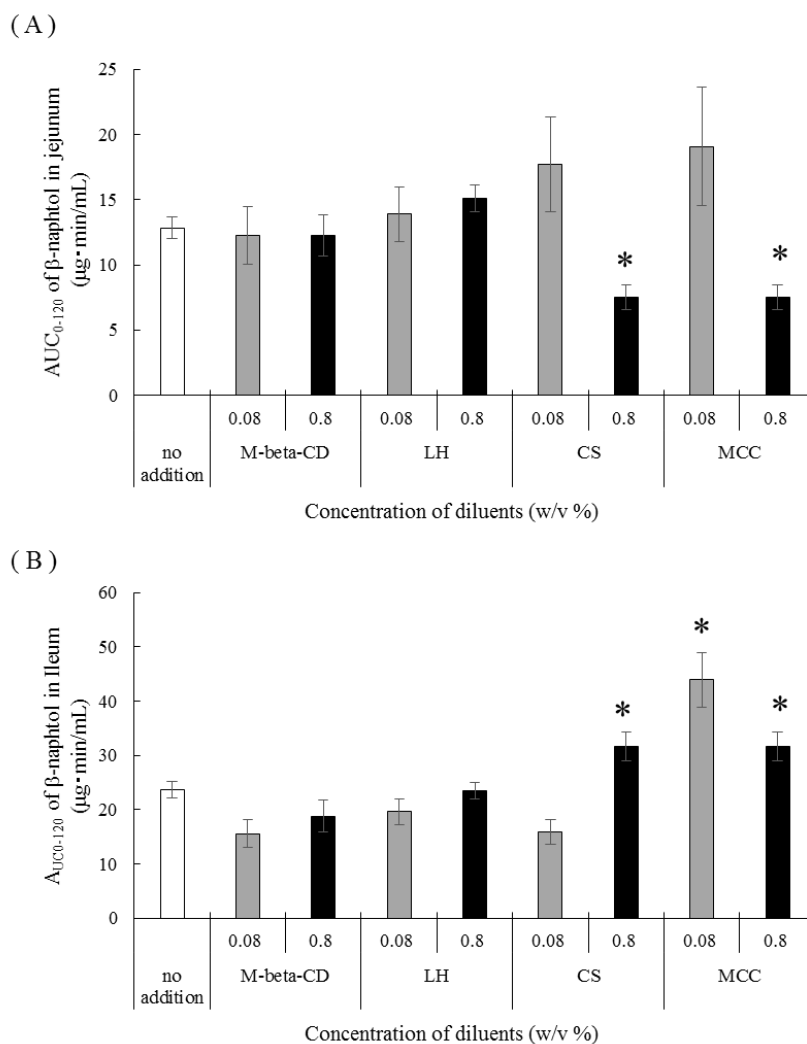


Fig-1: Changes in AUC₀₋₁₂₀ of β-naphthol in the jejunum (a) and ileum (b) with the co-existence of diluents. Data represent means and S.E. (n = 4-10 for each condition)

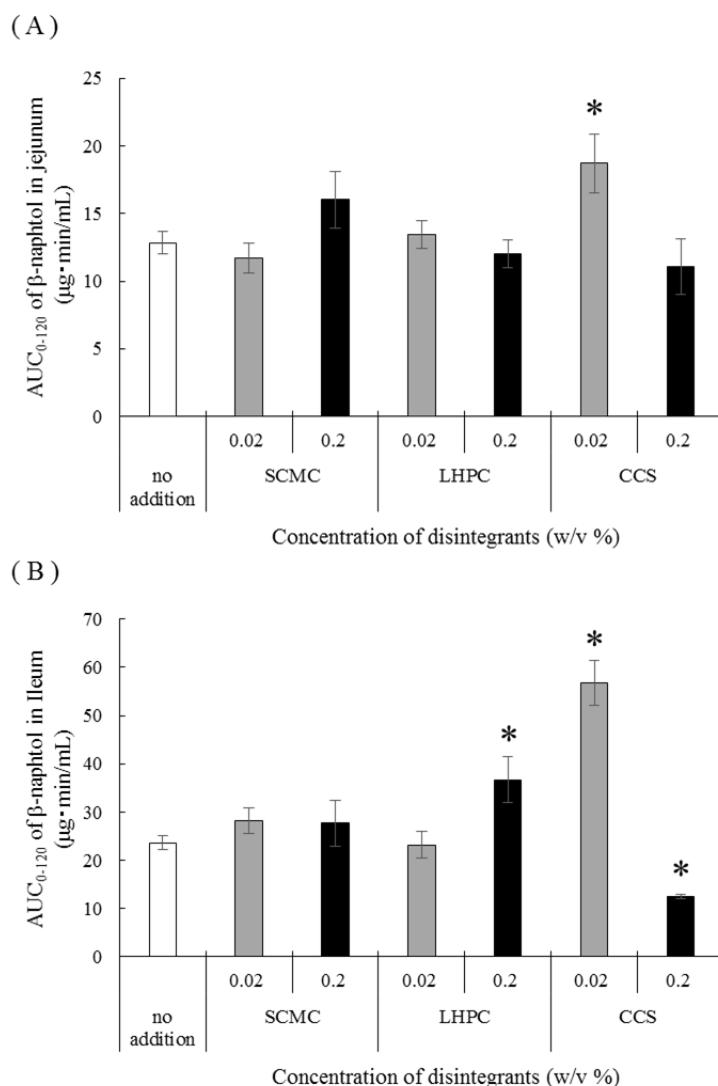
*P<0.05 vs. no additive condition.

Influence of disintegrants on the transcellular permeation of β-naphthol in the rat small intestine

Disintegrants were used at concentrations of 0.02 and 0.2% (w/v). Sodium carboxymethyl starch (SCMC), low substituted hydroxypropyl cellulose (LHPC), and croscarmellose sodium (CCS) were selected as disintegrants, and their effects on the gastrointestinal mucosal membrane were examined.

SCMC and LHPC did not significantly affect the membrane permeation of β-naphthol in the jejunum.

A high concentration of LHPC increased the AUC₀₋₁₂₀ of β-naphthol in the ileum. On the other hand, the AUC₀₋₁₂₀ of β-naphthol increased under the dosage condition of a low concentration of CCS (0.02 w/v %), but decreased under the high concentration condition (0.2 w/v %) in both the jejunum and ileum (Fig-2). This behavior is similar to that of MCC, but needs to be examined in more detail in future studies. Since no information is currently available on the effects of these disintegrants on transcellular permeation, these results are novel and important.



**Fig-2: Changes in the AUC₀₋₁₂₀ of β-naphthol in the jejunum (a) and ileum (b) with the co-existence of disintegrants. Data represent means and S.E. (n = 4-10 for each condition)
*P<0.05 vs. no additive condition**

Influence of binders on the transcellular permeation of β-naphthol in the rat small intestine

Binders were used at concentrations of 0.02 and 0.2% (w/v). Hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), povidone (K29/32), povidone (K90), and pullulan were selected as binders, and their effects on the gastrointestinal mucosal membrane were examined.

The AUC₀₋₁₂₀ of β-naphthol was slightly changed under the additive conditions of all types of binders. It was significantly increased by 0.2% (w/v) of HPC, 0.02% (w/v) of PVP (K90), and 0.02% (w/v) of pullulan in the jejunum, and significantly decreased by 0.02 and 0.2% of PVP (K90) and 0.02% of pullulan in the ileum (Fig-3). Binders alter the viscosity of a solution, and it currently remains unclear whether this may be attributed to changes in the viscosity of a drug solution. Further studies on binders that consider the composition of the solution are warranted.

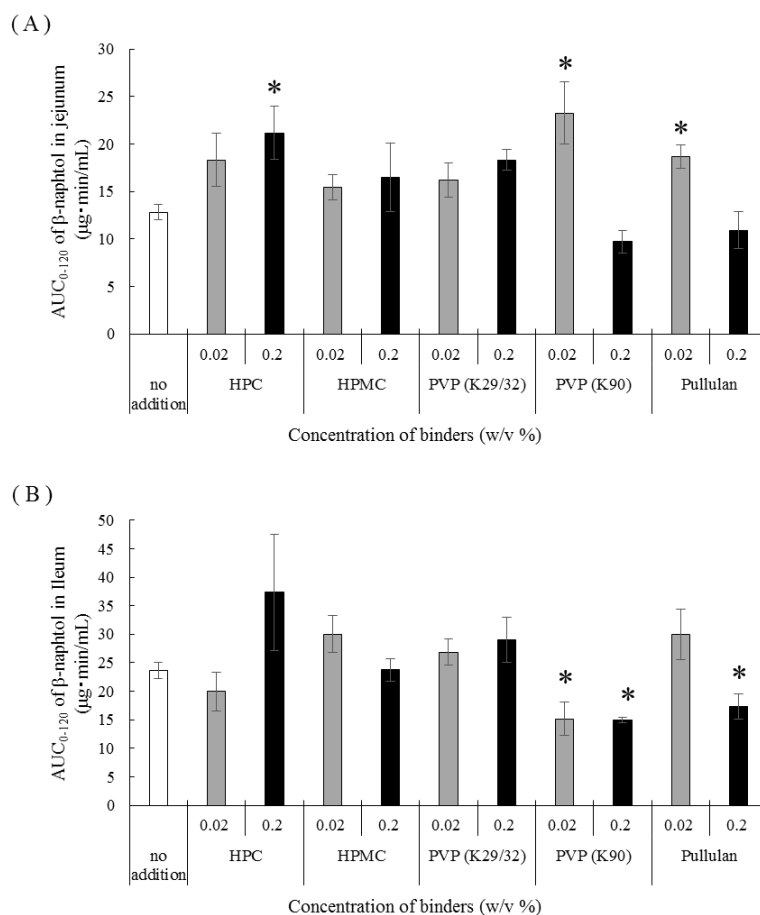
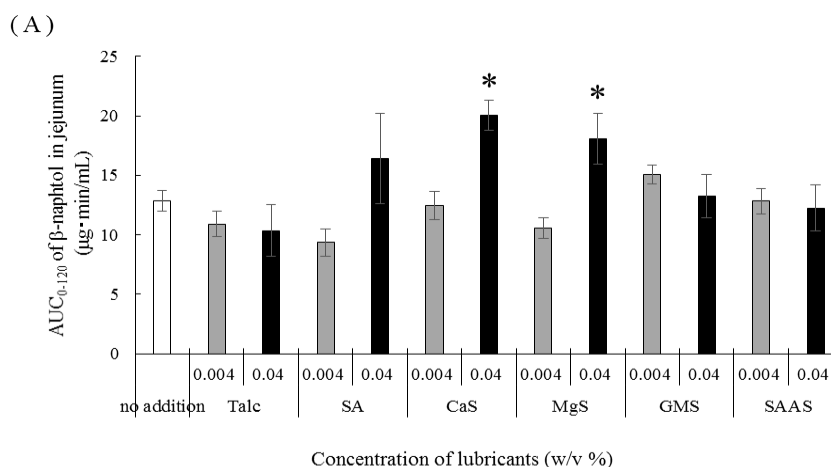


Fig-3: Changes in the AUC₀₋₁₂₀ of β -naphthol in the jejunum (a) and ileum (b) with the co-existence of binders. Data represent means and S.E. (n = 4-10 for each condition) *P<0.05 vs. no additive condition.

Influence of lubricants on the transcellular permeation of β -naphthol in the rat small intestine

Lubricants were used at concentrations of 0.004 and 0.04% (w/v). Talc, stearic acid (SA), calcium stearate (CaS), magnesium stearate (MgS), glyceryl monostearate (GMS), and soft anhydrous silicic acid (SAAS) were selected as lubricants, and their effects on the gastrointestinal mucosal membrane were examined.

In the jejunum, the AUC₀₋₁₂₀ of β -naphthol was significantly increased by 0.04% (w/v) of CaS and MgS. Under other conditions in the jejunum, increases and decreases in the AUC₀₋₁₂₀ of β -naphthol were both observed with the co-existence of lubricants. Similar results were obtained in the ileum. While 0.04% (w/v) of CaS and 0.04% (w/v) of SAAS significantly increased the AUC₀₋₁₂₀ of β -naphthol, 0.04% (w/v) of talc and 0.004% (w/v) of SAAS significantly decreased it (Fig-4).



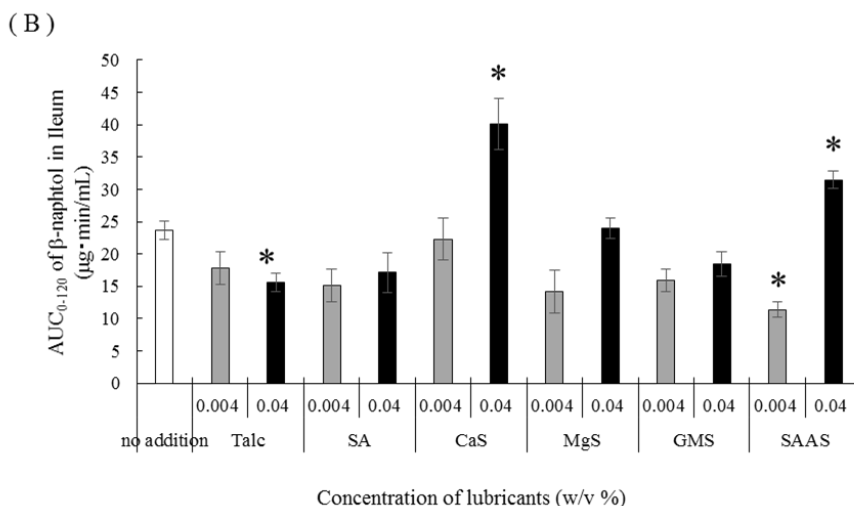


Fig-4: Changes in the AUC₀₋₁₂₀ of β-naphthol in the jejunum (a) and ileum (b) with the co-existence of lubricants. Data represent means and S.E. (n = 4-10 for each condition) *P<0.05 vs. no additive condition.

Influence of sustained release substrates on the transcellular permeation of β-naphthol in the rat small intestine

Sustained release substrates were used at concentrations of 0.08 and 0.8% (w/v). Ethyl cellulose (EC) and methyl cellulose (MC) were selected as sustained release substrates.

Although no significant changes in transcellular permeability were observed in the jejunum or ileum following the addition of MC at both concentrations, a significant increase was noted in the jejunum and ileum with the addition of EC (Fig-5). However, the effects of EC were not dependent on the concentration applied; therefore, further studies are needed to elucidate the underlying mechanisms.

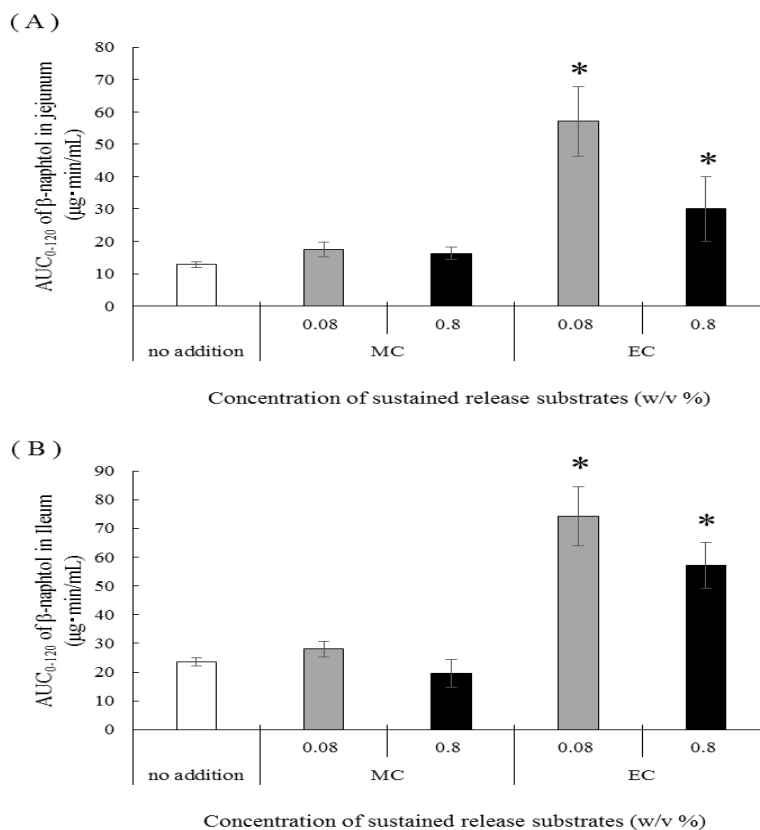


Fig-5: Changes in the AUC₀₋₁₂₀ of β-naphthol in the jejunum (a) and ileum (b) with the co-existence of sustained release substrates. Data represent means and S.E. (n = 4-10 for each condition) *P<0.05 vs. no additive condition.

Although the mechanisms of action of pharmaceutical excipients on membrane permeation were not elucidated in the present study, changes in the membrane permeation of transcellular markers with the co-administration of pharmaceutical excipients were demonstrated in Figures 1, 2, 3, 4, and 5. Moreover, since the effects of some pharmaceutical excipients were not dependent on concentration, further studies are warranted.

In the present study, membrane permeation experiments were only conducted with the co-existence of the substrate drug (β -naphthol) and pharmaceutical excipients; therefore, it currently remains unclear whether the changes observed in the absorption of the substrate drug were due to alterations in membrane permeability. The changes observed in the solubility of the substrate drug may be attributed to the pharmaceutical excipients used, and not to its membrane permeability. Although the underlying mechanisms were not clarified in the present study, the apparent permeation amount (absorption amount) of the substrate drug was affected by the co-existence of pharmaceutical excipients. Furthermore, since pharmaceutical excipients have been shown to affect drug transporters, such as BCRP [17-20], further studies are needed to investigate not only passive transport, but also changes in active transport for optimal drug administration design.

CONCLUSION

The present results demonstrated that many types of pharmaceutical excipients affected passive transcellular permeation in the rat small intestine. Moreover, the changes observed in the absorption amount of the substrate drug with the co-existence of pharmaceutical excipients were site-specific in the small intestine. In addition, some pharmaceutical excipients exerted effects that were not dependent on concentration. Based on these results, further detailed studies on pharmaceutical excipients and gastrointestinal absorption are needed for the safer and more effective use of pharmaceutical formulations.

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Conflicts of Interest: The authors declare no conflicts of interest.

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