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New findings on melatonin absorption and alterations by pharmaceutical excipients using the Ussing chamber technique with mounted rat gastrointestinal segments

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ABSTRACT

We examined how melatonin absorption was affected by pharmaceutical excipients using the Ussing chamber technique with mounted rat gastrointestinal (GI) segments. Melatonin absorption occurs throughout the GI tract, with the greatest absorption being in the rectum and ileum and the least in the stomach. Melatonin can be classified as a low permeability drug. P-glycoprotein (P-gp) does not affect melatonin absorption but transported rhodamine 123, a well-known P-gp substrate. The possibility of saturating P-gp by melatonin was excluded. Sodium cholate (0.5%) increased melatonin absorption, but decreased absorption at higher concentrations (1.0% and 5.0%). Sodium oleate (0.5% and 1.0%) consistently decreased melatonin absorption. Pharmaceutical excipients increased the absorption of Lucifer yellow (100 μg/mL), a paracellular probe but decreased the absorption of melatonin above the critical micelle concentration (cmc), suggesting that melatonin was transported mainly by transcellular pathway. Sodium cholate and sodium oleate, when above the cmc, resulted in micellar complexes as revealed by ^{1}H NMR spectra and particle size distribution. Histology tests showed mucosal damage of jejunum tissues in the presence of these excipients. The balance of tissue damage by the formation of micellar complexes could affect the melatonin absorption. This information on melatonin absorption behaviors and its modulation by pharmaceutical excipients can be used in further oral dosage formulations to affect circadian rhythm.

1. Introduction

Melatonin is an indoleamide hormone that is endogenously secreted by the pineal gland in the brain according to a day–night cycle (Lee et al., 1999). Melatonin acts as a circadian rhythm synchronizer and endogenous antioxidant (Pandi et al., 2006). Oral melatonin preparations are important since exogenous administration of melatonin can be clinically used to maintain/initiate sleep and treat circadian rhythm disorders (Lee et al., 1999). However, the bioavailability of orally administered melatonin preparations in human subjects is limited and variable (Lee et al., 1995). Melatonin is slightly soluble, has good permeability characteristics, and is in the class II category according to the Biopharmaceutics Classification System (Lee et al., 1997; Vlachou et al., 2006). Although the physical properties and numerous oral dosage forms of melatonin have been studied, an understanding of melatonin absorption is very limited. No information about the GI absorption of melatonin and its p-glycoprotein (P-gp) dependency is available.

Drug absorption is a key process governing the *in vivo* bioavailability of drugs. It is affected by the physicochemical properties of the drug, dosage form, biological state of the gastrointestinal (GI) tract, and co-administered food components (Lee et al., 1997; Wagner et al., 2001). In particular, pharmaceutical excipients can significantly modify physicochemical properties, intestinal permeability, and drug bioavailability when co-administered with a drug (Mithani et al., 1996; Vine et al., 2002; Sharma et al., 2005). Bile acids and fatty acids are generally recognized as safe (GRAS)-listed pharmaceutical excipients and are common components in food and dosage formulations. These excipients have been widely studied as absorption promoters and inhibitors of various drugs. Formation of stable micellar structure could explain changing drug absorption (Yamaguchi et al., 1986b; Sharma et al., 2005). However, no information on the potential modulation of melatonin absorption by these pharmaceutical excipients is available.

The aim of this work was to understand the *in vitro* absorption behaviors and P-glycoprotein dependency of melatonin using the Ussing chamber technique with mounted rat GI segments. The modulation of melatonin absorption by GRAS-listed sodium cholate and sodium oleate at different concentration levels was also examined.

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2. Materials and methods

2.1. Materials

Melatonin was purchased from Morepen (New Delhi, India). Absolute alcohol (99.9%) was purchased from Hayman (Witham, England). Sodium oleate, sodium cholate, and sodium chloride were purchased from Sigma (St. Louis, MO, USA). The saline solution (0.9% NaCl) was purchased from Choongwae Pharm. Inc. (Seoul, Korea). Lucifer yellow, Kreb's Ringer bicarbonate buffer and rhodamine 123 were purchased from Sigma–Aldrich (Seoul, Korea). Deionized water was used throughout the study. All other chemicals were of reagent grade and used without further purification.

2.2. Animal treatment

Male Sprague–Dawley rats weighing 300–350 g were supplied from Samtaco (Taconic, Korea). Rats were kept for a week or more before experiments to adapt to a circadian rhythm of 12 h day and night. Food and water were freely supplied to the rats, but only water was supplied for 24 h prior to experiments.

2.3. In vitro GI absorption of melatonin

Rats were sacrificed using ether. Following a midline incision, the GI tract was removed and individual segments were separated and placed in ice-cold oxygenated Kreb's Ringer bicarbonate buffer (pH 6.8). To minimize the damage of the tissues, the intestinal segments were isolated without stripping off the underlying muscle layer. The isolated GI segments of duodenum, jejunum, ileum, colon, and rectum were opened along the mesenteric border, rinsed with cold physiological saline to remove luminal contents. Care was taken to avoid the Peyers patches. The mucosa of the glandular portion of the stomach was carefully stripped from submucosa and serosa, leaving the mucosa intact for the use in the Ussing chamber (Harvard Apparatus Inc., USA). Prior to adding the test solution, the mucosal segments were equilibrated for approximately 20 min with 6 mL of preheated Kreb's Ringer solution (pH 6.8). The drug was added to the mucosal chamber as a solution (0.01%, w/v equivalent to 100 μg/mL) with or without pharmaceutical excipients, including sodium cholate or sodium oleate, at different concentrations. To fully explain the paracellular mechanism, the absorption of Lucifer yellow (100 μg/mL) was also performed in the jejunum with and without excipient (control).

To ensure oxygenation and agitation, a mixture of O_2/CO_2 (95%/5%) was bubbled through each compartment. The temperature was maintained at 37 °C with a circulating water bath during all the experiments.

2.4. P-gp dependency of melatonin

To investigate the contribution of P-gp efflux transporters on the absorption of melatonin, the melatonin transport was studied going both from mucosal side to serosal side (MS) and from serosal side to mucosal side (SM) of the jejunum, ileum, and colon. In order to examine the possibility of saturation of P-gp function, the bidirectional transport of melatonin across the ileum was examined at different concentrations: 7.5, 20 and 100 μg/mL. We also investigated the transport of 10 μg/mL rhodamine 123, a well-known P-gp substrate as the positive control.

2.5. Concentration determination of melatonin, rhodamine 123 and Lucifer yellow

Samples (200 μL) from the receptor chamber were collected at a sampling interval of 15 min for 90 min. The receptor phase was replenished with an equal volume of fresh medium after each sample was collected. The collected samples were centrifuged to remove any insoluble residue. Drug concentrations were determined by HPLC as previously described (Lee et al., 1999). However, the signals were monitored by fluorescence detector at the excitation and emission wavelengths of 290 and 348 nm, respectively. The concentration of Lucifer yellow and rhodamine 123 were measured by a spectrofluorimetric method. The excitation and emission wavelengths for Lucifer yellow were 418 and 512 nm and 485 and 546 nm for rhodamine 123, respectively.

2.6. Calculation of permeability coefficient

The apparent permeability coefficients, P_{app}, of excised intestinal segments for melatonin was calculated as follows (Martin and Martin, 2006).

$$P_{app} = Q/A \times C \times t$$

where Q is the total amount of the drug permeated throughout the incubation time, A is the diffusion area of the Ussing chamber, C is the initial concentration in the donor chamber, and t is the total time of the experiment.

2.7. Histological examination

After 90 min of the melatonin absorption test with or without the pharmaceutical excipients, jejunum tissues were used for histological examination. Upon completion of each experiment, the tissues were removed from the Ussing chambers and placed in 4% formalin. The samples were serially cross-sectioned at 5 mm thickness using a microtome and then stained with a Harris hematoxylin–eosin solution on glass slides. Histological examination was carried out at 100× magnification using an Olympus B microscope (Tokyo, Japan) and imaging software (Baumer Twain Ver. 1.0, Humin Tech Corp., Seoul) in the Department of Pathology, Kangwon National University Hospital (Chuncheon, Korea).

2.8. ^{1}H NMR analysis

The ^{1}H NMR spectra of pure compounds (melatonin, sodium oleate, and sodium cholate) and a mixture of melatonin with 0.5% and 1.0% (w/v) sodium oleate or 0.5%, 1.0%, and 5.0% (w/v) sodium cholate were recorded using a 600 MHz NMR spectrometer (Bruker Avance 600) with deuterium oxide as the solvent. The longitudinal relaxation time was measured by the inversion recovery method (π–τ–π/2). The longitudinal relaxation time (spin-lattice relaxation time) describes the exponential recovery of the equilibrium, longitudinal magnetization that is aligned with the applied magnetic field (Richardson et al., 2005).

2.9. Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical significance was determined by one way analysis of variance (ANOVA) tests using SPSS (version 16.0). LSD post hoc multiple comparison tests were then used to compare statistical significance at a 5% probability level ($p < 0.05$).

3. Results and discussion

3.1. Site dependence of melatonin absorption behavior

The site dependency of melatonin absorption through excised rat GI segments at 0.01% melatonin is given in Fig. 1. Melatonin was continuously absorbed and the absorption rate increased as

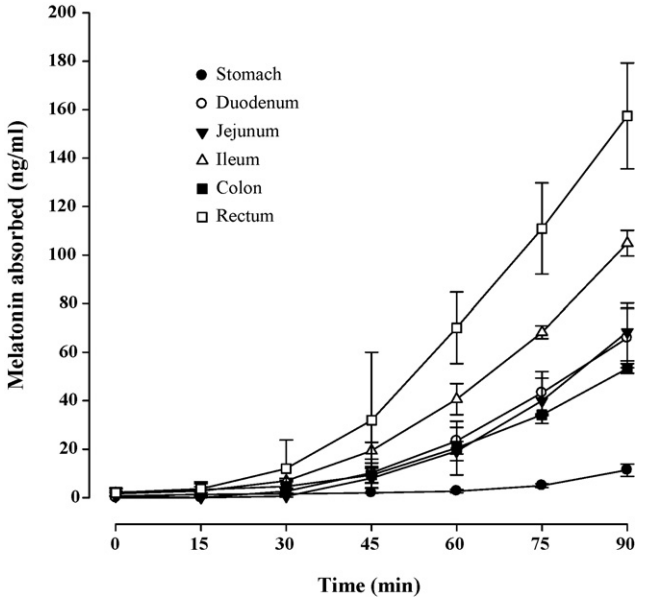


Fig. 1. Site dependency of melatonin (0.01%) absorption through excised rat gastrointestinal segments ($n = 4$).

a function of time. The cumulative absorption of a dose of 0.01% (100 μg/mL) melatonin in excised segments of the duodenum, jejunum and colon was similar at about 65.5 ± 3.4 ng/mL after 90 min. The cumulative absorption was the highest in the rectum (157.4 ± 20 ng/mL) followed by the ileum (104.8 ± 5.2 ng/mL) and three other sites (duodenum, jejunum, colon). Absorption in the stomach had the lowest value of 11.3 ± 2.6 ng/mL after 90 min.

Regional difference in intestinal drug permeability was attributed to the difference in epithelial surface area, pore radius of the paracellular pathway, mucus layer and regional membrane fluidity (Masaoka et al., 2006). The absorption of melatonin through the ileum was higher than that to the colon, following the order of epithelial surface area. The calculated values of the pore radii in the jejunum, ileum and the colon were 6.19, 5.31 and 6.23 Å, respectively; the order of melatonin absorption was ileum > jejunum > colon. The cellular membranes from proximal intestine were less fluid than distal one. However, the absorption of the drug in the proximal region was higher than that in the distal region except in the rectum, indicating that the regional membrane fluidity affected negligibly the regional difference of melatonin absorption. In addition, drug absorption is mainly affected by physicochemical properties of drugs, drug metabolism in the gastrointestinal mucosa, lipophilicity, unionized fraction of drugs, molecular size and drug solubility (Curatolo and Ochoa, 1994). Changing of these absorption parameters along the gastrointestinal tract may contribute to the differences in site-specific absorption of melatonin. Regional difference in the functional expression of P-gp might be one of the possible factors for the site-dependence absorption.

3.2. Bi-directional transport of melatonin

P-glycoprotein efflux pumps, predominantly located in the apical membranes of epithelia (e.g., small intestine, colon), significantly limit the oral absorption of many drugs (Collnot et al., 2007). To investigate the contribution of P-gp efflux transporters to the site-dependent absorption, bi-directional transport (from mucosal to serosal side (MS) and from serosal to mucosal side (SM)) of 0.01% melatonin across excised intestinal mucosa of the jejunum, ileum,

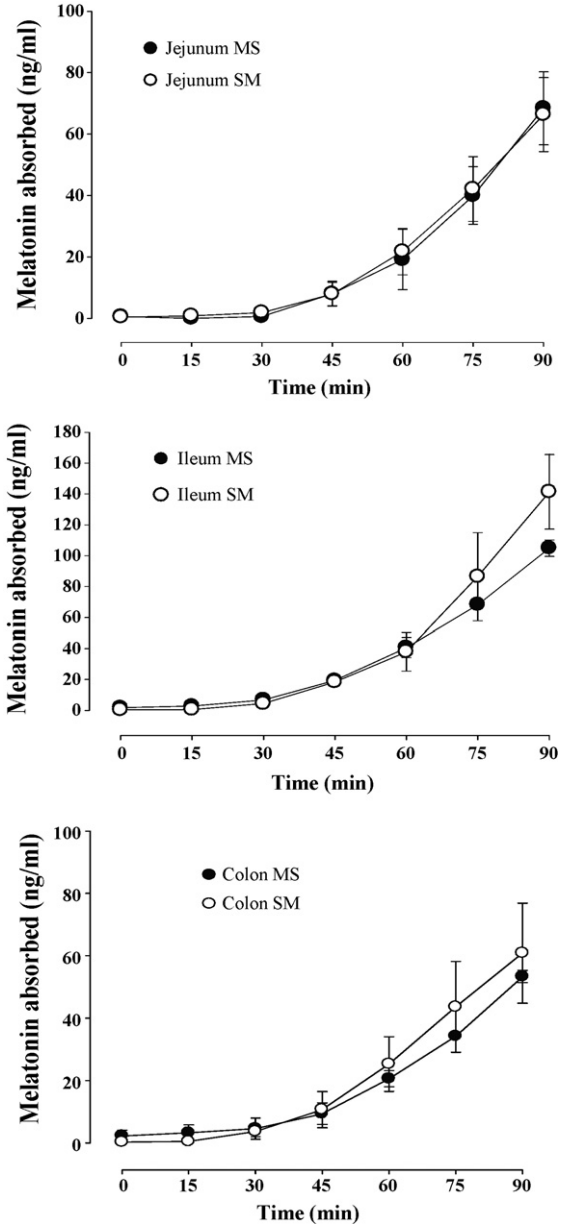


Fig. 2. Bi-directional transport of 0.01% melatonin across excised intestinal mucosa, from mucosal to serosal side (MS) and from serosal to mucosal side (SM).

and colon is given in Fig. 2. The transport of melatonin between SM and MS of the all three areas were almost identical for 90 min, suggesting no P-gp dependency for melatonin absorption at the tested concentration (0.01%).

In order to examine the possibility of saturation of P-gp function, we investigated the transport of rhodamine 123, a well-known P-gp substrate as the positive control. Bi-directional transports of 10 μg/mL rhodamine 123 (positive control) and melatonin at different concentrations (7.5, 20 and 100 μg/mL) across the ileum were illustrated in Fig. 3. The transport of 10 μg/mL rhodamine 123 in the efflux direction was greater than that in the absorption way (3126.5 ± 319.2 pg/mL versus 1052.3 ± 192.6 pg/mL after 90 min). The transport of melatonin at higher concentration was also greater than that of the lower concentrations at the both sides. However, the different concentrations did not induce the difference in the bi-directional transport of melatonin. Thus, P-gp efflux pumps do not affect melatonin absorption and the possibility of saturating P-gp by melatonin should be excluded. There is no drug secretion or any

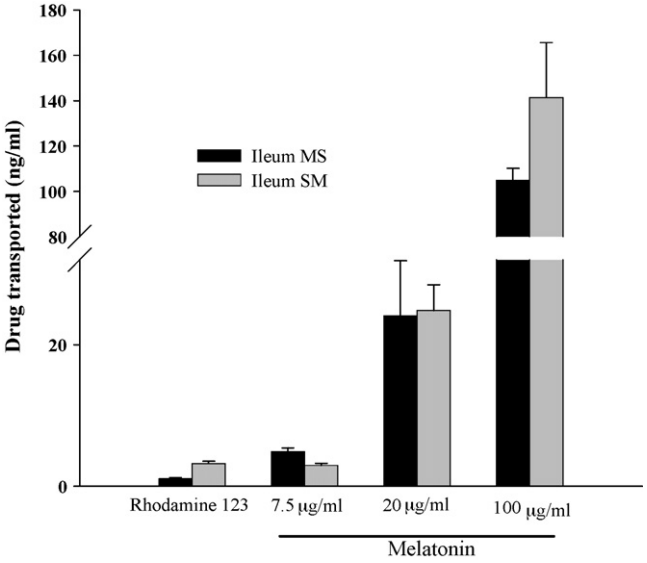


Fig. 3. Bi-directional transport of 10 μg/mL rhodamine 123 and melatonin at different concentrations across excised ileum from mucosal to serosal side (MS) and from serosal to mucosal side (SM).

efflux transporter implicated in the absorption of melatonin across intestinal membranes.

3.3. Effect of pharmaceutical excipients

The effect of sodium cholate on melatonin absorption through the jejunum is given in Fig. 4. Low (0.5%) sodium cholate increased the cumulative melatonin absorption compared to melatonin alone (85.6 ± 6.7 ng/mL and 65.5 ± 3.4 ng/mL, respectively) for up to 90 min. However, the levels of sodium cholate (1.0% or 5.0%) higher than cmc decreased melatonin absorption (48.9 ± 4.7 and 42.3 ± 7.4 ng/mL, respectively).

Bile salts were reported to enhance the intestinal absorption of many poorly water-soluble drugs, such as griseofulvin and salicylate (Yamaguchi et al., 1986a). However, sodium cholate inhibits nadolol absorption via stable micellar formation, decreasing uptake into the intestinal membrane (Yamaguchi et al., 1986a,b,c). Micelle formation of lipolytic products by bile salts is important for normal fat digestion and absorption, but the micellar complex is not

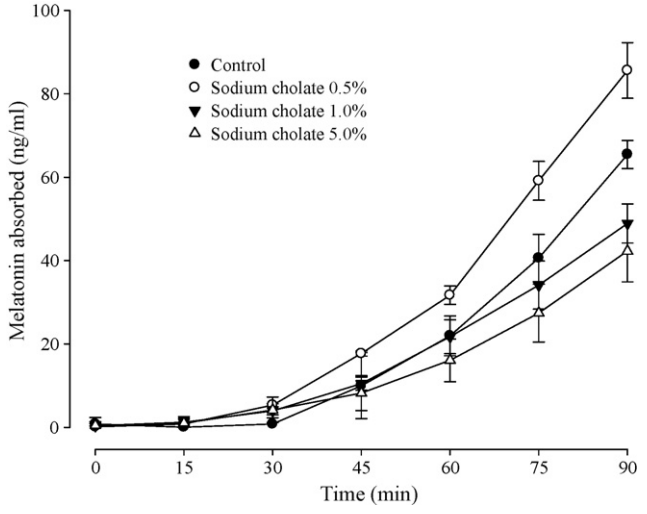


Fig. 4. Effect of sodium cholate concentrations on melatonin absorption in the jejunum. Control: drug absorption without excipient ($n = 4$).

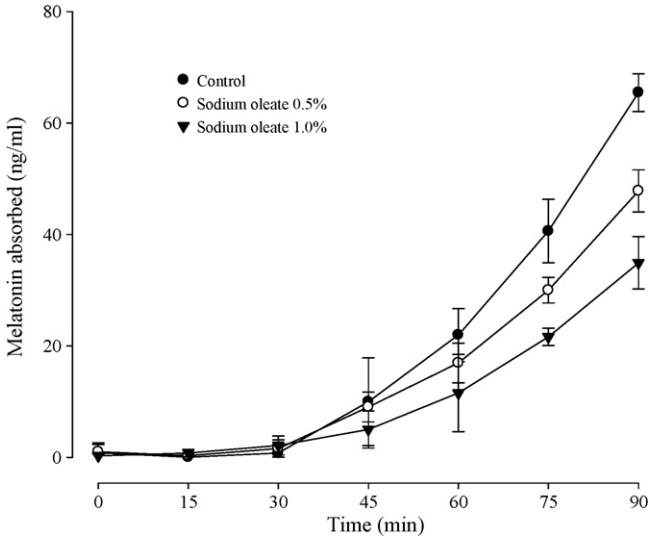


Fig. 5. Effect of sodium oleate concentrations on melatonin absorption in the jejunum. Control: drug absorption without excipient ($n = 4$).

absorbed due to its relatively high molecular weight and reduced lipophilicity (Yamaguchi et al., 1986b).

Fatty acids moderately change the active and passive transport properties of excised rat jejunum (Vine et al., 2002; Sharma et al., 2005). Micellar complexes of fatty acids with GI components and changes in drug solubility and partitioning might contribute to increased intestinal drug absorption (Dangi et al., 1998). Sodium oleate is also an absorption enhancer in the large intestine (Tomita et al., 1988). Interestingly, sodium oleate, a hydrophobic monounsaturated fatty acid, decreased melatonin absorption at 0.5% (47.8 ± 3.8 ng/mL) and 1.0% (34.9 ± 4.7 ng/mL) after 90 min (Fig. 5), with greater efficacy than sodium cholate. The experiment could not be performed at 5% sodium oleate because the salt is too viscous at this concentration.

Although fatty acids primarily enhance the intestinal absorption of hydrophilic drugs through the paracellular pathway (Zornoza et al., 2004), sodium oleate failed to enhance melatonin absorption at the tested concentrations. Thus, the drug might be transported via a transcellular pathway rather than the paracellular pathway. Sodium caprate not only enhance transcellular and paracellular absorption, but also reduce basolateral to apical secretion of a P-gp substrate, epirubicin (Lo and Huang, 2000). However, melatonin transport is independent of P-gp efflux transporters (see Fig. 2).

Table 1 summarizes *in vitro* melatonin permeability through excised rat intestine after 90 min. Sodium oleate decreased melatonin permeability at both concentrations, while sodium cholate increased the permeability at a low concentration but decreased the permeability coefficient at higher concentrations. Based on the in vitro permeability class of 20 drugs in rat intestine (Kim et al.,

Table 1
Permeability coefficients of melatonin across rat jejunum in the Ussing chamber using Kreb's Ringer buffer solution by co-incubation with excipients ($n = 4$).

Co-incubation	P_{app} (cm/s) × 10^{-6}
Control	2.31 ± 0.12
Sodium oleate 0.5%	1.69 ± 0.13*
Sodium oleate 1.0%	1.24 ± 0.10**
Sodium cholate 0.5%	3.03 ± 0.24*
Sodium cholate 1.0%	1.73 ± 0.17*
Sodium cholate 5.0%	1.50 ± 0.26*

* $p < 0.05$ compared with the control.
** $p < 0.01$ compared with the control.

2006), we established the dynamic range of the variation of permeability values with and without the excipients. For example, cimetidine ($0.105 \pm 0.06 \times 10^{-4}$ cm/s) was classified as a low permeability compound. Permeability values of melatonin with and without excipients were lower than that of cimetidine. Thus, melatonin can be classified as a low permeability drug.

3.4. Absorption-modulating mechanism by pharmaceutical excipients

We investigated the absorption of Lucifer yellow (100 μg/mL), a paracellular probe in the jejunum to fully explain the paracellular mechanism. Effect of sodium cholate and sodium oleate on the transport of Lucifer yellow, a non-P-gp substrate and a marker compound of paracellular transport across the jejunal membrane is shown in Fig. 6. The transport of 100 μg/mL Lucifer yellow (11.0 ± 2.5 ng/mL) significantly increased by the addition of 1.0% sodium oleate (30.8 ± 1.3 ng/mL) and sodium cholate at low (0.5%) and high (5.0%) concentration (27.5 ± 5.1 and 46.7 ± 1.4 ng/mL, respectively). It was also reported that the paracellular transport of Lucifer yellow increased by more than 25 fold in the presence of 3 mM bile salts due to the opening of tight junctions (Catalioto et al., 2008). Thus, sodium oleate and sodium cholate enhanced the paracellular transport at these concentrations due to the changes in membrane integrity and mucosal damages. However, these excipients decreased the absorption of melatonin above the cmc, suggesting that melatonin was transported mainly by transcellular pathway.

We also examined the histological changes in intestinal membranes and micellar complexes formation induced by the excipients. Fig. 7 shows histological images of the jejunum at 90 min after exposure to melatonin alone (control) or melatonin with different concentrations of excipients. Histological images of intestinal segments treated with sodium oleate (0.5%, 1.0%) and sodium cholate (0.5%, 1.0%, 5.0%) demonstrate denudation of villous tips and extrusion of single cells and groups of cells from villi. The extent of histological damage varied at different concentrations of excipients. Sodium cholate caused more tissue damaged at 0.5%

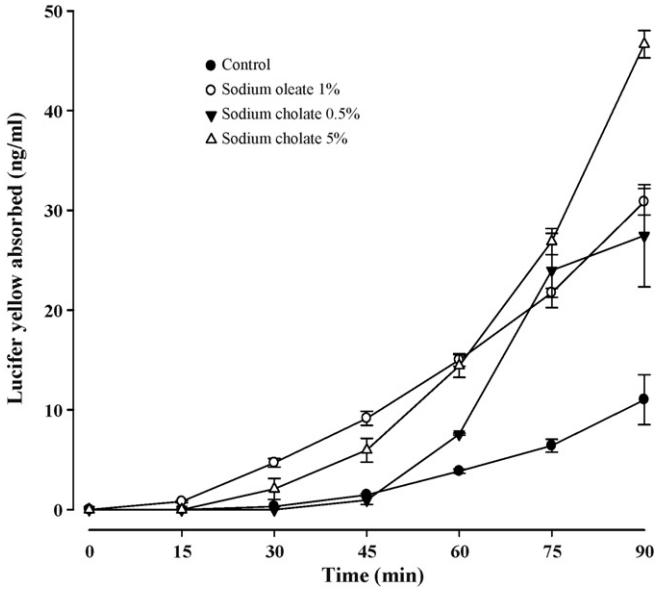


Fig. 6. Effect of sodium oleate (1.0%) and sodium cholate (0.5% and 5.0%) on the transport of Lucifer yellow (100 μg/mL) in the ileum. Control: drug absorption without excipient.

than at higher concentrations (1.0% and 5.0%). Local acute damage to the intestine was characterized by the sloughing of cells from the tip of the villi and by reduction and broadening of the villi (Curatolo and Ochoa, 1994). These histological changes in the intestinal tissue are the primary cause of enhanced drug absorption.

Tissue damage might result from altered composition of the excised rat intestinal membrane by releasing membrane components from intestinal tissue. In a previous study, sodium cholate at concentrations of 5 mM (0.215%), 10 mM (0.43%), and 20 mM (4.31%) released phospholipids and proteins by solubilizing the membrane component (Kojima et al., 1977). In addition, bile acids readily undergo auto-oxidation, producing free radicals that in turn

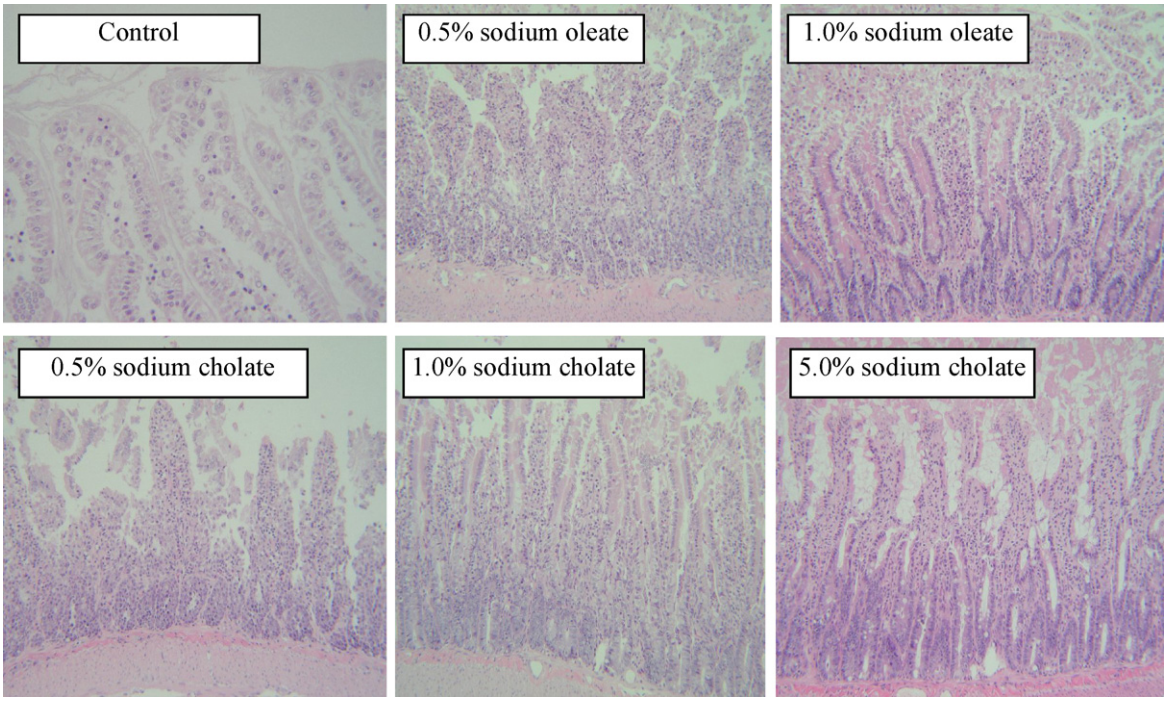


Fig. 7. Histological images of jejunum at 90 min after exposure to solutions of melatonin alone (control) or melatonin containing different concentrations of excipients.

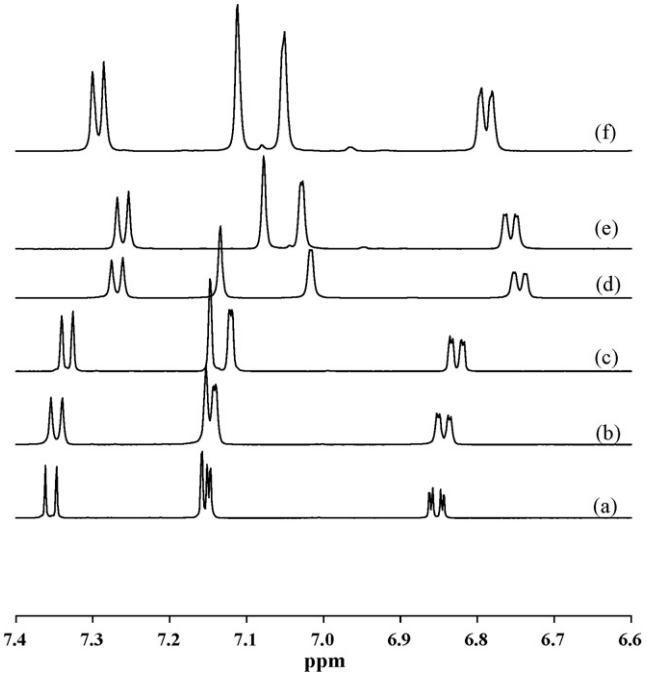


Fig. 8. ^{1}H NMR of melatonin (a) and the mixture of melatonin with 0.5% sodium cholate (b); 1.0% sodium cholate (c); 5.0% sodium cholate (d); 0.5% sodium oleate (e); and 1.0% sodium oleate (f).

cause oxidative damage to the small intestine epithelium (Tan et al., 1999). Fatty acid salts, such as sodium caprate, disrupt and detach the cell monolayer at 50 mM (0.96%), but no mucosal damage was observed *in vivo* (Cebrian et al., 2005). Melatonin absorption was decreased in the presence of 1.0% and 5.0% sodium cholate and 0.5% and 1.0% sodium oleate, although mucosal damage still occurred. One explanation is that tissue damage is compensated for by forming stable micellar complexes above the cmc.

To study molecular interactions of drugs with excipients for stable micellar complex formation, ^{1}H NMR analyses were performed. Fig. 8 shows the ^{1}H NMR chemical shifts of melatonin with and without excipients. Several characteristic peaks were observed in the melatonin spectrum, and the protons of the aromatic ring are observed at 7.15 ppm, a triplet peak integrated for three protons. This triplet peak was maintained when mixed with 0.5% sodium cholate, but it was separated in the mixture with 0.5% and 1.0% sodium oleate and 1.0% sodium cholate, and was broadly separated at 5.0% sodium cholate. The molecular motion of hydrophobic groups of melatonin was limited in D_2O, thus causing peak broadening of melatonin. In DMSO, the triplet peaks remained in all mixtures (data not shown). The longitudinal relaxation time measurement allowed us to identify the protons of melatonin in the mixtures (Table 2). In the presence of 1.0% sodium oleate and 5.0% sodium cholate, relaxation times relative to melatonin alone were shortened at aromatic ring protons and methoxy protons, while the relaxation times of protons of the side chain (C10, C11 proton signals) were unchanged or increased. In contrast, 0.5% sodium cholate significantly increased relaxation times for melatonin at C11 and C5. Thus, the aromatic ring protons of melatonin were more immobilized in the presence of higher concentrations of the excipients, whereas 0.5% sodium cholate did not immobilize these aromatic ring protons. Accordingly, the hydrophobic portions of sodium cholate or sodium oleate and melatonin interact with each other.

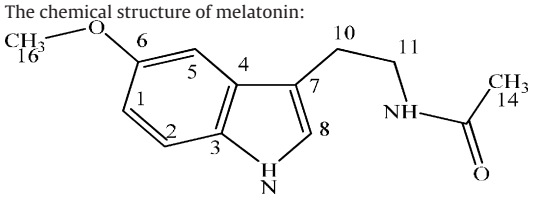
Micellar complexes are formed at these concentrations because the cmc of sodium oleate (0.146%) and sodium cholate (0.560%) are known. The micelles would form complexes with melatonin and immobilize the aromatic ring protons of melatonin in aqueous medium. A mixture of sodium taurocholate with sodium oleate at a lower concentration (0.25%) enhanced colonic absorption of cefmatazole by increasing membrane pore size (Tomita et al., 1988). Also, sodium caprate is an absorption-enhancing agent at concentrations in the vicinity of its cmc (Cebrian et al., 2005; Zornoza et al., 2004). However, higher concentrations in ^{1}H NMR analysis showed increased micellar formation at 1.0% and 5.0% sodium cholate and 0.5% and 1.0% sodium oleate and drug encapsulation, leading to decreased effective concentrations of the free drug for transport through membranes (Ingels et al., 2004). This complex formation occurred between sodium cholate and nadolol, where the longitudinal relaxation times of aromatic ring protons of nadolol calculated from ^{1}H NMR spectra were shortened by about 10% in the presence of sodium cholate (Yamaguchi et al., 1986c). Cyclosporine A transport decreased from fasted state simulated intestinal fluid after encapsulation or interaction of the drug with the sodium taurocholate/lecithin mixed micelles (Ingels et al., 2004). In another study, micellar formation at concentrations of 10–20% labrasol trapped the drug in the micelles with increasing concentration, leading to decreased transport of rhodamine 123 across the intestinal membranes at high concentrations of labrasol (Lin et al., 2007).

In addition to ^{1}H NMR analysis, particle size of the drug solution containing sodium oleate was measured by dynamic light scatter-

Table 2
Longitudinal relaxation time (s) and chemical shifts (ppm) of melatonin with and without pharmaceutical excipients.

	C10	C11	C16	C1	C5		C2
Melatonin alone	2.874 ppm 0.68 ± 0.06	3.406 ppm 0.54 ± 0.11	3.825 ppm 1.78 ± 0.27	6.858 ppm 4.19 ± 0.01	7.159 ppm 4.47 ± 0.74		7.349 ppm 11.46 ± 5.4
Melatonin + 1.0% sodium oleate	2.851 ppm 0.68 ± 0.04	3.408 ppm 0.61 ± 0.07	3.785 ppm 1.03 ± 0.17	6.795 ppm 1.48 ± 0.10	7.051 ppm 1.26 ± 0.23	7.114 ppm 2.04 ± 0.22	7.289 ppm 1.61 ± 0.14
Melatonin + 0.5% sodium cholate	2.874 ppm 0.65 ± 0.08	3.406 ppm 4.35 ± 0.11	3.822 ppm 1.25 ± 0.21	6.850 ppm 2.78 ± 0.31	7.154 ppm 18.89 ± 0.25		7.342 ppm 8.65 ± 0.13
Melatonin + 5.0% sodium cholate	2.830 ppm 0.67 ± 0.09	3.391 ppm 0.98 ± 0.16	3.805 ppm 0.80 ± 0.01	6.735 ppm 0.32 ± 0.07	7.016 ppm 1.80 ± 0.43	7.134 ppm 3.65 ± 0.05	7.263 ppm 7.15 ± 1.12

The chemical structure of melatonin:



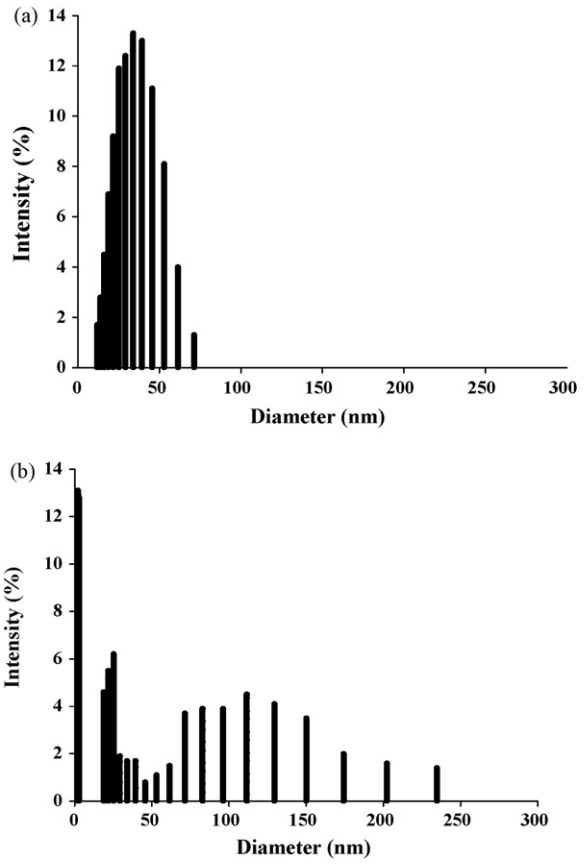


Fig. 9. Particle size of 0.5% (a) and 1.0% (b) sodium oleate in buffer (pH 6.8).

ing (Otsuka, Japan). Fig. 9 shows that sodium oleate forms micellar complexes. The 1.0% concentration gave a much wider and larger size distribution (120 nm) than the 0.5% concentration (40 nm). Regardless of tissue damage, the higher concentration of sodium oleate decreased melatonin absorption significantly because more drugs could be entrapped in the larger stable micelles.

4. Conclusions

Melatonin absorption occurs throughout the GI tract but shows absorption site dependency. The extent of absorption was highest in the rectum followed by the ileum. However, the absorption behaviors of melatonin are more complicated when simultaneously dosed with excipients. Melatonin absorption varied by the concentration of excipient. Tissue damage, formation of micellar complexes as characterized by ^{1}H NMR analysis, and particle size distribution could affect the melatonin absorption rate. For example, higher concentrations of sodium cholate and sodium oleate decreased melatonin absorption via formation of micellar complexes despite histological tissue damage. The new insights in absorption behaviors of melatonin and effects of common pharmaceutical excipients could be useful for oral dosing in clinical pharmacokinetics as well as designing future oral formulations.

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