Pharm Res (2014) 31:1676–1688
DOI 10.1007/s11095-013-1272-4

RESEARCH PAPER

Commonly Used Excipients Modulate UDP-Glucuronosyltransferase 2B7 Activity to Improve Nalbuphine Oral Bioavailability in Humans

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Received: 2 October 2013 / Accepted: 19 December 2013 / Published online: 14 February 2014

ABSTRACT

Purpose Nalbuphine (NAL) is a potent opioid analgesic, but can only be administered by injection. The major aim of this study was to develop an oral NAL formulation employing known excipients as UDP-glucuronosyltransferase 2B7 (UGT2B7) inhibitors to improve its oral bioavailability.

Methods Twenty commonly used pharmaceutical excipients were screened *in vitro* by using liver microsomes to identify inhibitors of UGT2B7, the major NAL metabolic enzyme. Tween 20 and PEG 400 were potent UGT2B7 inhibitors and both were co-administered (Tween-PEG) with NAL to rats and humans for pharmacokinetic and/or pharmacodynamic analyses.

Results In animal studies, oral Tween-PEG (4 mg/kg of each) significantly increased the area under the plasma NAL concentration-time curve (AUC) and the maximal plasma concentration (C_{max}) by 4- and 5-fold, respectively. The results of the pharmacodynamic analysis were in agreement with those of the pharmacokinetic analysis, and showed that Tween-PEG significantly enhanced the analgesic effects of orally administered NAL. In humans, oral Tween-PEG (240 mg of each) also increased NAL C_{max} 2.5-fold, and AUC by 1.6-fold.

Conclusions Tween-PEG successfully improved oral NAL bioavailability and could formulate a useful oral dosage form for patient's convenience.

KEY WORDS bioavailability · excipient · nalbuphine · pharmacodynamic · pharmacokinetic

ABBREVIATIONS

CET	Cold ethanol tail-flick
ESI	Electrospray ionization
FDA	Food and Drug Administration
GRAS	Generally recognized as safe
GSP	Galactose single point
HLM	Human liver microsome
IIG	Inactive ingredient guide
M3G	Morphine-3-glucuronide
NAL	Nalbuphine
PEG	Polyethylene glycol
PVP	Polyvinyl pyrrolidone
RLM	Rat liver microsome
Tween-PEG	The combination with equal dose of Tween 20 and PEG 400
UGT	Uridinyl diphosphate glucuronosyltransferase
UPLC–MS/MS	Ultra-high performance liquid chromatography tandem mass
WHO	World Health Organization

INTRODUCTION

Pharmaceutical excipients have long been considered inert substances, safe for use in drug formulations (1). Since the 1950s, the United States FDA has published lists of GRAS substances and the IIG in the Federal Register (2). The current GRAS and IIG lists identify hundreds of substances

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including many excipients that are universally added to foods, drugs, cosmetics, and nutraceuticals in daily use.

According to their physicochemical properties, excipients serve various functions in the drug manufacturing process, for example, as surfactants, stabilizers, co-solvents, and lipid-based vectors. However, several reports have identified many commonly used excipients that may significantly influence drug metabolism and disposition, even below the doses suggested as safe by the WHO or the FDA (2–4). Drug-excipient interactions have been confirmed by both *in vitro* and *in vivo* studies (5–7), in which it has been reported that excipients such as Cremophor, Tween 80, and Solutol HS15 can increase plasma levels of certain drugs, including digoxin, colchicine, and midazolam. Some excipients may modify drug pharmacokinetic properties through regulation of metabolic enzymes or membrane transporters (8–10).

Although there is a potential risk of excipient-active ingredients interactions during drug treatments, the proper use of common excipients may improve drug therapeutic outcomes by modulating enzyme/transporter activities. In recent years, the global market for pain-relief medication has diversified and undergone constant expansion (11). The design of convenient oral formulations of opioid analgesics, which address the low bioavailability afforded by oral administration, has become a popular area of pain-relief research because such formulations can improve patient compliance (12,13). Thus, we adapted the injectable opioid nalbuphine (NAL, Nubain®) to develop a new oral formulation, by employing excipients for improving oral bioavailability.

NAL is a semi-synthetic narcotic that is equipotent to morphine in clinical use (14). It can be administered to relieve moderate to severe pain in cases of conditions such as migraine headache, parturition, postoperative pain, and lower-back pain (15,16). With mixed agonist-antagonist activities on κ and μ opioid receptors, NAL also has the potential to be useful in opioid replacement therapy for drug addiction (17). NAL has a ceiling effect at doses higher than 0.5 mg/kg in men (18), which effectively prevents respiratory suppression. Thus, because of a low incidence of severe side effects and no association with drug addiction (18), NAL is not categorized as a controlled drug. Furthermore, owing to the fact that there are no reports of NAL-associated gastrointestinal bleeding or adverse renal effects, NAL could have advantages over non-steroidal anti-inflammatory drugs for general self-medication.

Because of its low oral bioavailability, NAL cannot be administered orally (19,20). The drug is effectively absorbed in the gastrointestinal tract, and its poor oral bioavailability predominantly results from an effective first-pass metabolism (14). UDP-glucuronosyltransferase 2B7 (UGT2B7), an enzyme belonging to the phase II metabolic system, is the primary enzyme for NAL biotransformation, producing nalbuphine-3-glucuronide as the major metabolite (21,22). Several studies have investigated the usefulness of orally-administered NAL in the relief of acute and chronic pain associated with dental extraction, orthopedic surgery, and cancer treatment (23–25). However, the clinical application of NAL has remained restricted to parenteral dosage forms because of its substantial first-pass metabolism.

In this study, we performed *in vitro* screening of 20 commonly used excipients in human and rat liver microsomes with 4 concentrations to examine their enzyme modulation effects. The excipients identified as effective enzyme inhibitors *in vitro* were then chosen as candidates for oral administration with NAL in rats. The analgesic effects of orally administered NAL with the selected excipients were also evaluated in a pharmacodynamic study. Based on the results obtained from the animal studies, we conducted an exploratory clinical trial in 12 healthy volunteers with 3 different excipient combinations to evaluate the novel oral NAL formulations.

MATERIALS AND METHODS

Materials

NAL was supplied by Yung-Shin Pharmaceutical Ind. Co., Ltd. (Taichung Hsien, Taiwan). Morphine, morphine-3-glucuronide and naloxone were purchased from the Narcotic Bureau, Department of Health, Executive Yuan of Taiwan. All chemicals and solvents were of analytical or liquid chromatography-mass spectrometry (LC–MS) grade. Acetonitrile, methanol, and diethyl ether were obtained from Merck (Darmstadt, Germany). Series products of Tween and PEG were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solutol HS15, Cremophor EL, and Cremophor RH40 were gifts from BASF Co., Ltd. (Taipei, Taiwan). All other excipients were supplied by Lotus Pharmaceutical Ind. Co., Ltd. (Taipei, Taiwan). Water was prepared using a Milli-Q water purification system (Millipore Corporation, Billerica, MA, USA).

Methods

Screening for UGT2B7 Inhibitors

Human and Rat Liver Microsome Preparation. With approval from the Ethics Committee of Tri-Service General Hospital Institutional Review Board (Taipei, Taiwan), human liver samples were obtained from 6 male liver surgery patients; informed consent of the patients was obtained before obtaining the samples. Rat liver samples (10 g) were obtained from 8-week-old Sprague–Dawley® (SD) rats weighing 250±20 g. The procedure for microsome preparation followed that described in a previous report, involving the use of a differential centrifugation technique with slight modifications (26). The liver microsomes prepared were harvested and resuspended in an equal volume of phosphate buffer (0.1 M, pH 7.4). The levels

of liver microsomal protein were quantified using the Lowry method. The final human liver microsomes (HLMs) and rat liver microsomes (RLMs) prepared exhibited protein concentrations of approximately 20 and 25 mg/mL, respectively, and were stored at −80°C prior to use.

In Vitro Metabolism Study. Morphine is a well-known specific substrate for determining UGT2B7 activity (27). Morphine-3-glucuronide (M3G), a major conjugated metabolite of morphine, is generated by UGT2B7 in the gut and the liver. We used morphine as a probe for *in vitro* screening of 20 commonly used excipients in the HLM and RLM systems. The excipients' inhibitory effects on UGT2B7 were determined by measuring the glucuronidation of morphine to M3G.

Morphine was incubated at an initial concentration of 500 μM in the presence of a range of excipient concentrations (0.425%, 0.0425%, 0.00425%, or 0.000425% w/v). The tricyclic antidepressant diazepam (85 μM) was used as the positive control (28). Assays were conducted in phosphate buffer (0.1 M, pH 7.4) containing $MgCl_2$ (2.5 mM), alamethicin (10 μg/mL), and uridine diphosphate glucuronic acid (2.5 mM) at 37°C. Incubations were run for 30 min, and then stopped by addition of cold acetonitrile (500 μL), vortexed, and placed on ice. After addition of naloxone (20 μg/mL), the samples were centrifuged at 4,000×g for 10 min, and the supernatant (200 μL) was then dried under a stream of nitrogen at 40°C before M3G analysis.

M3G was quantitatively determined by LC-MS analysis using a silica column (15×4.6 mm, 3 μm) with a mobile phase of 10 mM sodium acetate in acetonitrile (25% v/v, pH 4.4). The flow rate was maintained at 0.4 mL/min and the retention times for M3G and the internal standard (IS), naloxone, were 4.6 and 1.3 min, respectively.

The effects of a 1:1 mixture of Tween 20 and PEG 400 on HLMs and RLMs enzyme activity were also investigated under the aforementioned conditions, using concentrations ranging from 4.25×10^{-5}% to 0.425% (w/v) to determine the IC_{50} for UGT2B7 inhibition.

Animal Studies of Orally Administered NAL

Animals. Male SD rats with body weights between 250 and 300 g were purchased from the National Laboratory Animal Center (Taipei, Taiwan). Surgically modified male SD rats for the portal vein administration study were obtained from BioLASCO Taiwan (Taipei, Taiwan) and housed one animal per cage. All rats were housed in a room with air and humidity control, with a 12-h light-dark cycle. The rats had *ad libitum* access to food and water throughout the experiments, all of which were conducted in accordance with the Principles of Laboratory Animal Care (National Institutes of Health) for the treatment of animals.

Pharmacokinetic Studies in Rats. We used equal doses of Tween 20 and PEG 400 (2, 3, 4, 8, or 12 mg/kg), named Tween-PEG, to determine these excipients' effects on the oral bioavailability of NAL in the rats. The rats were anesthetized using ether and connected to a jugular catheter for periodic blood sample collection, for up to 8 h following dose administration. In the dose escalation studies, the rats in the study group ($n\geq6$ per group) orally received 20 mg/kg NAL combined with a range of Tween-PEG in 0.9% saline. The rats in the control group ($n=16$) received oral NAL only.

To elucidate the excipient's mechanism of action, separate groups of rats were orally dosed with NAL (20 mg/kg) and the study group was administered Tween-PEG (4 mg/kg of each in a total volume of 5 mL/kg) via their portal vein (i.p.v.), while the control group received no Tween-PEG i.p.v. Twelve blood samples (approx. 0.25 mL each) were collected from the jugular vein cannula prior to dosing, and at 5, 10, 20, 40, 60, 120, 180, 240, 360, 480, and 720 min after drug administration. Blood samples were collected in microcentrifuge tubes containing 20 μL of 10 IU heparin. After collection, the isolated plasma samples were centrifuged at 13,000×g for 10 min, and stored at −80°C until assay.

Rat Plasma Sample Preparation. A simple liquid-liquid extraction was applied for NAL extraction from plasma samples. Briefly, a 0.1-mL aliquot of rat plasma sample was spiked with 50 μL of IS (naloxone: 250 ng/mL) before adding 50 μL of 1 N Na_2CO_3 (pH 10). Extraction solvent (6 mL *n*-hexane:isoamyl alcohol = 9:1) was added and the sample was vortexed for 3 min and centrifuged at 3,000×g for 5 min. The upper organic phase was transferred to a clean glass tube. The solvent was evaporated to dryness at 40°C under a gentle stream of nitrogen (Zymark® MA, USA). The residue was reconstituted in 100 μL of the mobile phase and vortexed for 30 s. Finally, the reconstituted solution was transferred to autosampler vials, and 5 μL was injected into the ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) system for NAL analysis (described below).

Pharmacodynamic Studies in Rats. Pharmacodynamic studies were conducted to compare the analgesic effects of orally administered NAL with those of intramuscular (i.m.) NAL injection in rats. The cold ethanol tail-flick (CET) latency test was employed in accordance with previously described procedures (29). A custom-designed circulation system (BL-110; Yie-der, Taipei, Taiwan) was used for testing with ethanol at −20°C. All animals ($n=6$ per group) were tested at 15, 25, and 35 min prior to medication, to obtain an average baseline CET latency. Each animal therefore served as its own control, and experimental values were compared with the pre-administration baseline values for each rat. The rats in the parenteral dosing groups received 2.5, 5, or 10 mg/kg NAL i.m. in the contralateral hind legs. The non-parenteral groups

were orally administered with 50, 100, or 200 mg/kg NAL concomitantly with Tween-PEG (4 mg/kg of each). The tail-flick latencies of antinociceptive thresholds were measured over 4 h at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after drug administration. To minimize damage to each animal's tail, a predetermined cutoff time of 40 s was used, and we defined this as the maximum latency. The antinociceptive effects of NAL were converted to percentages of maximum possible analgesia (% MPA) using the following formula:

$$\%\ \mathrm{MPA} = [(\mathrm{Test}-\mathrm{Baseline})/(\mathrm{Cutoff}-\mathrm{Baseline})] \times 100$$

Human Clinical Trial

Participants. Twelve male participants, aged 22–35 years, were recruited from the local community by referral and advertisement. The participants were in good general health according to routine medical screening, which included a physical examination, 12-lead electrocardiogram, hematology, and urinalysis testing. The study was approved by the Ethics Committee of Tri-Service General Hospital Institutional Review Board (Taipei, Taiwan) and conducted according to the ethical principles of the Declaration of Helsinki. All volunteers gave written informed consent prior to participation in the study.

Pharmacokinetic Studies of Orally Administered NAL in Healthy Volunteers. We conducted a single-center, open-label and parallel design pilot clinical trial to investigate the pharmacokinetic properties of a single oral dose of NAL (66 mg) administered with or without 3 different combinations of Tween-PEG in healthy volunteers. Similarly to the pharmacokinetic study in rats, equal amounts of Tween 20 and PEG 400 (120, 240, or 360 mg) were administered to determine their effects on oral NAL bioavailability in humans. Each participant was encouraged to complete at least 2 of the 4 study periods. The clinical study was performed at the Clinical Research Center of Tri-Service General Hospital. In addition to the general tests performed during the screening visit, residual liver function was tested for all participants after an overnight fast using the GSP method (30). Approximately 24 mL of blood was collected as a blank sample before the start of each study period. After dosing, participants remained at the Clinical Research Center for 24 h. Blood samples (8 mL) were collected at 5, 10, 15, 20, 30, and 45 min, and again at 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after administration of the study medication. Blood plasma was obtained by centrifuging blood samples at 1,500×g for 10 min. Samples were stored at −80°C until required for analysis.

Human Plasma Sample Preparation. NAL and its metabolites were extracted from human plasma samples by using a simple and rapid solid-phase extraction method. Briefly, a 0.5 mL aliquot of human plasma was spiked with 50 μL of IS (naloxone, 250 ng/mL). The sample was vortexed for 10 s, and then centrifuged at 12,000×g for 10 min. The supernatant was loaded onto a methanol pre-activated Oasis HLB 1 mL (30 mg) extraction cartridge (Milford, MA, USA) that had been washed with 1 mL of 0.5% (v/v) methanol in water. NAL and its metabolites were eluted from the cartridge with 1 mL of 40% (v/v) acetonitrile in isopropanol. The samples were heated to dryness at 40°C under a gentle stream of nitrogen, to remove traces of eluent. The residue was reconstituted with 100 μL of mobile phase and vortexed for 30 s. Finally, the reconstituted solution was transferred to autosampler vials and conditioned at 4°C. Sample solutions (3 μL) were injected into the UPLC–MS/MS system for analysis, as described below.

Analytical Assay of NAL

NAL and naloxone were analyzed using UPLC (Waters Acquity^{TM} Milford, MA, USA) coupled to a Biosystems-Sciex API 3000 series triple-quadrupole mass spectrometer (Foster City, CA, USA) with an electrospray ionization (ESI) interface. Chromatographic separation was conducted using a C18 column (Waters Acquity UPLC BEH C18, 2.1×100 mm, 1.7 μm), and a mobile phase comprising solvent A (2 mM ammonium formate and 0.1% formic acid in water) and solvent B (2 mM ammonium formate and 0.1% formic acid in acetonitrile). The total run time was 8.5 min and the column temperature was maintained at 35°C. The gradient program governing the mobile phase composition and the elution flow rate was as follows: 85% A at 0.25 mL/min (0−3 min); 85%−10% A at 0.25−0.3 mL/min (3−3.5 min); 10% A at 0.3 mL/min (3.5−5.5 min); and 10%−85% A at 0.3−0.25 mL/min (5.5−6 min). The final mobile phase composition was maintained for 2.5 min to stabilize the chromatographic system. The retention times of NAL and naloxone were 2.8 and 1.6 min, respectively.

Tandem MS acquisition was performed in positive ESI mode, and a multiple reaction monitoring mode was used for quantification of analytes. The precursors to production ion transitions in the analytes were 358.1→340.1 for NAL and 328.3→310.3 for naloxone. The ion-source temperature was maintained at 420°C, and the ion spray voltage was set to 5.0 kV. The dwell times were set to 250 ms per channel for all determined compounds. Analyst 1.4.2 software (Applied Biosystems-Sciex; Foster City, CA, USA) was used to collect and process the MS/MS data.

Statistical Analysis

Plasma NAL concentration *versus* time data were analyzed by applying a non-compartmental pharmacokinetic model, using WinNonlin 5.0.1 software (Pharsight, Mountain View, CA, USA). The statistical significance of data obtained from the pharmacokinetic and pharmacodynamic studies was determined using one-way analysis of variance (ANOVA). All results are expressed as means ± standard error, and were analyzed using SPSS software (version 10; SPSS Inc., Chicago, IL, USA). Statistical significance was determined by $p<0.05$.

RESULTS

Assay Conditions for NAL and its Metabolites

The specific UPLC–MS/MS analytical method used for NAL determination was fully validated according to the United States FDA criteria for Bioanalytical Method Validation. The standard calibration curves for NAL were linear ($r^2 \geq 0.996$) over the concentration range of 0.5–50 ng/mL. The precision and accuracy of quality control (QC) sample determinations were all within an acceptable range, as determined from the relative standard deviation and relative error. The within-run and between-run precisions were 1.7%−10.2% and 2.6%−8.6%, respectively, and the within-run and between-run accuracies were -6%–13.3% and -3.4%−7.1%, respectively. The QC standards were acceptable, and did not deviate by more than ±15% from nominal. These findings indicated that the method was precise and accurate within a concentration range that was relevant for this study. Thus, the method was sufficiently reliable for analysis of the pharmacokinetic samples in this study.

Excipient-Mediated Modulation of UGT2B7 Activity

The relative effects of the 20 commonly used excipients on UGT2B7 activity were determined *in vitro* using HLMs and RLMs incubated with a specific substrate (morphine). The percentage UGT2B7 inhibition was determined for a series of excipient concentrations, in the presence of 0.5 mg/mL of microsomal protein (Table I). Diazepam, a high-affinity UGT2B7 inhibitor, restricted 50% of morphine glucuronidation in HLMs and 75% in RLMs at a concentration of 85 μM, and it was therefore used as the positive control for *in vitro* screening.

The anionic surfactant, sodium lauryl sulfate, exhibited the greatest impact on morphine metabolism, almost completely halting UGT2B activity in HLMs and RLMs. Although other excipients were categorized as nonionic surfactant or co-solvent, and were generally considered non-irritating, some exerted potent effects on UGT2B activity. At a concentration of 0.425%, several excipients inhibited UGT2B activity by over 50%. In order of decreasing potency, these were: Tween 20 > Cremophor EL > Cremophor RH > PEG 400 > Tween 80 > Solutol H15. It should also be noted that two preservatives commonly used in drug formulations, propyl paraben and methyl paraben, almost completely inhibited UGT2B activities at a concentration of 0.425%, but showed moderate effects at lower concentrations. Carbohydrate polymers, PVP K90F, and PVP K30 exerted no significant impact on UGT2B activity at concentrations below 0.0425%.

Determination of Tween-PEG IC_{50}

To determine an appropriate initial dose of Tween-PEG for the *in vivo* studies, the Tween-PEG IC_{50} value was determined by analyzing the effects of a series of Tween-PEG concentrations on UGT2B7 activity. Tween-PEG strongly inhibited UGT2B7 activity in both HLMs and RLMs in a concentration-dependent manner (Fig. 1). Surprisingly, the IC_{50} values for Tween-PEG determined in HLMs and RLMs were similar, at concentrations of 0.00419% and 0.00415%, respectively. Compared with the enzyme inhibition effects produced by Tween 20 or PEG 400 alone, a combination of these excipients decreased the metabolism of morphine by an order of magnitude, presumably as a result of a synergistic effect between them.

Effects of Tween-PEG on NAL Oral Bioavailability in Rats

The *in vitro* IC_{50} determination confirmed that Tween-PEG potently inhibited the activity of human and rat liver UGTs. We therefore conducted *in vivo* experiments to determine how a range of Tween-PEG formulations affected NAL oral bioavailability. We determined pharmacokinetic parameters (Table II) in rats given oral NAL alone (control group), or NAL accompanied by various combinations of Tween-PEG. Figure 2 shows the NAL plasma concentration *versus* time for these groups of animals. An oral dose of 4 mg/kg Tween-PEG significantly increased NAL oral bioavailability, producing an approximately 5-fold increase in peak plasma concentration (C_{max}) and a 4-fold increase in the area under the plasma concentration-time curve (AUC_t or AUC_∞), compared to the corresponding values in the control group. The data presented in Table II showed no significant change in the terminal elimination rate constant (k), and there were therefore no significant differences in NAL half-life ($T_{1/2}$) between groups. These data suggested that Tween-PEG reduced the metabolism of NAL by about 80% in the drug absorption phase. However, once NAL was absorbed, these inhibitors did not significantly affect its clearance.

We conducted Tween-PEG dose escalation studies to evaluate their influence on oral NAL bioavailability in rats. In

Table I The Effects of 20 Commonly Used Excipients on UGT2B Activity

Excipients (Pharmaceutical classification)	Reacted Concentration (%, w/v)	UGT2B7 (HLM) Inhibition (%)[b]	UGT2B1 (RLM) Inhibition (%)[b]	Pharmaceutical uses
Diazepam[a]	0.0024	47.7 ± 2.3	75.0 ± 4.3	–
Sodium lauryl sulfate (anionic surfactant)	0.425	100.0 ± 0.0	100.0 ± 0.0	Emulsifier, wetting agent, solubilizer
	0.0425	100.0 ± 0.0	100.0 ± 0.0	
	0.00425	85.5 ± 1.5	73.5 ± 5.5	
	0.000425	9.6 ± 4.3	11.9 ± 9.6	
Propyl paraben (preservative)	0.425	100.0 ± 0.0	100.0 ± 0.0	Antimicrobial, antifungal, antibacterial
	0.0425	98.9 ± 0.4	82.6 ± 3.1	
	0.00425	47.9 ± 5.0	41.7 ± 3.7	
	0.000425	5.9 ± 7.3	17.3 ± 2.4	
Tween 20 (non-ionic surfactant)	0.425	93.9 ± 1.0	91.2 ± 1.5	Emulsifier, wetting agent, solubilizer
	0.0425	62.9 ± 2.1	64.9 ± 2.0	
	0.00425	19.2 ± 13.5	16.8 ± 10.2	
	0.000425	1.4 ± 2.8	5.4 ± 4.7	
Methyl paraben (preservative)	0.425	99.7 ± 0.1	65.9 ± 2.0	Antimicrobial, antifungal, antibacterial
	0.0425	59.4 ± 6.0	49.6 ± 1.8	
	0.00425	23.2 ± 9.7	13.9 ± 2.8	
	0.000425	11.9 ± 1.2	22.7 ± 7.1	
Cremophor EL (non-ionic surfactant)	0.425	81.0 ± 6.2	66.2 ± 3.1	Solubilizer, emulsifier, wetting agent
	0.0425	70.9 ± 4.9	48.1 ± 3.3	
	0.00425	68.5 ± 12.9	32.2 ± 1.6	
	0.000425	63.9 ± 5.9	37.6 ± 9.9	
Cremophor RH40 (non-ionic surfactant)	0.425	77.3 ± 0.5	71.8 ± 1.8	Emulsifier, solubilizer, dispersant
	0.0425	81.6 ± 3.5	79.3 ± 0.8	
	0.00425	67.2 ± 4.1	46.8 ± 2.3	
	0.000425	66.5 ± 7.5	33.3 ± 5.3	
Tween 80 (non-ionic surfactant)	0.425	67.5 ± 2.0	72.3 ± 1.7	Surfactant, emulsifier, solubilizer, wetting agent
	0.0425	54.7 ± 5.3	52.3 ± 1.4	
	0.00425	19.9 ± 5.0	9.7 ± 3.5	
	0.000425	6.5 ± 28.2	-0.5 ± 3.4	
Solutol H15 (non-ionic surfactant)	0.425	51.0 ± 3.8	65.9 ± 2.0	Emulsifier solubilizer, wetting agent
	0.0425	46.1 ± 2.6	49.6 ± 1.8	
	0.00425	13.9 ± 3.9	13.9 ± 2.8	
	0.000425	-1.2 ± 3.9	22.7 ± 7.1	
Tween 40 (non-ionic surfactant)	0.425	36.0 ± 4.1	37.4 ± 2.4	Emulsifier, solubilizer, wetting agent
	0.0425	40.0 ± 5.1	22.8 ± 3.4	
	0.00425	10.2 ± 7.5	-6.4 ± 4.4	
	0.000425	0.6 ± 15.4	-6.6 ± 3.5	
Sodium benzoate (preservative)	0.425	20.8 ± 16.9	16.9 ± 2.0	Antimicrobial, antifungal,
	0.0425	7.3 ± 1.7	4.6 ± 1.8	
	0.00425	6.7 ± 3.2	3.9 ± 2.8	
	0.000425	0.9 ± 2.1	2.7 ± 7.1	
Pluronic F68 (non-ionic surfactant)	0.425	13.8 ± 4.2	5.3 ± 2.6	Emulsifier, solubilizer, wetting agent
	0.0425	-3.8 ± 7.0	-19.0 ± 2.3	
	0.00425	-6.3 ± 4.0	-13.9 ± 4.4	
	0.000425	-9.7 ± 3.7	-7.1 ± 1.5	
PEG 2000 (co-solvent)	0.425	3.4 ± 0.6	8.8 ± 4.9	Tablet coating, emulsifier, dispersant, lubricant
	0.0425	4.4 ± 3.1	-0.4 ± 11.2	
	0.00425	4.4 ± 5.9	0.6 ± 9.1	
	0.000425	5.2 ± 1.5	0.3 ± 4.4	
PEG 400 (co-solvent)	0.425	74.2 ± 12.7	71.0 ± 8.4	Coating agent, solvent, lubricant, suppository base
	0.0425	52.8 ± 7.7	44.6 ± 1.9	

Table I (continued)

Excipients (Pharmaceutical classification)	Reacted Concentration (%, w/v)	UGT2B7 (HLM) Inhibition (%)[b]	UGT2B1 (RLM) Inhibition (%)[b]	Pharmaceutical uses
	0.00425	37.3 ± 2.9	18.1 ± 3.2	
	0.000425	18.8 ± 4.9	10.0 ± 4.9	
Pluronic F127 (non-ionic surfactant)	0.425	-3.6 ± 13.1	53.9 ± 1.1	Emulsifier, solubilizer, wetting agent
	0.0425	-8.3 ± 1.8	24.4 ± 8.1	
	0.00425	-2.7 ± 9.9	-9.7 ± 3.6	
	0.000425	-6.7 ± 9.6	-4.5 ± 6.2	
Myrj 52 (non-ionic surfactant)	0.425	13.9 ± 12.1	18.1 ± 7.2	Emulsifier, solubilizer, wetting agent
	0.0425	17.5 ± 1.3	14.2 ± 3.2	
	0.00425	12.6 ± 0.5	7.9 ± 4.3	
	0.000425	5.1 ± 9.1	2.3 ± 0.8	
Sorbitol (carbohydrate polymer)	0.425	4.3 ± 2.8	14.0 ± 5.6	Sweetner, humectants, cryoprotectant
	0.0425	3.0 ± 4.4	6.1 ± 5.0	
	0.00425	7.5 ± 1.6	5.8 ± 4.8	
	0.000425	0.2 ± 7.9	3.7 ± 6.4	
Tween 60 (non-ionic surfactant)	0.425	21.8 ± 6.6	27.3 ± 1.0	Emulsifier, solubilizer, wetting agent
	0.0425	23.2 ± 12.3	26.5 ± 3.7	
	0.00425	-3.8 ± 3.0	-0.6 ± 2.6	
	0.000425	-7.7 ± 5.6	-3.7 ± 4.3	
Starch acetate (carbohydrate polymer)	0.425	9.5 ± 23.7	8.4 ± 2.7	Filler, binder, disintegrant, adsorbent, diluent
	0.0425	-8.5 ± 4.0	4.8 ± 1.7	
	0.00425	1.1 ± 4.6	3.3 ± 2.9	
	0.000425	5.2 ± 12.3	1.8 ± 4.9	
PVP K90F (synthetic polymer)	0.425	45.2 ± 13.6	20.0 ± 9.4	Dispersing and suspending agent, binder, stabilizer
	0.0425	42.2 ± 11.5	27.1 ± 7.3	
	0.00425	-5.1 ± 6.1	26.7 ± 4.3	
	0.000425	-8.4 ± 5.4	26.3 ± 3.5	
PVP K30 (synthetic polymer)	0.425	54.1 ± 15.9	34.4 ± 3.1	Dispersing and suspending agent, binder, stabilizer
	0.0425	32.1 ± 18.9	35.9 ± 8.8	
	0.00425	7.6 ± 6.8	31.5 ± 2.6	
	0.000425	4.2 ± 1.3	31.9 ± 6.7	

[a] Diazepam is used as positive control at concentration of 85 μM

[b] The results are represented as mean ± S.D. for separate triplicate determinations

contrast to the *in vitro* results, we did not observe a linear dose-dependency *in vivo* and the optimal dose of Tween-PEG in rats was 4 mg/kg of each excipient.

Portal Vein Administration of Tween-PEG Improved NAL Oral Bioavailability

To investigate the impact of Tween-PEG on liver UGT2B7 activity specifically, the influence of excipients on intestinal NAL absorption was eliminated by treating rats with Tween-PEG via intra-portal vein (i.p.v.) injection, with NAL administered orally. The pharmacokinetic profile shown in Fig. 3 revealed that Tween-PEG i.p.v. administration was associated with a substantial increase in plasma NAL concentration. The C_{max} increased from 85.5 ng/mL in the controls to 927.5 ng/mL in the rats treated with Tween-PEG i.p.v. Similarly, AUC_t values increased from 154.0 to 1264.9 h·ng/mL (Table III). This indicated significant enhancement of NAL oral bioavailability, with an approximately 8-fold increase in AUC_t and AUC_∞, compared to the control group. These data indicated that oral NAL bioavailability was increased more effectively by Tween-PEG i.p.v. administration than by Tween-PEG oral administration, reflecting the importance of NAL first-pass metabolism in the liver. Furthermore, Tween-PEG i.p.v. did not significantly alter the pharmacokinetic profile of NAL in the elimination phase ($T_{1/2}$ and CL). Because of the lack of permanent drug-excipient interactions, Tween-PEG can be therefore considered as safe when given by oral administration.

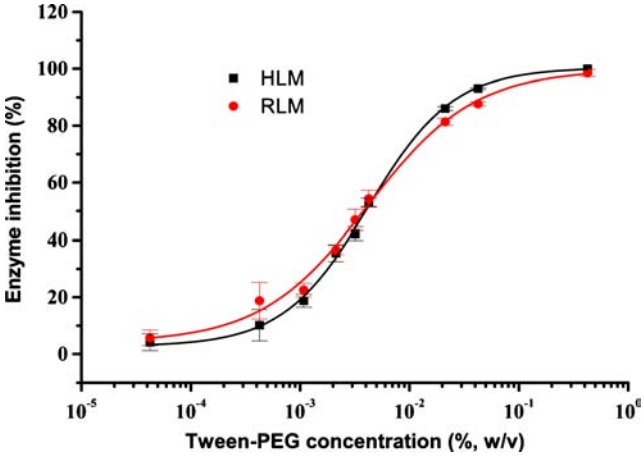

Fig. 1 The IC_{50} of Tween-PEG (a 1:1 mixture of Tween 20 and PEG 400) was determined in HLMs and RLMs using the concentrations indicated. The UGT2B inhibitory effects of Tween-PEG were expressed as a percentage of control morphine-3-glucuronide production. Data represent the mean ± S.D. for separate triplicate determinations.

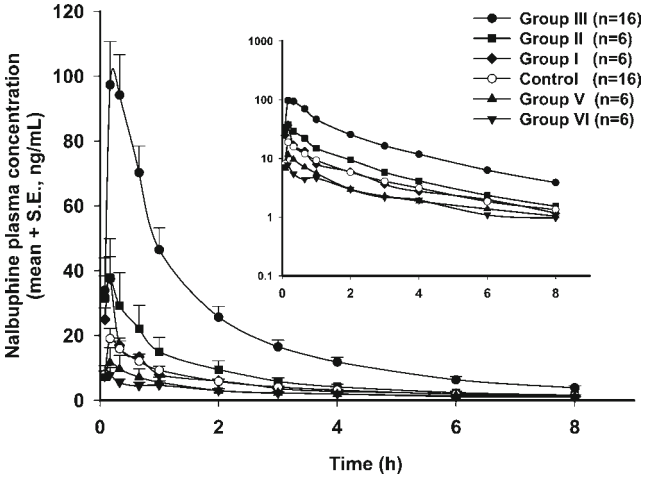

Fig. 2 Mean plasma concentration-time profile in SD rats after oral administration of 20 mg/kg NAL, with or without oral Tween-PEG (Groups I to VI) from time 0 to 8 h. The symbols represent the following treatment groups: (*white circle*) NAL only; (*black diamond*) NAL + Tween-PEG 2 mg/kg; (*black square*) NAL + Tween-PEG 3 mg/kg, (*black circle*) NAL + Tween-PEG 4 mg/kg; (*black up-pointing triangle*) NAL + Tween-PEG 8 mg/kg and (*black down-pointing triangle*) NAL + Tween-PEG 12 mg/kg. Insert plot is depicted in semi-log scale.

Antinociceptive Effects of Oral NAL in Rats

As shown in Fig. 4, rats administered with i.m. injections of NAL at doses of 2.5, 5, or 10 mg/kg, or with oral NAL (50, 100, or 200 mg/kg) plus Tween-PEG (4 mg/kg of each) showed similar trends in % MPA data obtained in CET tests. The antinociceptive response was evaluated by calculating the AUC (%MPA *versus* time) and the duration for which the MPA was greater than 50% for each group (Table IV). Statistical analysis of the data indicated that within each administration route, there were significant dose-dependent increases in the AUC. There were no statistically significant differences between groups treated with the novel oral NAL formulation (50 mg/kg) *versus* i.m. NAL (2.5 mg/kg); oral NAL (100 mg/kg) *versus* i.m. NAL (5 mg/kg); or oral NAL (200 mg/kg) *versus* i.m. NAL (10 mg/kg).

Tween-PEG Effects on NAL Oral Bioavailability in Humans

We successfully completed an exploratory trial of oral NAL bioavailability in 12 healthy volunteers (8 subjects in Group I and III). In the control group, participants were orally administered 66 mg of NAL alone, whereas the experimental groups

Table II Pharmacokinetic Parameters Following Oral Administration of NAL (20 mg/kg) in the Presence or Absence of Different Doses of Tween -PEG in SD Rats

PK parameter	Control NAL ($n=16$)	Group I NAL + Tween-PEG 2 mg/kg ($n=6$)	Group II NAL + Tween-PEG 3 mg/kg ($n=6$)	Group III NAL + Tween-PEG 4 mg/kg ($n=16$)	Group V NAL + Tween-PEG 8 mg/kg ($n=6$)	Group VI NAL + Tween-PEG 12 mg/kg ($n=6$)
C_{max} (ng/mL)	21.1 ± 13.3	37.5 ± 16.7	56.0 ± 22.4*	111.9 ± 49.4***	11.7 ± 11.2	9.0 ± 7.6*
T_{max} (hr)	0.3 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.2	0.2 ± 0.0	0.3 ± 0.3
AUC_t (hr·ng/mL)	36.8 ± 13.7	38.1 ± 7.7	60.0 ± 33.7	168.2 ± 81.0***	22.3 ± 12.0*	18.3 ± 9.6*
AUC_∞ (hr·ng/mL)	42.8 ± 14.9	41.8 ± 9.0	65.3 ± 33.3	181.8 ± 85.7***	27.7 ± 11.6*	22.7 ± 10.2*
k (1/hr)	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
$T_{1/2}$ (hr)	2.9 ± 1.0	2.3 ± 0.8	2.4 ± 0.4	2.3 ± 0.6	3.7 ± 0.9	3.5 ± 0.9
Cl/F (L/hr/kg)	536.5 ± 227.2	499.3 ± 115.8	377.8 ± 183.3	140.0 ± 73.3***	806.1 ± 249.0*	583.4 ± 53.9
V/F (L/kg)	2206.8 ± 1114.8	1645.1 ± 607.4	1352.1 ± 809.0	469.8 ± 286.1***	4408.7 ± 2027.4*	5386.7 ± 2717.3*

Tween-PEG: the oral dosage combined with equal dose of Tween 20 and PEG 400, respectively

Statistic method: one-way ANOVA test

Post Hoc test: LSD

*$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with control group

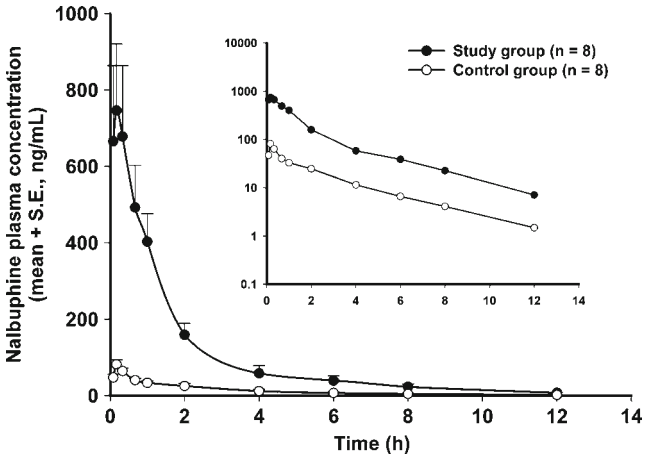

Fig. 3 Mean plasma concentration-time profile in SD rats after oral administration of 20 mg/kg NAL with (study group) or without (control group) intraportal vein administration of Tween-PEG (4 mg/kg each of Tween 20 and PEG 400) from time 0 to 12 h. The symbols represent the following treatment groups: (*white circle*) NAL and (*black circle*) NAL + Tween-PEG i.p.v. Insert plot is depicted in semi-log scale.

(Table V, Groups I, II, and III) were co-administered a range of Tween-PEG doses. Table V describes these oral formulations and the numbers of subjects in each group. No severe side effects arose during this clinical trial. The mean plasma concentration-time profile for NAL in each study period is shown in Fig. 5, and the pharmacokinetic parameters are listed in Table V. Oral NAL (66 mg), combined with Tween-PEG (240 mg of each excipient) was the optimal formulation among those studied. The C_{max} and the AUC for NAL in Group II, which received this optimal formulation, were nearly 2-fold and 1.5-fold higher, respectively, than the corresponding values seen in the controls.

Table III Pharmacokinetic Parameters Following Oral Administration of NAL (20 mg/kg) with or Without Intra-Portal-Vein Administration of Tween-PEG (4 mg/kg Each of Tween 20 and PEG 400) in SD Rats

PK parameter	Control group NAL ($n=8$)	Study group NAL + Tween-PEG ($n=8$)
C_{max} (ng/mL)	85.5 ± 31.9	927.5 ± 523.5**
T_{max} (h)	0.2 ± 0.1	0.2 ± 0.2
AUC_t (h·ng/mL)	154.0 ± 90.9	1264.9 ± 663.9**
AUC_∞ (h·ng/mL)	159.9 ± 91.3	1294.2 ± 671.1**
k (1/h)	0.3 ± 0.1	0.3 ± 0.1
$T_{1/2}$ (h)	3.2 ± 2.0	2.7 ± 0.6
Cl/F (L/h/kg)	172.7 ± 102.6	19.2 ± 8.6**
V/F (L/kg)	984.1 ± 108.2	74.8 ± 40.3*

Tween-PEG: the oral dosage combined with equal dose of Tween 20 and PEG 400, respectively

Statistic method: T-test

*$p < 0.05$, **$p < 0.01$ compared with control group

DISCUSSION

In addition to influencing the pH of gastrointestinal fluids, gastrointestinal transit time, and membrane permeability, excipients may also alter drug bioavailability by affecting drug metabolism and efflux (31). In the present study, we selected Tween 20 and PEG 400 from 20 common excipients screened for UGT2B7 inhibition to determine their effects on oral bioavailability of NAL in rats and humans. Tween 20 and PEG 400 appeared to have synergistic effects on UGT2B7 activity *in vitro* and were combined for testing *in vivo*. These selected excipients are non-ionic surfactants, with strongly hydrophilic components. Partially because of their low toxicity, they often serve as lubricants, and as solubilizing and wetting agents in pharmaceutical formulations. The WHO recommendation for acceptable daily human intake of Tween 20 and PEG 400 is 25 mg/kg (32) and 10 mg/kg (33), respectively. These excipients showed appropriate safety profiles under the broad dose ranges suggested by the WHO, in addition, Tween 20 and PEG 400 have been estimated to be suitable for manufacturing soft oral capsules. Because of the non-irritating properties, safety profile, and potential effects on UGT2B7 activity of Tween 20 and PEG 400, the Tween-PEG combination was selected for NAL oral administration studies, even though these excipients did not exhibit the strongest effects on UGT2B7 activity during *in vitro* screening.

Compared to control, pharmacokinetic studies in rats suggested that oral administration of Tween-PEG (4 mg/kg) resulted in 4- and 5-fold higher NAL oral bioavailability and C_{max}, respectively. The mechanism by which Tween-PEG increased NAL plasma concentrations may involve inhibition of membrane-bound UGT2B7, which is distributed throughout the gastrointestinal tract, from the esophagus to the colon (34). After oral administration of NAL, the large lumen surface area in the intestinal tract facilitates contact with membrane-bound UGT2B7, which initiates a conjugation reaction. At this stage, Tween-PEG may protect against extensive NAL metabolism by inhibiting UGT2B7 activity. Alternatively, surfactant-induced instability may alter the integrity or fluidity of cell membranes, modifying cell membrane function (35,36) and thereby enhancing NAL absorption. The positive effects of Tween-PEG on NAL oral bioavailability were observed in both humans and rats. Oral administration of NAL, accompanied by an optimal dose of Tween-PEG, produced an immediate increase in the NAL plasma concentration during the drug absorption phase. The aforementioned processes may contribute to increased oral bioavailability of NAL administered with Tween-PEG.

Because of their physicochemical properties, the absorption of excipients from the gastrointestinal tract is relatively low. To elucidate the precise effects of Tween-PEG on liver NAL metabolism, we administered Tween-PEG i.p.v. to rats

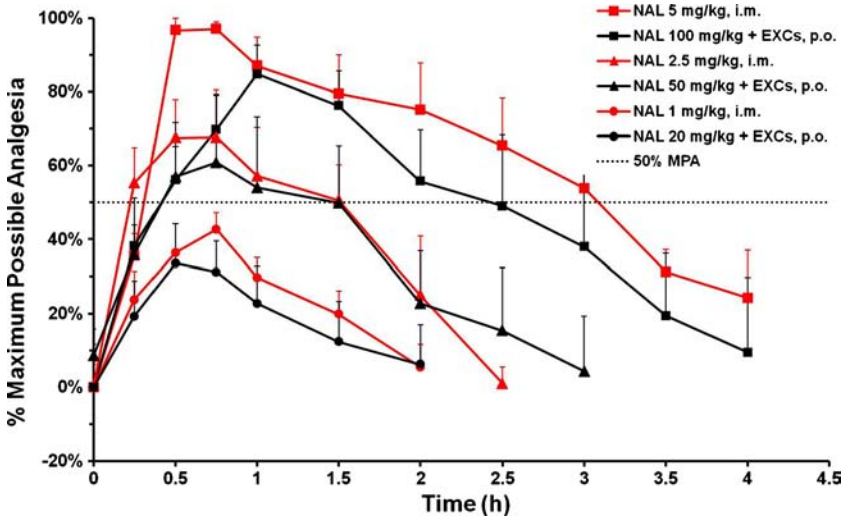

Fig. 4 Pharmacodynamic study of analgesic effects using the cold ethanol tail-flick test (CET) in SD rats following oral (p.o.) administration of NAL combined with Tween-PEG (4 mg/kg each of Tween 20 and PEG 400) or NAL intramuscular (i.m.) injection ($n = 6$ in each group). Symbols represent the following groups: (*red square*) NAL 5 mg/kg; (*black square*) NAL 100 mg/kg + Tween-PEG; (*red up-pointing triangle*) NAL 2.5 mg/kg; (*black up-pointing triangle*) NAL 50 mg/kg + Tween-PEG; (*red circle*) NAL 1 mg/kg; and (*black circle*) NAL 20 mg/kg + Tween-PEG.

that were orally dosed with NAL. After Tween-PEG i.p.v. administration (4 mg/kg of each Tween 20 and PEG 400), NAL oral bioavailability increased approximately 8-fold, and C_{max} increased by a factor of 11, compared to that in the controls. Pharmacokinetic data indicated that in the absence of an intestinal barrier, Tween-PEG effectively modulated liver enzyme function, and reduced the hepatic metabolism of NAL. These data indicated that the poor absorption of orally administered Tween-PEG limited its effects on NAL metabolism. However, Tween-PEG did not significantly change NAL clearance (CL) or elimination half-life ($T_{1/2}$) after i.p.v. administration. This suggested that as expected, the inhibitory effects of Tween-PEG on UGT2B7 were both temporary and reversible. Once Tween-PEG distributed to the systemic circulation, its concentration was probably too low to affect UGT2B7 activity. Tween-PEG can therefore be considered as an ideal adjuvant for an oral NAL formulation as it fulfilled the primary condition that a reliable and safe modulator should not influence the original pharmacokinetic profile of an active component.

Improvement of NAL analgesic effects after oral administration with Tween-PEG was expected from the results of these pharmacokinetic studies. Because the absolute oral bioavailability of NAL in rats is approximately 1% (37), oral dosage must be 100 fold higher than parenteral (i.v. or i.m.) dosage to achieve a comparable analgesic effect. Because Tween-PEG provided an almost 5-fold increase in NAL oral bioavailability, we anticipated that this difference could be reduced by a factor of 5. We therefore tested whether a 20-fold oral dosage of NAL accompanied by 4 mg/kg Tween-PEG achieved similar analgesia as NAL administered by i.m. injection in rats. As shown in Fig. 4, CET tests revealed that the analgesic effects of oral NAL plus Tween-PEG treatment were slightly lower than the effects seen for the dose-relative groups receiving NAL i.m. administration. However, there were no significant differences between the AUC (%MPA

Table IV Pharmacodynamic Study of NAL in SD Rats ($n = 6$ Per Group) by the CET Tests

	Group I[b]		Group II[b]		Group III[b]	
	i.m. NAL 5 mg/kg	p.o. NAL 100 mg/kg + Tween-PEG[a]	i.m. NAL 2.5 mg/kg	p.o. NAL 50 mg/kg + Tween-PEG[a]	i.m. NAL 1 mg/kg	p.o. NAL 20 mg/kg + Tween-PEG[a]
$AUC_{0\text{-}t}$ (h·sec)	119.5 ± 14.4	96.4 ± 29.0	67.6 ± 35.2	61.7 ± 18.1	33.8 ± 4.2	30.6 ± 2.9
Duration above 50% MPA (hr)	2.6 ± 0.5	1.9 ± 0.4	1.0 ± 0.5	0.8 ± 0.3	–	–

[a] Tween-PEG: the oral dosage combined with equal dose (4 mg/kg) of Tween 20 and PEG 400, respectively

[b] There was no significant difference within groups

Statistic method: one-way ANOVA test

Post Hoc test: LSD

*$p < 0.05$, **$p < 0.01$ compared with control group

Table V Pharmacokinetic Parameters Following Oral Administration of NAL (66 mg) in the Presence or Absence of Different Doses of Tween-PEG Combination in Human Subjects

PK parameter	Control NAL ($n=12$)	Group I NAL + Tween-PEG 120 mg ($n=8$)	Group II NAL + Tween-PEG 240 mg ($n=12$)	Group III NAL + Tween-PEG 360 mg ($n=8$)
C_{max} (ng/mL)	21.6 ± 6.5	30.3 ± 13.8	54.1 ± 17.2***	27.7 ± 9.2
T_{max} (h)	0.9 ± 0.7	1.0 ± 1.2	0.5 ± 0.2	0.4 ± 0.1
AUC_t (h·ng/mL)	96.9 ± 37.8	122.8 ± 55.0	159.1 ± 52.1**	95.5 ± 33.3
AUC_∞ (h·ng/mL)	111.7 ± 44.3	148.0 ± 71.2	177.8 ± 56.1**	108.9 ± 36.9
k (1/h)	0.10 ± 0.03	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.03
$T_{1/2}$ (h)	9.5 ± 2.5	9.9 ± 2.3	8.6 ± 3.1	9.7 ± 3.0
Cl/F (L/h)	682.5 ± 279.0	574.9 ± 376.0	417.3 ± 168.1*	683.8 ± 274.7
V/F (L)	9369.6 ± 4415.0	8815.1 ± 7431.3	4877.1 ± 1663.4**	9496.5 ± 4956.7

Tween-PEG: the oral dosage combined with equal dose of Tween 20 and PEG 400, respectively

Statistic method: one-way ANOVA test

Post Hoc test: LSD

*$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with control group

versus time) or duration of time for which MPA was more than 50% MPA in the oral and i.m. groups (Table IV). These data indicated that the combined use of Tween-PEG not only increased NAL oral bioavailability but also improved its analgesic effects. Thus, optimization of NAL oral bioavailability by concomitant administration of an optimal dose of Tween-PEG could produce similar pharmacodynamic effects as the parenteral administration routes currently used in the clinic.

The absolute oral bioavailability of NAL is nearly 15% in humans and a 66 mg oral dose is therefore equivalent to approximately 10 mg administered by i.v. or i.m. injection. A comparison of the results of the present human study with our previous data for 10 mg NAL i.m. administration in healthy volunteers (data not shown) showed that the two plasma concentration-time plots almost completely overlapped. Thus, even in the absence of excipients that can alter oral bioavailability, 66 mg NAL taken orally was equivalent to 10 mg administered by i.m. injection. In agreement with previous literature (20), orally administered NAL had a $T_{1/2}$ of approximately 8 h in humans, longer than the 4-h $T_{1/2}$ observed for i.m. injection. We conclude that oral administration of NAL increases analgesic efficacy compared with parenteral forms of administration, thereby increasing its potential benefits and improving patient compliance. The preliminary results obtained from the present study revealed that the optimal dose of Tween-PEG increased the C_{max} of NAL by a factor of 2, providing a 50% increase in oral bioavailability. The elimination rates of NAL in different treatment groups were approximately equal, indicating that CL was not changed significantly (Table V). Thus, 3 different doses of the combined Tween-PEG treatment did not significantly affect the disposition of NAL in humans.

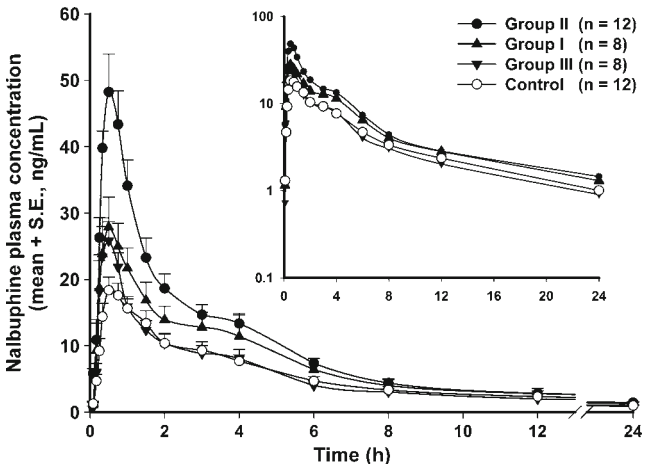

Fig. 5 Mean plasma concentration-time profile in human subjects after an oral dose of 66 mg NAL with or without concomitant Tween-PEG from time 0 to 24 h. The symbols represent the following treatment groups: (*white circle*) NAL; (*black up-pointing triangle*) NAL + Tween-PEG (120 mg of each); (*black circle*) NAL + Tween-PEG (240 mg of each); and (*black down-pointing triangle*) NAL + Tween-PEG (360 mg of each). Insert plot is depicted in semi-log scale.

Nonetheless, different doses of Tween-PEG exerted distinct effects on NAL absorption. In dose escalation studies in both humans and rats, we adjusted the oral doses of Tween-PEG to maximize NAL oral bioavailability. NAL plasma levels did not increase in a Tween-PEG dose-dependent manner. Increasing the Tween-PEG dosage produced bell-shaped profiles for NAL bioavailability, indicating that higher levels of Tween-PEG actually hindered its intestinal absorption in both rats and humans. This phenomenon was consistent with previous reports in the literature (1). In our animal studies, Tween-PEG (4 mg/kg each of Tween 20 and PEG 400) provided the greatest enhancement in NAL oral bioavailability compared with other dose combinations. With a Tween-PEG

oral doses of 12 mg/kg, NAL oral bioavailability was only approximately half that in the control group. Similarly, human participants orally administered with 66 mg NAL plus 240 mg Tween-PEG exhibited the greatest improvement in NAL oral bioavailability, compared with the controls. We reasoned that large doses of the excipient may form micelles (3), which would entrap NAL, and consequently reduce drug availability for uptake in the gastrointestinal tract (38,39). These data demonstrated that the optimization of the oral dosage of Tween-PEG was essential to provide the significant improvement in NAL oral bioavailability.

In addition to improving drug solubility and gastrointestinal absorption, certain commonly used excipients increase drug bioavailability by modulating the activities of membrane transporters or metabolic enzymes. The excipients may significantly interfere with the enzymes or receptors and lead to excipient-drug interactions. Therefore, we cannot ignore the safety issues associated with high-dose excipient intake, even though they are generally regarded as safe. The careful estimation of excipient doses must be considered in future studies involving their concomitant administration to improve oral drug bioavailability. Currently, excipient-drug interactions have been exploited in several medications to normalize the drug's therapeutic effects and reduce their inter-individual variance. Because of their safety record and ubiquitous application, the use of excipients at safe and optimal doses is an effective strategy for the improvement of drug therapeutic outcomes.

CONCLUSION

The combination of Tween 20 and PEG 400 significantly increased NAL oral bioavailability in both rats and humans. Concomitant use of oral NAL with an optimal dose of Tween-PEG may assist NAL absorption by regulating UGT2B7 activity in the gastrointestinal tract and liver. The present study showed that the use of excipients was an effective strategy to minimize the metabolism of NAL, enabling development of a novel oral NAL formulation. Most importantly, the use of excipients to facilitate the conversion of a non-oral medication to oral use will have significant benefits for patients and dramatically increase drug compliance.

ACKNOWLEDGMENTS AND DISCLOSURES

This study was funded by The Department of Health, Executive Yuan of Taiwan (No. DOH97-TD-I-111-DD002). No conflict of interest to be declared by authors.

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