Route-Dependent Metabolism of Morphine in the Vascularly Perfused Rat Small Intestine Preparation[1]

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Received September 24, 1999; accepted December 13, 1999

Purpose. 1. To compare the disposition of tracer morphine ([^{3}H]M) following systemic and intraduodenal administration in the recirculating, rat small intestine preparation in absence or presence of verapamil (V), an inhibitor of P-glycoprotein. 2. To develop a physiological model to explain the observations.

Methods. A bolus dose of [^{3}H]M was added to the reservoir or injected into the duodenum of the rat small intestine preparation. V (200 μM in reservoir) was either absent (control studies) or present. Intestinal microsomal, incubation studies were performed to evaluate the effect of V on morphine glucuronidation.

Results. After systemic administration, [^{3}H]M was not metabolized but was exsorbed into lumen. By contrast, both [^{3}H]M and the 3β-glucuronide metabolite, [^{3}H]M3G, appeared in reservoir and lumen after intraduodenal administration. A physiologically-based model that encompassed absorption, metabolism and secretion was able to describe the route-dependent glucuronidation of M. The presence of V resulted in diminished levels of M3G in perfusate and lumen and mirrored the observation of decreased glucuronidation in microsomal incubations. Verapamil appeared to be an inhibitor of glucuronidation and not secretion of M.

Conclusions. M was secreted and absorbed by the rat small intestine. Route-dependent glucuronidation of M was explained by physiological modeling when M was poorly partitioned in intestinal tissue, with a low influx clearance from blood and a even poorer efflux clearance from tissue. The poor efflux rendered a much greater metabolism of M that was initially absorbed from the lumen. V increased the extent of M absorption through inhibition of M glucuronidation.

KEY WORDS: morphine; intestine; metabolism; secretion; absorption; route-dependent metabolism.

INTRODUCTION

The intestine is the first substantial barrier that retards drug entry into the body following oral ingestion and regulates the flow of substrate to other first-pass organs—the liver and the lung. The tissue is noted for its absorptive function and for the various transporters for drugs and metabolites—organic anions and cations—(1), and for its metabolic action due to the presence of conjugating enzymes and cytochrome P450 3A for oxidative metabolism (2–9). In addition, drug exsorption occurs with the 170 kDa P-glycoprotein (Pgp) that mediates the efflux of drugs back to the lumen (10–12), thereby effectively reducing accumulation of absorbed drug. These enzymatic and secretory activities associated with the intestinal tissue or lumen have been the foci of recent investigations since they render poor oral bioavailability and mask the favorable absorbability of drugs. However, there have been only a few reports that comprehensively summarize the simultaneous influence of these factors on the overall absorption of drugs (13–16).

"Route-dependent" intestinal metabolism has been observed. We and others have detected a greater intestinal extraction ratio and extent of metabolism with luminal dosing vs. "systemic" dosing in the perfused, rat small intestine preparation. Metabolism was noted for enalapril (17), acetaminophen (18) and morphine (19) when given luminally, whereas metabolites were absent when the drug dose was added to the reservoir for systemic delivery. A greater conversion of the prodrug (−)aminocarbovir to (−)carbovir existed with luminal drug delivery *vs.* systemic delivery (20). These results from the vascular intestinal perfusion model mimicked those observed for midazolam hydroxylation in man, in whom extensive first-pass metabolism was noted with oral dosing (extraction ratio of 0.43), with much of the formed primary metabolite, 1-hydroxy midazolam, reaching the hepatic portal blood. However, a low intestinal extraction ratio (0.09) was estimated in anhepatic patients undergoing liver transplantation (6,15). The intestinal CYP3A4 levels in man correlated well with rates of midazolam 1′ and 4-hydroxylation but failed to show a relation with the erythromycin breath test (21) that is routinely given intravenously to define liver CYP3A4 levels (22). The exclusivity of the intravenous erythromycin test for hepatic but not intestinal CYP 3A function translates to the inaccessibility of the systemically administered substrate to the intestinal mucosal (CYP3A4) enzymes. That enzymes for preabsorptive intestinal metabolism are present on enterocytes facing the lumen and inaccessible to drugs in the circulation has been used to explain the overall findings. A general hypothesis has been put forth to explain route-dependent intestinal metabolism—intestinal drug metabolism behaves as if it were a preabsorptive event occurring predominantly during absorption, but little or no intestinal removal occurs from drug in the systemic circulation due to the inaccessibility of enzymes (13).

The proper characterization of drug behavior in the intestine will undoubtedly result in improved oral drug therapy. In the present communication, we chose morphine (M), a substrate that is glucuronidated by the rat intestine (23,24) and effluxed by Pgp in both intestinal cell culture (25) and in knockout mice (26) as the model substrate to examine the roles of intestinal metabolism and exsorption on the net absorption of drugs. The vascularly perfused rat small intestine preparation was utilized to examine the possibility of route-dependent metabolism. In this preparation, the effect of enterohepatic circulation is eliminated and the native architecture of the small intestine is preserved, thereby allowing for the simultaneous examination of intestinal metabolic, absorptive and secretory processes. In the perfusion system, M exhibited little binding to albumin (unbound fraction in plasma = 0.89 ± 0.07), although a slight

[1] This work was supported by the Medical Research Council of Canada (MA9104 and MOP-36457) and was presented in part at the ASPET meeting in San Diego, 1997 and the AAPS meeting in Boston, 1997.

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0724-8741/00/0300-0291$18.00/0

red cell partitioning [λ or (RBC concentration/unbound concentration in plasma) = 1.2 ± 0.75] was noted with the medium (19). Moreover, inclusion of a Pgp inhibitor such as verapamil or V (27) into the perfusate was easily conducted for exploration of the potentially important inhibition of Pgp. In the present studies, we hypothesized that V increased the apparent absorption of orally administered M by decreasing the exsorption of M into the intestinal lumen. The events were viewed by the newly developed physiological model.

MATERIALS AND METHODS

Chemicals and Reagents

Unlabeled (−)M, M3G, and ethylmorphine were obtained from the National Institute on Drug Abuse (NIDA, Rockville, MD). [N-Methyl-^{3}H](−)morphine (or [^{3}H]M, specific activity 2.5 mCi/mmol) was purchased from New England Nuclear Co., Boston, MA. The radiochemical purity exceeded 96% as confirmed by high performance liquid chromatography (HPLC). Verapamil HCl (V), uridine 5′-diphosphoglucuronic acid (UDPGA, trisodium salt), and bovine serum albumin (BSA, fraction V) were obtained from Sigma Chemical Co. (St Louis, MO, USA). All of the solvents used were of HPLC grade (Caledon Labs, ON, Canada), and all other chemicals were of the highest quality grade available.

Vascularly Perfused Rat Small Intestine

Male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada; 300–350 g), which were fed *ad libitum*, allowed free access to water, and housed under artificial light on a 12-hour light-dark cycle in accordance to approved protocols of the University Animal Committee, served as intestine donors. Eighteen hours before surgery, the animals were fasted, having access to only a 2% aqueous glucose solution. Before surgery, they were anesthetized with an intraperitoneal dose (50 mg/kg) of sodium phenobarbital.

The surgical procedure and the perfusion apparatus were identical to those described previously (17,18). The perfusate consisted of 20% washed, fresh bovine RBC (a kind gift from Ryding Regency Meat Packers Ltd, Toronto, ON, Canada), 4% BSA, 5 mmol/l glucose and a complement of 20 amino acids in Krebs-Henseleit bicarbonate (KHB) solution buffered to pH 7.4, and was oxygenated with carbogen (95% oxygen-5% carbon dioxide, Matheson, Mississauga, ON, Canada). Perfusate (reservoir volume of 200 ml) entered the intestine through the superior mesenteric artery (SMA) at a flow rate of 8 ml/min and exited through the portal vein in a recirculating fashion. An outflow catheter was placed near the ileocecal end to divert luminal contents (or exudate) out of the lumen into a 12 ml polypropylene tube for the duration of the experiment (120 min) to monitor the exsorption for mass balance considerations. Following surgery, each preparation was stabilized for 20 min with blank blood before switching to a reservoir of known volume (200 ml) for the experiment. Throughout the experiment, perfusate pH and pressure at the SMA were monitored; the pH was adjusted by altering the inflow of oxygen or carbogen. Blood perfusate (1.5 ml) were removed from the reservoir at 0, 2, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 min and stored at −20°C prior to the quantitation of [^{3}H]M and [^{3}H]M3G by HPLC. At the conclusion of each experiment, the reservoir volume was recorded. The intestinal lumen was washed thrice with 2 ml of ice-cold saline introduced at the duodenal end, and the total washings were added to the contents exuded during the 120 min of perfusion. The intestinal tissue was further rinsed gently and homogenized for analysis of radioactivity.

Study Design

For systemic administration, labeled M (25,600 ± 6500 dpm/ml) was perfused through the superior mesenteric artery for a duration of 2 h (n = 4). For intraduodenal administration, a bolus dose of [^{3}H]M ($26 \pm 5.2 \times 10^6$ dpm in 0.5 ml physiological saline solution) was injected into the proximal duodenum. In the control study, V was absent in the blood perfusate (n = 4). In the verapamil study, V was added to the reservoir and allowed to equilibrate for 20 min prior to bolus intraduodenal administration of [^{3}H]M (n = 5). The concentration of V (200 μM) which was chosen for the inhibition study was similar to that used to block Pgp exsorption in intestinal segments (10). The radioactive contents of the intestinal fluid and homogenized intestinal tissue were determined by liquid scintillation counting.

Sham experiments (without intestine) were conducted to ascertain the extent of adherence of [^{3}H]M to tubing. For the morphine sham, 5×10^6 dpm [^{3}H]M was added to recirculating reservoir perfusate with sampling (100 μl) of the reservoir at regular intervals during the 120 min of perfusion.

Intestinal Microsomes

The effect of V on M glucuronidation was also investigated *in vitro*. Intestinal microsomes were prepared to examine the effect of V (0, 50, 100, 200 μM) on the glucuronidation of tracer [^{3}H]morphine. The preparation of intestinal microsomes from the male Sprague-Dawley rat (previously fasted for 18 h) entailed the initial washing of the intestinal lumen with ice-cold physiological saline solution, opening of the intestine along its length, and removal of the mucosal layer by scraping with a glass-microscope slide. The mucosal scrapings were then homogenized with four volumes of ice-cold buffer (0.05 M Tris-HCl pH 7.4 containing 0.25 M sucrose and 1 mM EDTA). The homogenate was subjected to differential centrifugation at 9000 × g for 20 min and at 105,000× g for 60 min at 4°C in a refrigerated ultracentrifuge to obtain the microsomal fraction.

Preliminary studies had indicated that no difference existed for morphine glucuronidation with a detergent activated (Triton X-100 and $MgCl_2$/Brij 56) and native (no detergent) microsomal system. Hence, the latter (native microsome) preparation was employed. The composition of the incubation mixture (total volume 1 ml) was based on that used by del Villar *et al.* (23) where microsomal protein (2.5 mg/ml) and UDPGA (5 mM) were mixed with [^{3}H]M (400,000 dpm) and V (0, 50, 100, 200 μM) in Tris-HCl buffer (50 mM). Samples (200 μl) were removed at 0, 5, 10, 15 min for deproteinization of the microsomal protein with 5 M perchloric acid (50 μl); the supernatant was analyzed immediately by HPLC. The protein content of the reaction mixture was determined according to the method of Lowry *et al.* (28) with use of varying concentrations of BSA as standards. Microsomal incubations were conducted in duplicate for each V concentration. A control incubation (no UDPGA) was also included.

Sample Analysis

M and M3G

A specific HPLC assay was used for the quantification of [^{3}H]M and [^{3}H]M3G by radioelution, with ethylmorphine, the internal standard, being detected by UV at 280 nm. Whole blood perfusate samples (500 μl) were spiked with 50 μl of ethylmorphine (100 μg/ml); the sample was deproteinized with 50 μl of perchloric acid (5 M), vortexed and centrifuged. Two hundred μl of the supernatant was subjected to reverse phase chromatographic separation using a C_{18} column (Beckman Ultrasphere ODS 5 μm, 4.6 mm × 25 cm) with a mobile phase described by Svensson and co-workers (29), buffered to a pH of 3.1. The HPLC system consisted of a Shimadzu pump LC6A, UV spectrophotometric detector SPD-6A, autoinjector SIL-6A and system controller SCL6A. A gradient system (from 10% to 40% acetonitrile) was delivered at a flow rate of 1 ml/min. Typical retention times for M3G, M and ethylmorphine were 5.5, 15 and 19 min, respectively. A calibration curve consisting of varying known amounts of [^{3}H]M or [^{3}H]M3G as standards was constructed in similar fashion.

The quantitation of [^{3}H]M or [^{3}H]M3G in microsomal samples was conducted by HPLC radioelution on the day of the experiment. The supernatant (50 μl) obtained from the microsomal incubation mixture after perchloric acid treatment was analyzed immediately for both [^{3}H]M and [^{3}H]M3G. Luminal samples were centrifuged, and 100 μl was counted for radioactivity while another 100 μl was injected directly onto the HPLC; the eluted fractions associated with [^{3}H]M and [^{3}H]M3G were collected. For these samples, no internal standard was included.

Modeling with Physiological Model

A physiologically-based model was utilized to describe the disposition of M and its metabolite M3G in the rat intestine (Fig. 1). In this model, the intestine is composed of three compartments: intestinal blood, tissue, and lumen. Drug is delivered under constant blood flow (Q_I) into the intestinal blood via the SMA and exits the intestine into the portal vein. Influx of M into the intestinal tissue from the blood is characterized by the transport clearance parameter, CL1. Once M traverses the basolateral membrane from systemic blood, it undergoes biotransformation to M3G (denoted by the intestinal metabolic clearance, CL11) or is effluxed across the basolateral (denoted by transport clearance CL2) and the luminal (denoted by exsorption intrinsic clearance CL3) membranes. The absorption intrinsic clearance of M from the intestinal lumen is denoted by CL4, and its transit out of the lumen, CL12. M3G, once formed in the intestinal tissue, can either efflux out to the perfusate blood (CL10) or be excreted into the lumen (CL7), where there exist deconjugation of the glucuronide metabolite (with CL5) and reglucuronidation of M (with CL6). The influx and efflux clearances for M3G across the basolateral membrane are denoted by CL9 and CL10, respectively. Mass balance relationships were developed to describe events occurring during the traverse of M and M3G across the intestine (see Appendix).

Fitting

Since incomplete recovery was observed with oral administration (Table 1), the sum of the amounts of [^{3}H]M and [^{3}H]M3G in perfusate, luminal fluid, tissue and the amounts sampled, all expressed as % dose, was raised to 100% so as to facilitate the simultaneous fitting of data from intravenous and intraduodenal administration. Each component was raised proportionately. Since binding of tracer M is constant and should not contribute to kinetic changes, the effects of binding of M to albumin and red cells were neglected in the fitting procedure. Volumes and flows were assigned; the volume terms were nonessential in the fitting procedure, since mass balance was presented in terms of amounts (Table 2). Due to published accounts on the lack of deglucuronidation of M3G to M (31) and absence of M glucuronidation to M3G in lumen in our systemic studies, CL5 and CL6 were set to zero.

Fitting was performed with differential equations shown in the appendix (Eqs. A1 to A10) with the program, Scientist® (Micromath, Salt Lake City, Utah). Initial estimates were obtained with the Simplex method, then, least square optimization was performed on the sets of data of M (systemic and duodenal administration) obtained in absence of verapamil. The optimized parameters for transport and absorption were kept constant for subsequent data fitting of the oral data in the presence of V; only the parameters for secretion and metabolism were allowed to vary.

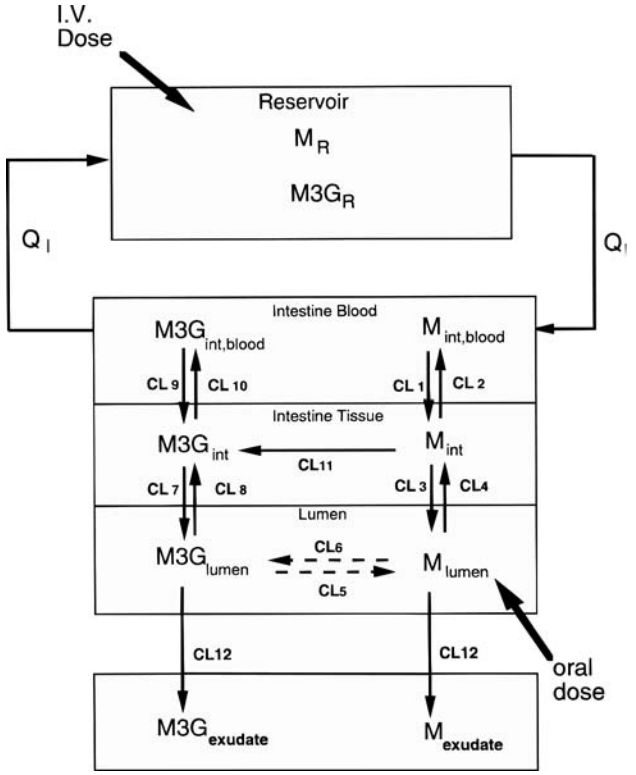

Fig. 1. Schematic representation of the recirculating, vascularly perfused intestine preparation as viewed by the traditional, physiologically-based model to describe absorption, metabolism, and secretion. The model consists of the reservoir (R), intestine tissue (int), intestine tissue blood (int,blood), luminal (lumen) compartments and an "exuduate" compartment for collection of contents leaving the intestine at the ileocecal cannula. See text for details.

Data Analysis

The uncorrected, observed data were reported and presented as mean ± S.D. in Table 1. Statistical comparison of

Table 1. Experimental Parameters for Systemic and Intraduodenal Administration of Labeled Morphine, in the Absence and Presence of Recirculating Verapamil HCl (200 μM) in the Vascularly Perfused Rat Small Intestine

Study Parameters	Systemic morphine (n = 4) no verapamil	Intraduodenal morphine (n = 4) no verapamil	Intraduodenal morphine (n = 5) with verapamil
Rat weight (g)	286 ± 35	292 ± 24	283 ± 28
Intestine weight (g)	5.4 ± 0.5	3.9 ± 0.6	4.0 ± 0.8
Flow rate (ml/min/g)	1.5 ± 0.4	2.1 ± 0.3	2.0 ± 0.4
Hematocrit	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
Volume recovery (%)	86 ± 9	88 ± 4	88 ± 5
Recovery (% dose)			
Perfusate	92.2 ± 13.9	29.2 ± 3.6	28.3 ± 13.6
Morphine	92.2 ± 13.9	16.2 ± 3.6	26.3 ± 16.0
M3G	ND[b]	13.0 ± 3.5	2.4 ± 2.8[a]
Luminal Fluid	6.0 ± 0.5	41.5 ± 2.7	46.4 ± 13.4
Morphine	6.0 ± 0.5	35.0 ± 3.5	45.2 ± 13.1
M3G	ND[b]	5.4 ± 0.84	1.2 ± 0.4
Intestinal Tissue	NM[c]	5.8 ± 3.6	4.8 ± 2.1
Total Recovery	98.2	76.5	79.4

Note: Data are presented as mean ± S.D.
[a] Significantly different ($p < 0.05$) with and without verapamil for intraduodenal morphine dosing.
[b] Not detected.
[c] Not measured.

data was carried out with the Student's t-test; a p value of 0.05 was viewed as significant.

RESULTS

Intestinal Viability

The viability of the vascularly perfused rat small intestine preparation was similar to that previously characterized in our laboratory (32). For the present studies, surgery was completed by 20 min with minimal handling of the intestine. The volume of the reservoir remaining at the end of each study (expressed as a percent of total volume) was constant across all experimental arms (Table 1). Perfusion pressure measured at the SMA remained relatively constant during the perfusion study with average values at 120 min that were generally within 10% of the pressure at the start of the experiment. Sudden, large deviations in pressure, often the result of occlusion of the inlet catheter by intestinal tissue, were easily rectified by gently repositioning the intestine. Data from preparations with steady rise in pressure that resulted in "leaky" preparations and low volume recovery were excluded from analysis.

Intestinal Absorption, Metabolism, and Secretion of Tracer Morphine

Sham experiments conducted with the recirculation of perfusate through the apparatus in absence of the intestine confirmed the lack of adsorption of [^{3}H]M to the tubing of the perfusion system. Route-dependent M glucuronidation was observed. Following systemic administration, an initial decrease, possibly associated with distribution and a slow removal, was observed (Fig. 2A). The loss of [^{3}H]M from the perfusate blood was accounted for by the appearance of [^{3}H]M in the intestinal lumen (6.0 ± 0.5% dose). However, [^{3}H]M3G was absent in perfusate blood or lumen (Table 1). By contrast, both [^{3}H]M (16.2 ± 3.6% dose) and [^{3}H]M3G (13.0 ± 3.5% dose) were detected in perfusate when [^{3}H]M was administered intraduodenally (Fig. 2B). At the conclusion of the 2-h perfusion study, 35 ± 3.5% and 5.4 ± 0.8% of the dose were recovered from the intestinal lumen as M and M3G, respectively, and 5.8 ± 3.6% of the dose was detected in intestinal tissue (Table 1).

Effect of Verapamil on Morphine Absorption, Metabolism, and Secretion

When [^{3}H]M was administered intraduodenally against circulating levels of V in the intestine preparation, [^{3}H]M absorption was greater (26.3 ± 16% dose into perfusate) but the extent was highly variable such that the increase in absorption failed to reach significance ($p > 0.05$, compared to that in absence of V). However, glucuronidation of M was significantly reduced by V (8.4% dose to 3.6% dose within two hours, summed amounts of lumen and perfusate; $p < 0.05$). The [^{3}H]M3G appearing in perfusate (13% vs. 2.4% dose, without and with V) was significantly lower with V ($p < 0.05$; Table 1; Fig. 3). Again, due to the variability among preparations, the amounts of [^{3}H]M3G recovered in luminal fluid, though lower in the presence of V (1.2% dose vs. 5.4% dose), were not statistically different from those in absence of V (Table 1).

Intestinal Microsomal Incubations

Intestinal microsomal incubations of [^{3}H]morphine were conducted for 15 min since preliminary studies indicated linear formation of [^{3}H]M3G over the time period. [^{3}H]M3G was formed only when UDPGA was present, and M3G was not observed in control incubations where UDPGA was absent (Fig. 4). V reduced the formation of [^{3}H]M3G in the intestinal

Table 2. Assigned and Fitted Parameters for the Physiologically-Based Model (Fig. 1) for Systemic (n = 4) and Intraduodenal Administration of Morphine, in Absence and Presence of Verapamil in the Recirculating, Vascularly Perfused Rat Small Intestine[a]

Assigned flows (ml/min)		
Intestinal blood flow rate (Q_I)[b]	8	
Assigned volumes (ml)		
Reservoir volume (V_R)[b]	200	
Total blood volume of intestine ($V_{int,blood}$)[c]	1.6	
Intestinal tissue volume (V_{int})[d]	3.0	
Intestinal luminal volume (V_{lumen})[e]	2	
Fitted parameters (ml/min)	Without V	With V
CL1	0.513 ± 0.077[f]	0.513
CL2	0.0232 ± 0.010	0.0232
CL3	0.0714 ± 0.087	*0.0692* ± 0.0033
CL4	0.191 ± 0.271	0.191
CL5	0[g]	0[g]
CL6	0[g]	0[g]
CL7	0.253 ± 3.08	*0.313* ± 0.207
CL8	0.0323 ± 0.74	0.0323
CL9	~0	~0
CL10	0.688 ± 3.97	0.688
CL11	0.0210 ± 0.010	*0.0039* ± 0.0008
CL12	0.0651 ± 0.085	0.0651
Weighting	1	1/observation
r^2	0.997	0.999
MSC[h]	4.59	6.27

[a] Data for intravenous (n = 4) and intraduodenal (n = 4) injections of M without V were fitted simultaneously with equations shown in Appendix for the model. Fitting of data in the presence of V (intraduodenal morphine) was performed by varying the metabolic and secretory intrinsic clearances (underlined parameters); all other parameters obtained previously were kept constant.
[b] Experimental value in intestinal perfusion experiments.
[c] Estimated based on 20% tissue volume.
[d] Estimated value based on Harrison and Gibaldi (30) wherein 10 ml was used for a 360 g rat (including cecum and stomach).
[e] From perfusion studies.
[f] Standard deviation of parameter estimates.
[g] Assigned.
[h] Model selection criterion—the greater the number, the better the fit.

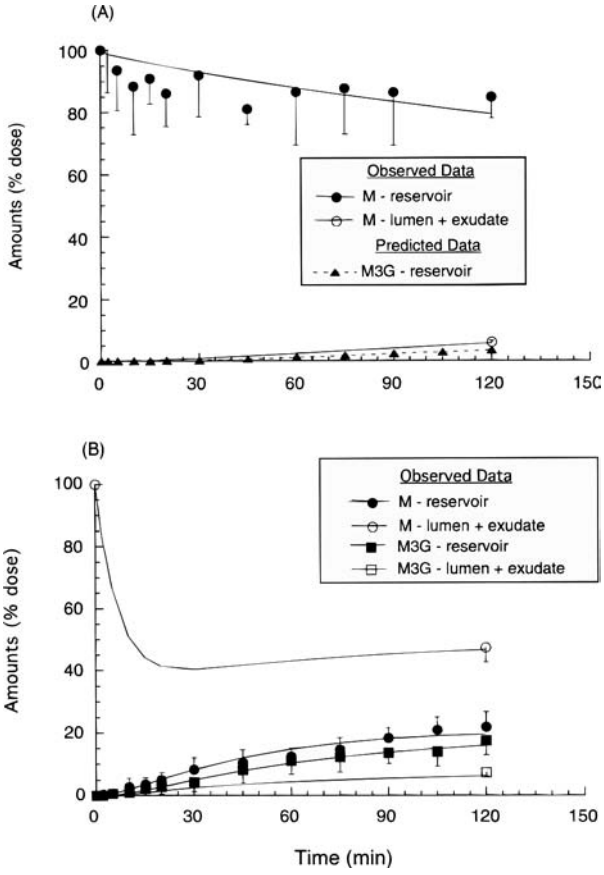

Fig. 2. The fits (——) of the intravenous (A) and oral (B) data on morphine (M) and morphine 3β-glucuronide (M3G) to the physiologically-based model in Fig. 1. Note that M3G was not detected in reservoir or lumen after systemic dosing (A) despite that the model predicted low levels of M3G formed (--▲--).

microsomal incubation mixture in a concentration-dependent fashion ($p < 0.05$). There was, however, no significant difference in [^{3}H]M3G formation rates among the various concentrations of 50, 100 and 200 μM of V (Fig. 4). In the absence of V, the rate of formation of M3G was 0.104% ± 0.06% of the initial concentration per min per mg protein (mean ± S.D., n = 4), and decreased to 0.005% ± 0.003% initial concentration/min/mg protein in the presence of 200 μM of verapamil.

Fitting

Fits to Data (without Verapamil). The optimized parameters obtained from the simultaneous fitting of the systemic and oral data (without V) to the physiological model are summarized in Table 2. Least-square fitting was best with a weighting scheme of unity, and the resultant fits generally yielded good correlation with the M data. An adequate fit to [^{3}H]M was observed for intraduodenal administration (Fig. 2B), but a slight systematic trend was observed for the fit to the intravenous [^{3}H]M data (Fig. 2A). The metabolic data on [^{3}H]M3G in perfusate and lumen were generally well described following both intravenous and intraduodenal dosing. The parameters for morphine were well estimated (standard deviations were less than estimates). The influx and efflux transport clearances for M (CL1 and CL2) differed greatly (0.51 and 0.0232 ml/min, respectively), suggesting a high tissue partitioning ratio for morphine (CL1/CL2 = 22). The secretory intrinsic clearance (CL3 or 0.0714 ml/min) was three times of the metabolic intrinsic clearance (CL11 or 0.021 ml/min). An appreciable absorptive intrinsic clearance of M (0.191 ml/min) and the low clearance for gastrointestinal transit (CL12 of 0.065 ml/min) were obtained, suggesting a high fraction of dose absorbed [F_{abs} = CL4/(CL4 + CL12) = 0.75]. There was, however, more uncertainty associated with metabolite parameters, shown by the large standard deviations of the parameter estimates (Table 2, Fig. 2), and this is due to overparametization. The parameters for transport for M3G (CL9 and CL10) were almost zero and 0.69 ml/min, respectively, suggesting a poor partitioning of M3G into the intestine (CL9/CL10 ≈ 0). The secretory intrinsic and absorption intrinsic clearances of M3G were 0.25 and 0.032 ml/min, respectively, suggesting poor absorption/exchange of M3G across the

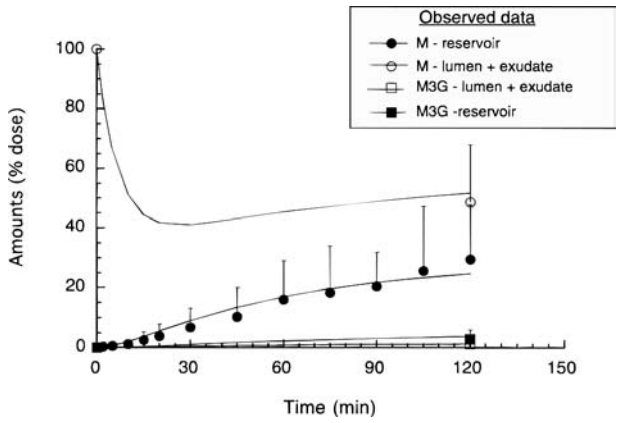

Fig. 3. The disposition of morphine (M) and morphine 3β-glucuronide (M3G) after an intraduodenal, tracer dose of morphine given at 20 min after the recirculation of 200 μM verapamil (present in the reservoir) and the fit to the data (——).

intestinal tissue. A high secretory clearance of M3G (CL7 or 0.253 ml/min) was obtained. These parameters derived from fitting for M3G were less reliable since the metabolite was not given.

Fit to Morphine Data (with Verapamil). The fit to the intraduodenal data in the presence of V adequately described the observed data (Fig. 4). The secretory clearances of M and M3G were not altered for both models (Table 2); however, the metabolic clearance of M (CL11) was greatly attenuated by V. The reduced metabolic intrinsic clearance was due to the noticeable decrease in M3G formation, and the finding correlated well with results from the *in vitro* microsomal incubation studies.

DISCUSSION

The present studies were designed to illustrate the dynamics of metabolism and secretion in the overall absorption of drugs. Morphine proved to be a good candidate for examination of the interplay of these factors in the perfused rat small intestine preparation since it was absorbed, secreted, and metabolized. From the present studies with morphine, we found that glucuronidation occurred following intraduodenal administration,

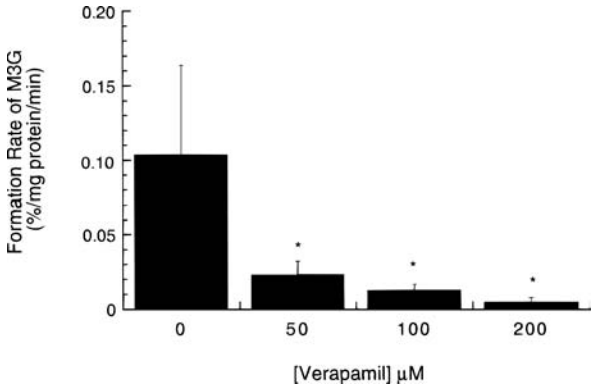

Fig. 4. Effect of verapamil on rates of glucuronidation of morphine at tracer concentration in intestinal microsomes.

an observation that is in agreement with other *in vitro* and *in vivo* findings that the rat intestine tissue was capable of glucuronidating M (23,24,33,34). However, route-dependent metabolism of morphine was observed and M3G was noticeably absent during the systemic delivery of M to the small intestine preparation.

To address the observation, a physiologically-based model was developed to describe the morphine and metabolite data after intravenous and intraduodenal administration. The present example encompassed secretion of morphine and transit of drug into the collecting tube (or exudate compartment). Solutions to the equations for the area under the curve of drug, and extents of metabolism and secretion were obtained in a recent report (35). In absence of secretion or loss to the lumen, the ultimate extent of metabolism of morphine is expected to be identical for both intravenous and intraduodenal administration in the perfused intestine preparation, regardless of the partitioning characteristics of drug (CL1 and CL2). This view could be readily supported by a simple simulation based on the mass transfer equations presented in the Appendix (data not shown).

Loss of drug in lumen ($CL12 > 0$) will contribute to reduced drug absorption by affecting the fraction absorbed (F_{abs}). The fraction of dose of M absorbed was estimated to be very high (0.75 according to fitted results), and was consistent with reports on the almost complete absorption of M from the intact intestine (36,37). Our observation on the low bioavailability of M for the vascularly perfused rat small intestine preparation was likely due to the high secretion of M and loss by gastrointestinal transit (Table 2). A role of Pgp for the secretion of M has been implicated. Callaghan and Riordan (25) suggested that M interfered with the transport function of Pgp in cultured cells whereas Schinkel and co-workers (26) demonstrated, using both *in vitro* cellular transport studies and *mdr1a* 'knockout' mice, that M was marginally transported by Pgp. A recent study with brain capillary endothelial cells also suggest that M efflux in brain was inhibited by the Pgp inhibitor, GF120918 (38). The present studies indeed revealed that M absorption was increased with V. The amount of M absorbed (% dose) was apparently increased when verapamil was added to the system, although the changes were insignificant due to variability (Table 1). Upon modeling of the data, however, drug secretion seemed to remain unaltered by V. The role of Pgp for the secretion of M by the intestine was therefore deemed unimportant, as also suggested in a recent study on rat *in vivo* morphine kinetics, which remained unperturbed with the Pgp inhibitor, GF120918 (39). The underlying change was with inhibition of M glucuronidation and not on secretion, as originally envisioned (Table 2, Fig. 3), and improved absorption of M in the presence of V was attributed to reduced metabolism. The reduced ability of the perfused intestinal preparation to glucuronidate M in the presence of V (from 18.4 to 3.6% dose; Table 1 and Fig. 3) was confirmed by microsomal incubations *in vitro* (Fig. 4). The present study suggests that whereas verapamil is ineffective in inhibiting M secretion, its potency as an inhibitor of glucuronidation, as suggested in earlier studies on inhibition of AZT glucuronidation (40), is more substantial. The reduced intestinal glucuronidation in the presence of V appears to be the major factor that contributed to increased morphine absorption (16% to 26 dose, Table 2).

Although there were notable levels of M3G accumulated in the reservoir after the intraduodenal dose (Fig. 2B), M3G

was not detected after intravenous administration despite that low levels of M3G were predicted to exist in reservoir (Figs. 2A). There were at least two explanations for the observed data. One explanation is the poor influx clearance of morphine from blood to tissue (CL1 or 0.51 ml/min, Table 2) but the even poorer efflux (CL2 or 0.0232 ml/min) clearance for M. The value of CL1 is much lower in relation to blood flow (8 ml/min), rendering a sluggish entry and ablating the extent of intestinal metabolism with systemic dosing; the entire system is not sensitive to the presence of enzymes (high or low metabolic intrinsic clearance, simulations not shown). With oral drug absorption, the drug needs to gain entry to the circulation; the very slow efflux (CL2 = 0.0232 ml/min) leads to entrapment of the absorbed morphine species, conducing to a greater extent of M3G formation with oral dosing. Another reason is the low metabolic intrinsic clearance in relation to the higher secretory intrinsic clearance (ratio is 0.29), revealing that M is primarily secreted, albeit not *via* Pgp.

In summary, the vascularly perfused rat small intestine preparation proves to be a useful tool for examination of the absorptive, secretory and metabolic activities of the intestine on drug bioavailability. The data led to the development of a physiologically-based model that viewed the processes of absorption, metabolism and secretion, and organ flow and transport simultaneously. The data showed route-dependent metabolism of morphine in the perfused rat small intestine preparation, an observation that was explained by the physiological model based on poor partitioning of drug into tissue (low value of CL1) and a even poorer efflux from tissue (very low value of CL2). The route-dependent metabolism was addressed and the inhibition of morphine metabolism and not secretion by verapamil was revealed. There persisted various aspects on the modeling that needed improvement-the systematic fit to the morphine data (Fig. 2A). It is anticipated that the present model could be refined to better the description of intestinal drug metabolism and secretion (35).

APPENDIX

The mass transfer equations describing the changes in the amounts of morphine (M) and morphine-3β-glucuronide (M3G) in the lumen (subscripted lumen), intestinal tissue (subscripted int) and intestinal blood (subscripted int,blood) per unit time (t) according to the scheme depicted in Fig. 1, are shown below. The conversion of M to M3G takes place in the intestinal tissue with metabolic intrinsic clearance CL11, and in lumen with intrinsic clearance CL6. The luminal contents flowed into the collecting tube (exudate). For the IV case, CL11 = 0 since glucuronidation was not observed. Other CL terms denote transport (CL1 and CL2 for M and CL9 and CL10 for M3G), the intrinsic clearances for secretion (CL3 and CL7 for M and M3G, respectively), or absorption (CL4 and CL8 for M and M3G, respectively). Both M and M3G are exuded from the small intestine with the clearance, CL12.

For M and M3G in reservoir (R) compartment,

$$\frac{dM_R}{dt} = Q_I \frac{M_{int,blood}}{V_{int,blood}} - Q_I \frac{M_R}{V_R} \quad (A1)$$

$$\frac{dM3G_R}{dt} = Q_I \frac{M3G_{int,blood}}{V_{int,blood}} - Q_I \frac{M3G_R}{V_R} \quad (A2)$$

For M and M3G in intestinal blood (int,blood) compartment

$$\frac{dM_{int,blood}}{dt} = Q_I \frac{M_R}{V_R} - CL1 \frac{M_{int,blood}}{V_{int,blood}} + CL2 \frac{M_{int}}{V_{int}} - Q_I \frac{M_{int,blood}}{V_{int,blood}} \quad (A3)$$

$$\frac{dM3G_{int,blood}}{dt} = Q_I \frac{M3G_R}{V_R} - CL9 \frac{M3G_{int,blood}}{V_{int,blood}} + CL10 \frac{M3G_{int}}{V_{int}} - Q_I \frac{M3G_{int,blood}}{V_{int,blood}} \quad (A4)$$

For M and M3G in intestine (int) tissue compartment,

$$\frac{dM_{int}}{dt} = CL1 \frac{M_{int,blood}}{V_{int,blood}} - (CL2 + CL3 + CL11) \frac{M_{int}}{V_{int}} + CL4 \frac{M_{lumen}}{V_{lumen}} \quad (A5)$$

$$\frac{dM3G_{int}}{dt} = CL11 \frac{M_{int}}{V_{int}} + CL9 \frac{M3G_{int,blood}}{V_{int,blood}} - (CL7 + CL10) \frac{M3G_{int}}{V_{int}} + CL8 \frac{M3G_{lumen}}{V_{lumen}} \quad (A6)$$

For M and M3G in gastrointestinal lumen (lumen) compartment,

$$\frac{dM_{lumen}}{dt} = CL3 \frac{M_{int}}{V_{int}} - (CL4 + CL6 + CL12) \frac{M_{lumen}}{V_{lumen}} + CL5 \frac{M3G_{lumen}}{V_{lumen}} \quad (A7)$$

$$\frac{dM3G_{lumen}}{dt} = CL7 \frac{M3G_{int}}{V_{int}} - (CL5 + CL8 + CL12) \frac{M3G_{lumen}}{V_{lumen}} + CL6 \frac{M_{lumen}}{V_{lumen}} \quad (A8)$$

For M and M3G in exudate (exudate) compartment

$$\frac{dM_{exudate}}{dt} = CL12 \frac{M_{lumen}}{V_{lumen}} \quad (A9)$$

$$\frac{dM3G_{exudate}}{dt} = CL12 \frac{M3G_{lumen}}{V_{lumen}} \quad (A10)$$

The amounts of M in exudate and lumen were summed to provide the total amount collected in the sampling tube at 120 min. The same was done for M3G.

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