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

Margaret M. Doherty and K. Sandy Pang

Received September 24, 1999; accepted December 13, 1999

Purpose. 1. To compare the disposition of tracer morphine (\([1^H]M\)) 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 \([1^H]M\) was added to the reservoir or injected into the duodenum of the rat small intestine preparation. V (200 \(\mu\)M 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, \([1^H]M\) was not metabolized but was exsorbed into lumen. By contrast, both \([1^H]M\) and the 3β-glucuronide metabolite, \([1^H]M3G\), 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 inaccessibility of enzymes (13). 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 inaccessibility of enzymes (13). 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 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 limited binding to albumin (unbound fraction in plasma = 0.89 ± 0.07), although a slight
red cell partitioning \([\lambda] \text{ or (RBC concentration/unbound concentration in plasma)} = 1.2 \pm 0.75\) was noted with the medium (19). Moreover, inclusion of an 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 \([\text{H}]\)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 \textit{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 (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 carbon dioxide. 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 \(-20^\circ\text{C}\) prior to the quantitation of \([\text{H}]\)M and \([\text{H}]\)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 \(\pm 6500\) dpm/ml) was perfused through the superior mesenteric artery for a duration of 2 h (n = 4). For intraduodenal administration, a bolus dose of \([\text{H}]\)M (26 \(\pm 5.2 \times 10^4\) 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 \([\text{H}]\)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 \([\text{H}]\)M to tubing. For the morphine sham, \(5 \times 10^5\) dpm \([\text{H}]\)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 \textit{in vitro}. Intestinal microsomes were prepared to examine the effect of V (0, 50, 100, 200 μM) on the glucuronidation of tracer \([\text{H}]\)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 \(\times\) g for 20 min and at 105,000 \(\times\) 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 \textit{et al.} (23) where microsomal protein (2.5 mg/ml) and UDPGA (5 mM) were mixed with \([\text{H}]\)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 \textit{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.