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FIRST PASS EFFECT OF 1-NAPHTHOL IN THE GASTRIC MUCOSA

STUDIES WITH ISOLATED EPITHELIUM AND CULTURED MUCOUS CELLS OF GUINEA PIG

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Abstract—Disposition of 1-[^{14}C]naphthol was investigated in stripped gastric mucosal segments mounted in Ussing chambers. During 2-hr incubations, naphthol was glucuronidated (44–55% of added dose) and sulfated (7–15%). When naphthol was added to the luminal fluid at pH 7.0, conjugates were released with equal velocity to the luminal and to the vascular side, but with a luminal pH of 3, conjugates appeared predominantly on the vascular side. When naphthol was added to the vascular side (both sides pH 7), conjugates appeared predominantly on the vascular side. Cultured gastric mucous cells formed naphthol glucuronide and naphthol sulfate at a ratio of 9:1. These conjugates were transiently accumulated within cells up to 300-fold followed by slow release into the medium. In conclusion, the intact gastric mucosa is able to conjugate 1-naphthol at neutral and acidic luminal pH. The data suggest that ingested phenolic compounds might be modified by a gastric first pass effect.

The stomach is the first organ contacting orally administered drugs. The classic studies of Schanker *et al.* [1] showed that weakly acidic drugs are extensively absorbed from the acidic gastric lumen. Gastric absorption also depends on drug lipophilicity [1, 2], intragastric pH [3, 4] and time of residence in the stomach [5], but the knowledge of other factors affecting gastric drug absorption is still insufficient. In particular, there is no information on whether drugs undergo a gastric first pass effect similar to with the intestinal first pass effect.

1-Naphthol is a commonly used model drug for the assessment of glucuronidation and sulfation. It undergoes an extensive first pass effect in the small intestine [6–9]. We have found recently that it is conjugated also by mucosal preparations of the stomach [10]. In order to obtain information about the pharmacokinetic consequences of gastric conjugation, we studied here the disposition of 1-naphthol and its conjugates in an intact gastric epithelial preparation and in cultured mucous cells. The results indicate that 1-naphthol undergoes a pH-dependent gastric first pass effect.

MATERIALS AND METHODS

Materials. 1-[^{14}C]Naphthol (54 mCi/mmol) was from the Radiochemical Center Amersham Buchler (Braunschweig, F.R.G.). 1-Naphthol, minimum essential medium (MEM‡) and fetal calf serum were from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Silica gel G thin layer foils were from Merck (Darmstadt, F.R.G.). The other chemicals were obtained at the highest purity from Merck.

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‡ Abbreviation: MEM, minimum essential medium.

Animals. Male Pirbright-white guinea pigs (280–350 g) fed with industrial diets (Altromin®) and water *ad lib.* were obtained from Hagmann (Exertal, F.R.G.). Animals were killed by cervical dislocation. The stomachs were removed quickly, opened along the major curvature and washed twice with cold sterile saline. Gastric mucus was carefully removed with sterile pads and the mucosa washed again.

Ussing chamber. Specimens were dissected from the fundic region of the gastric wall. Muscularis and serosa were carefully stripped off (except in one preliminary experiment with unstripped mucosa). These epithelial sheets were mounted in conventional Ussing chambers in a design described previously [11] with an exposed surface of 0.5 cm^2. Both the luminal and vascular sides were bathed in 10 mL MEM, pH 7.0, stirred by a stream of carbogen at 37°. In one experiment luminal pH was adjusted to 3.0 with 150 mM HCl. Transepithelial potential difference (PD_t) and conductance (G_t) were measured continuously by an automatic microprocessor-controlled voltage clamp (AC Copy AG, Aachen, F.R.G.) under open-circuit conditions to control integrity of the epithelial barrier. Data were printed out in 60-sec intervals. After the 60-min equilibrium period, the mean PD_t (± SD) amounted to −12 ± 3 mV with regard to the blood side and G_t amounted to 23 ± 6 mS/cm^2 (N = 10). Both values remained stable until the end of the experiments, were independent of naphthol addition to either the mucosal or the serosal side and were also not affected by luminal acidification to pH 3. After the 60-min equilibration period, 1-[^{14}C]naphthol (25 μL, dissolved in 20% ethanol; 0.2 μCi) was added at a final concentration of 10 μmol/L to either the luminal side or the vascular side. Samples of 200 μL were withdrawn from each compartment every 15 min during the following 120 min and added to 400 μL

of methanol/chloroform (3:1); after shaking 600 μL chloroform were added. At the end of the experiment, the mucosal tissue was homogenized at 0° in 400 μL methanol/chloroform (3:1) for 5 min. Then 600 μL chloroform and 180 μL MEM were added to achieve separation of organic and aqueous phases.

Cultured mucous cells. Gastric mucosal cells were isolated with a collagenase–pronase method and mucous cells enriched (about 70%) by zonal elutriation as described by Soll [12] and modified by Sewing *et al.* [13]. Mucous cells (6×10^6) were suspended in 2.5 mL sterile culture medium [MEM, containing Hank's salts supplemented with 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 25 mM $NaHCO_3$, 10% Fetal calf serum, gentamycine (5 μg/mL) and amphothericin B (2.5 μg/mL)], spread on 6-cm plastic culture dishes (TC quality; Greiner, Nürtingen) and incubated in an atmosphere of 5% CO_2 in 100% humidity at 37°. After 48 hr of culture, the culture medium was withdrawn and replaced by 1.5 mL MEM. 1-[^{14}C]Naphthol (10 μL in 20% ethanol; 0.08 μCi) was added at a final concentration of 10 μmol/L and incubation continued at 37°. At the times indicated, the medium (1.5 mL) was transferred quickly to 3 mL of a mixture of ice-cold ethanol/chloroform (3:1) and shaken immediately. Later, 0.4 mL of each mixture were transferred to 0.6 mL chloroform. The cell monolayers were washed immediately for 1 sec with 4 mL ice-cold MEM, stopped with 0.3 mL methanol, scraped into the methanolic solution and transferred in a polyethylene vial; then 0.7 mL of chloroform and 0.2 mL MEM were added to extract conjugates. Cell protein on culture dishes averaged 0.52 ± 0.4 mg protein. Intracellular concentrations of conjugates were estimated with the assumption that 1 mg protein corresponds to 4 μL cell water [14].

Analysis of 1-naphthol conjugates. Fifty microlitres of the aqueous and organic phase of the chloroform/methanol extracts were counted for the determination of unconjugated (organic phase) and conjugated (aqueous phase) naphthol. Then 50 μL of the aqueous phases were applied to silica gel G TLC foils and developed in *n*-butanol: 0.01 M Tris: propionic acid (75:14:1.1). The R_f values were 0.26 for naphthol glucuronide and 0.69 for naphthol sulfate. The radioactive bands were quantified in a radioactivity scanner (Rita 90, Raytest, Straubenhardt, F.R.G.).

Each experiment in the Ussing chamber was performed with five samples from five different animals. Cell culture experiments were performed with five cell preparations; coefficients of variation were <15%.

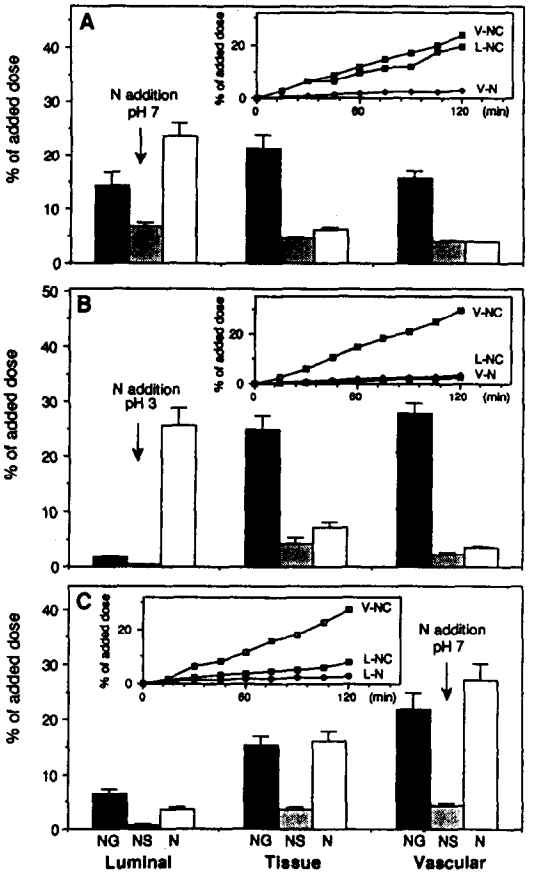

Fig. 1. Transfer and conjugation of naphthol in the Ussing chamber. (A) Luminal–vascular site transfer at pH 7. 1-[^{14}C]Naphthol (10 μM) was administered to the luminal fluid of the Ussing chamber with luminal and vascular sides at pH 7. Insert: time courses of transmucosal transfers. V-NC, vascular side naphthol conjugates; L-NC, lumina side naphthol conjugates; V-N, vascular side unconjugated naphthol; L-N, luminal side unconjugated naphthol. Bars: distribution of naphthol glucuronide (NG), naphthol sulfate (NS) and unconjugated naphthol (N) inluminal fluid, tissue specimen and vascular fluid after 120 min. Values are the means of duplicate determinations in five mucosal preparations ± SD. (B) Luminal–vascular site transfer at pH 3. Same procedure as in A but with luminal fluid at pH 3. (C) Vascular–luminal site transfer. Same procedure as in A but with naphthol added to the vascular side.

RESULTS

Three types of experiment were performed with isolated epithelial sheets in the Ussing chamber, differing with regard to pH and the site of substrate addition. In all three cases, no more than 4% of added 1-naphthol was transferred across the epithelium unchanged, and between 53 and 67% of naphthol was conjugated in 2 hr.

When 1-naphthol was added luminally at pH 7.0, a total of 66.5% of added radioactivity was conjugated, 23% of conjugates being naphthol sulfate and 77% naphthol glucuronide (Fig. 1A). Approximately equal amounts of conjugates were released to the luminal side and the vascular side. When 1-naphthol was added luminally at pH 3.0 (Fig. 1B) total conjugation was almost unchanged (60%) but sulfate conjugation was decreased (12%) and conjugates were transferred predominantly to the vascular side. Finally, when 1-naphthol was added to the vascular side (Fig. 1C), total conjugation and in particular sulfate conjugation were slightly decreased compared to luminal addition, and

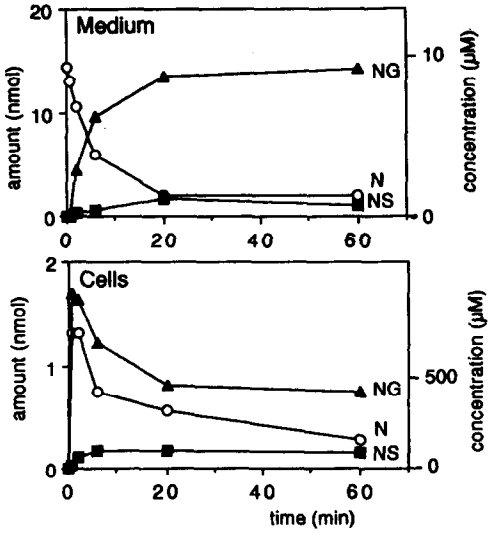

Fig. 2. Disposition of 1-naphthol in cultured gastric mucous cells. [^{14}C]Naphthol was added to cultured gastric mucous cells at a final concentration of 10 μM at 37° in MEM while shaking gently. Radioactivity was analysed in the medium and cells. NG, naphthol glucuronide; NS, naphthol sulfate; N, naphthol. Data are expressed as amounts (left y-axis) and calculated concentrations (right y-axis). Values are the means of duplicate determinations of a representative experiment.

conjugates were released predominantly to the vascular side.

In all three types of experiments, the tissue samples stored between 31 and 39% of added radioactivity, mainly as conjugates. In a preliminary experiment with unstripped mucosa, luminal side release was normal but vascular side release was diminished strongly from 22% to 4% and the tissue fraction rose corresponding to 53% (not shown).

Cultured gastric mucous cells efficiently conjugated 1-naphthol with a glucuronide/sulfate ratio of about 9:1 (Fig. 2). Naphthol glucuronide was accumulated transiently within cells up to 300-fold (calculated intra-/extracellular concentration gradient). However, even after 60 min, when steady state concentrations were almost reached, the intracellular concentration of both conjugates exceeded extracellular concentration by about 50-fold.

DISCUSSION

Despite the great importance of the stomach as the first storage organ for ingested drugs, previous studies on gastric drug biotransformation are scarce [15–23] and did not investigate the possibility of a gastric first pass effect. In fact, the complex blood supply of the stomach causes technical problems in performing pharmacokinetic studies *in situ* or with vascularly perfused preparations. We, therefore, used a well controlled isolated gastric epithelial preparation *in vitro*, which has been used previously in studies of the barrier function [24]. To reduce the problem of artificial diffusion obstacles, muscularis and serosa were stripped off and only the epithelial layer with adherent submucosa was installed in the Ussing chamber. Transmucosal potential difference and conductance remained constant over 2 hr, indicating functional integrity of the epithelial barrier throughout the experiments, whether naphthol was added to the luminal or the vascular side.

When 1-naphthol was added luminally especially at acidic pH, vascularly appearing drug was conjugated predominantly, indicating that 1-naphthol undergoes a gastric first pass effect. However, conjugates were also secreted to the luminal side indicating presystemic elimination. Finally, 1-naphthol added to the vascular side was also conjugated suggesting a role of the stomach in systemic biotransformation. Thus, the pharmacokinetic behaviour of 1-naphthol in the gastric epithelium is analogous to that in intestinal preparations [6, 7, 25, 26]. To our knowledge the present paper is the first demonstration of such effects in the stomach.

Rat intestinal sacs [6] and perfused intestine [7, 26] release 1-naphthol glucuronide predominantly towards the vascular side but 1-naphthol sulfate towards the lumen. In contrast, guinea pig jejunal [9] and gastric (present results) preparations showed no clear vectorial release, suggesting that there are species differences. However, jejunal and gastric guinea pig preparations exhibited administration site-dependent shifts in that vascular administration was associated with decreased sulfation and the increased release of glucuronide to the vascular side. The shifts might be caused by a presumed polar distribution of conjugating enzymes or conjugate-releasing carriers within mucous cells, which is the major naphthol-conjugating cell type [10]. In addition, it is possible that surface mucous cells, which are approached first by luminally administered 1-naphthol, produce a different conjugate pattern than gastric gland mucous cells, which are approached first by vascularly administered 1-naphthol. However, at present we have no information about the isozymes in these cell types as compared to the liver.

Low luminal pH had a negligible effect only on conjugation rates, but a clear enhancing effect on the transfer of conjugates to the vascular side. This suggests that the well known phenomenon of increased absorption of acidic drugs from an acidic gastric lumen [1] also holds for newly formed conjugates.

In the present experiments, the tissue concentrations of 1-naphthol and its glucuronides were higher than in comparable experiments with intestinal mucosal preparations [9]. This may be due partly to the lower 1-naphthol concentration (10 vs 50 μM) used herein, and partly to remaining artificial submucosal barriers. A further explanation is given by the cellular kinetics of 1-naphthol. We studied these kinetics in cell cultures which allow rapid accurate assessment of drug distribution between intra- and extracellular space. Glucuronidation rates were identical, sulfation rates about 40% lower than in freshly isolated cells [10]. Both conjugates showed persistently high intracellular concentrations, exceeding the extracellular concentrations more than 50-fold. This may explain the high tissue levels at

the end of the Ussing chamber experiments. Furthermore, the transient intracellular concentration peak of naphthol glucuronide suggests that glucuronide release may be a rate-limiting step in the transepithelial transport of the compound.

The present work demonstrates the suitability of isolated epithelial sheets and cultured mucous cells for studying gastric mucosal pharmokinetics. The latter system represents a new tool in gastrointestinal drug metabolism since attempts to culture differentiated enterocytes or colonocytes have failed [27]. This culture system will also be useful for studying induction phenomena [28] and for identifying the presumed carriers for cellular conjugate release.

In conclusion, 1-naphthol is handled by the gastric epithelium in a similar way as by the intestinal epithelium. Thus, in addition to the liver and intestine, the stomach may play a significant role both in the first pass effect and the systemic conjugation of drugs. More studies are required to assess further the influence of the stomach on drug pharmacokinetics.

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