Conjugation of 1-naphthol in human gastric epithelial cells

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Abstract

The biotransformation of xenobiotics is essential to the maintenance of the body's integrity. Mucosal biotransformation has been well documented in the small and large intestine of animals and humans but whether the gastric mucosa plays a role in detoxifying ingested compounds remains largely unknown. The conjugation of the model phenolic compound, 1-naphthol, by human gastric epithelial cells was assessed in vitro. Freshly isolated and cultured epithelial cells were prepared from surgical specimens obtained from patients undergoing total gastrectomy for cancer. Cell preparations were incubated with 1-¹⁴C-naphthol over 1 hour and the glucuronide and sulphate conjugates formed were separated by thin-layer chromatography. Conjugation of 1-naphthol was observed with both freshly isolated and cultured cells. In freshly isolated cells, the 1 hour turnover of 1 μ M 1-naphthol to its glucuronide and sulphate conjugates averaged 19% and 10% respectively. At higher 1-naphthol concentrations, both types of conjugate were formed at about the same rate, up to saturation (apparent $V_{\max}$ = 0.07 nmol/mg protein/minute, and apparent K_m = 40 μ M). In cultured cells, the 1 hour turnover of 1 μ M 1-naphthol to its glucuronide and sulphate conjugates averaged 35% and 8% respectively. These results suggest that the human gastric mucosa is a detoxifying organ, and that its role with regard to chemical carcinogenesis and drug first pass metabolism deserves further assessment.

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The organism protects itself from potentially harmful foreign compounds by oxidative and conjugative biotransformation reactions that can occur as early as in the gastrointestinal tract.¹⁻⁵ We eat phenolic compounds daily, in amounts of several hundred mg, as natural plant components (flavonoids, anthocyanes), contaminants or additives (spices, colours, antioxidants), or drugs (oestrogens, paracetamol, salbutamol), and their detoxification is thus of crucial importance.

It has been reported in animals and humans that the mucosa of the small and large intestine is able, to a considerable extent, to detoxicate phenols by conjugation.⁴⁻¹³ In contrast, the role of the stomach in biotransformation reactions has been considered negligible, probably because some authors failed to detect significant oxidative activity in gastric mucosa,¹⁴ despite the fact that enzymes involved in glucuronidation had earlier been found in the gastric mucosa of rodents.¹⁵⁻¹⁶ We recently reported, however, considerable glucuronidation and sulphation activity in the

guinea pig gastric epithelium.¹⁷ This present study provides evidence that human gastric epithelial cells also exert an important conjugative effect on the phenolic compound 1-naphthol.

Methods

PATIENTS AND CELL PREPARATION

Surgical specimens of gastric mucosa were obtained from 10 patients (aged 42-78 years) who required total gastrectomy for cancer. Adenocarcinomas were located in the antrum in seven patients, in the corpus in two patients, and at the oesogastric junction in one patient. Patients who had previously received antimitotic or enzyme inducing drugs and those with severe atrophic gastritis were excluded.

Cell isolation procedure

Specimens (4-6 cm²) were taken from a macroscopically healthy part of the corpus region of the freshly removed stomach, at least 8 cm from the tumour margin, and were placed in ice cold Hank's buffer. Gastric mucosal cells were isolated according to the method described by Soll *et al.*,¹⁸ modified by Sewing *et al.*,¹⁹ and adapted for human tissue by Varrentrapp *et al.*²⁰

Briefly, after removing gastric mucus with sterile pads, mucosa from the fundic region was scraped off, suspended in buffer A (70 mM NaCl, 20 mM NaHCO₃, 1.5 mM Na₂HPO₄, 5 mM KCl, 1.5 mM MgCl₂, 5 mM glucose, and 50 mM HEPES), cut into small pieces, and centrifuged (5 minutes at 200 g). The pelleted material was resuspended in 40 ml of an isolation medium consisting of buffer A supplemented with 1.15 mg collagenase type I (from *Clostridium histolyticum*; Sigma Chemical Co, St Louis, MI, USA), and 20 mg collagenase (from *Cl histolyticum*; Serva, Heidelberg, Germany). The suspension was preincubated for 10 minutes at 37°C and gassed with carbogen while stirring. The pH was kept at 7.4 by adding a solution of 5% TRIS. After this 10 minute preincubation, the tissue fragments were pelleted (2 minutes at 200 g) and incubated in 40 ml of isolation medium supplemented with 10 mg pronase E (from *Streptomyces griseus*, Serva), 25 mg hyaluronidase (Sigma), and 0.1% bovine serum albumin. After 30 minutes, the incubation was stopped, cells were filtered through a nylon cloth (mesh width 30 μ m), and were washed three times in buffer A supplemented with 25 mM HEPES and 0.1% bovine serum albumin. The isolated cells were centrifuged (5 minutes at 200 g). Cell viability was checked by trypan blue exclusion test and cells were used only if viability exceeded 90%.

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Cell suspensions were used either for immediate incubation or cultured.

Cell culture procedure

The procedure was adapted from that previously used in the guinea pig.²¹ Cells were suspended in sterile culture medium: minimum essential medium (MEM) containing Hank's salts supplemented with 20 mM HEPES, 25 mM NaHCO₃, 10% fetal calf serum (FCS), gentamycin (5 µg/ml), and amphotericin B (2.5 µg/ml). The cell count was estimated in a Türk haemocytometer. About 6×10^6 cells were spread on 6 cm plastic culture dishes (TC quality, Greiner, Nürtingen, Germany). The dishes were precoated with 300 µl of a 1:10 diluted collagen type I solution (Sigma). The dishes were placed in the incubator at 37°C in an atmosphere of 5% CO₂ in 100% humidity. The culture medium was changed daily.

CONJUGATION OF 1-NAPHTHOL BY HUMAN GASTRIC CELLS

The ability of human gastric mucosa to conjugate phenolic compounds was assessed with the model compound 1-naphthol, which is known to be detoxicated only by conjugation (glucuronidation and sulphation) in varied animal and human tissues.^{4,6,7,10,17}

Aliquots of freshly isolated cells (approximately 1 mg total cell protein, about 5×10^6 cells) or 4 day old cultures were incubated in 2 ml MEM at 37°C. After a 10 minute preincubation period, 1-¹⁴C-naphthol (0.5 µCi, 54 mCi/mmol, Amersham, Braunschweig, Germany) and cold 1-naphthol (Sigma) were added (1–900 µM final concentration). At various times over 1 hour, 200 µl samples were taken (three to five samples), without volume replacement. Samples were immediately denatured with 400 µl of a methanol:chloroform (3:1) mixture and shaken for 5 minutes. The extraction was terminated by adding 600 µl chloroform, resulting in an aqueous upper phase containing conjugates and an organic lower phase containing unconjugated

naphthol. Aliquots (20 µl) from the resulting aqueous and organic phases were taken and evaporated. After the addition of 2 ml of scintillator, radioactivity was counted in a Hewlett Packard liquid scintillation counter to determine the whole amount of conjugates formed and the remaining unconjugated naphthol. To determine the proportion of each type of conjugate, aliquots (50 µl) of the aqueous phase were applied to silica gel thin layer chromatography foils, and 1-naphthol glucuronide was separated from 1-naphthol sulphate in a solvent system consisting of n-butanol:0.01 M TRIS:propionic acid (75:14:1:1). The R_f values were 0.26 for 1-naphthol glucuronide and 0.69 for 1-naphthol sulphate.^{6,17} The radioactive bands were detected in a radioactivity scanner (Rita 90, Raytest, Straubenhardt, Germany) and the peaks integration was performed according to standardised parameters. The protein content of preparations was assayed by a modified Biuret method.

Results

The phenolic compound 1-naphthol was efficiently conjugated by human gastric epithelial cells in both types of preparations. In freshly isolated cells, the turnover of 1-naphthol into its conjugates over the 60 minutes of incubation averaged 29% of the added dose at 1 µM, and 3% at 100 µM, with complete saturation when the concentration was increased up to 900 µM. Both glucuronide and sulphate conjugates were formed at about the same rate (Fig 1). Apparent Michaelis constants, estimated from a Lineweaver-Burk plot, were similar for both reactions: the apparent V_{max}=0.07 nmol.mg protein⁻¹.min⁻¹, and apparent K_m=40 µM.

The 4 day old gastric cell cultures exhibited confluent islets of monolayers, and in some instances there were already confluent monolayers containing mainly mucous cells.²⁰ In cell cultures, the turnover of 1 µM 1-naphthol to its conjugates averaged 43 (13)% (mean (SEM)) of the added dose over 60 minutes (Fig 2), with glucuronidation exceeding sulphation.

Discussion

The rapid conjugation of phenolic compounds in the gastrointestinal tract and the liver has been considered to be a defence mechanism of the organism against intruding toxicants.^{1,2,4,5} The phenolic compound 1-naphthol is efficiently absorbed and at the same time more than 90% is conjugated by the intestine of rodents.⁴ 1-naphthol conjugating activities have also been described in normal and tumorous human colon^{9,10} and 4-nitrophenol-glucuronosyltransferase activity has been detected at varied levels in all parts of the human small and large intestine.⁸

The present study shows, for the first time, that this defence mechanism is also active in human gastric epithelium. When one considers that the gastric storage times of nutrients²² or drugs²³ may be up to 6 hours or more, the gastric conjugative activity in humans may be relevant to the inactivation of nutritional compounds and therapeutic drugs.

Figure 1: 1-Naphthol glucuronidation and sulphation in isolated human gastric epithelial cells. Freshly isolated human gastric cells (approximately 1 mg cell protein) were incubated in 2 ml minimal essential medium at 37°C in presence of 1-¹⁴C-naphthol (0.5 µCi). The final concentration was 0.5 µM (stars), 1 µM (open squares), 3 µM (open triangles), 7 µM (open circles), 20 µM (closed squares), 50 µM (closed triangles), and 100 µM (closed circles); 200 µl samples were assayed for glucuronide and sulphate conjugates as described.⁶ Data are means from six cell preparations.

Figure 2: Conjugation of 1-naphthol ($1 \mu\text{M}$) in cultured human gastric epithelial cells. The culture medium of 4 day old cultures was replaced by 2 ml of minimal essential medium and the cultures were incubated at 37°C in ambient air with $1 \mu\text{M}$ $1\text{-}^{14}\text{C}$ -naphthol. Samples were taken and processed as in Figure 1. Data are mean values (SEM) from four series of cultures.

The total rate of conjugation of 1-naphthol observed in our experiments with isolated human gastric cells was in the same range as those reported for the large intestine, but somewhat lower.¹⁰ When $1.5 \mu\text{M}$ 1-naphthol was incubated with isolated human colonic crypts, the turnover rate reached 55% over 40 minutes, with about 0.25 nmol conjugates/mg protein already being formed at 20 minutes. The apparent V_{max} derived from additional incubations with human colonic crypts amounted to 0.2 nmol/mg protein/minute, which is about twice the value estimated from the present data with isolated gastric cells. Similarly, total turnover rates ranging from 50–70% over 60 minutes were observed when $1 \mu\text{M}$ 1-naphthol was incubated with isolated human enterocytes (unpublished observations). Pacifici *et al.*²⁴ also reported that nuclear and microsomal fractions of human small intestinal mucosa were able to glucuronidate 1-naphthol efficiently but sulphation was not studied by these authors.²⁴ In freshly isolated human gastric cells, glucuronidation slightly exceeded sulphation at low naphthol concentrations, but both reactions occurred at about the same velocity at higher concentrations. In contrast, sulphation of 1 to $120 \mu\text{M}$ 1-naphthol always predominated glucuronidation (3:1) in human colonic crypts¹⁰ as well as in cultured non-tumorous human colon.⁹ Similar differences in glucuronidation/sulphation patterns between the stomach and the colon have been observed in the guinea pig.^{6,17}

In the present experiments with cultured gastric cells, the rates of conjugation, especially of glucuronidation, were higher, on a protein content basis, than in freshly isolated cells. This

may be a result of the predominance of mucous cells in cultures,^{20,21} as these were the most efficiently conjugating cell type in the guinea pig gastric mucosa.¹⁷ In addition, upregulation of glucuronidation and downregulation of sulphation after a few days of culture may have a role, as has been observed for 1-naphthol conjugation in cultured human hepatocytes.²⁵

The present results illustrate further the suitability of isolated and cultured cells for studying epithelial biotransformation reactions.^{9,10,26,27} The procedure used here yielded isolated gastric cells of high viability that could be cultured easily. It would be a significant advance if these suspensions and cultures of gastric cells proved adequate for investigating the effects of nutritional inducers on gastric biotransformation.^{28,29}

The stomach has long been forgotten in drug metabolism studies. The finding of considerable gastric drug metabolising activity in the present investigation, confirming our recent animal studies,¹⁷ should encourage new efforts to determine the role of gastric metabolism not only in early detoxication of ingested chemicals and drugs but also in chemical carcinogenesis.³⁰

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