Biotransformation enzymes in human intestine: critical low levels in the colon?

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Abstract

Biotransformation or drug-metabolising enzymes have an important function in the detoxication of ingested toxic, carcinogenic, or tumour promoting compounds. Enzyme activity and isoenzyme composition of three biotransformation systems: glutathione S-transferase, uridine diphosphate-glucuronosyltransferase, and cytochrome P-450 were studied in normal small and large intestinal mucosa from three kidney donors. The activity of most drug-metabolising enzymes decreases slightly from proximal to distal small intestine, whereas in the mucosa of the large intestine a sharp fall in activity was observed. The isoenzyme composition for each of the three biotransformation systems changed from the small to the large intestine. Class Alpha glutathione S-transferases were not expressed in the colon, in contrast to the small intestine where both Alpha and Pi class isoenzymes are present. In addition, with monoclonal antibodies fewer protein bands for UDP-glucuronosyltransferases and cytochrome P-450 were detected in the colon. In the small intestine both isoforms P-450, and P-450, were present, whereas in the colon only reduced amounts of cytochrome P-450, could be visualised. For UDP-glucuronosyltransferase, 53 and 54 kDa proteins could be detected in the small intestine, but in the colon there was only weak staining of the 54 kDa band. In the normal human colon enzymes are less active and there are fewer isoenzymes present in the mucosa than in the small intestine. This implies a lower level of the detoxifying potential in the colon, which might be important in regard to the high rates of carcinogenesis in the colon.

Biotransformation is the sum of all chemical reactions that alter the nature, water solubility, and distribution of non-nutritive compounds that are potentially toxic or carcinogenic. Such compounds may enter the body as food components, food additives, or drugs. The gastrointestinal tract often is the route of entry of such harmful molecules and the gastrointestinal mucosa can be very active in biotransformation. Data on human intestinal biotransformation enzymes are, however, relatively scarce. The presence of both phase I and phase II biotransformation enzymes has been shown. Compared with the liver, however, the specific activity of these enzymes in small intestinal mucosa may be lower, apart from glutathione S-transferase.

From the small to the large intestine the activity of glutathione S-transferase seems to decrease. The activity of UDP-glucuronosyltransferase decreases both in small and large intestine, whereas in the coloncytochrome P-450 decreases.

Figure 1: Longitudinal distribution of intestinal biotransformation activities. Activities for cysteine, glutathione S-transferase (GST) microsomal 4-nitrophenol uridine diphosphate-glucuronosyltransferase (UDPGT), and bilirubin UDPGT were determined as indicated under Methods. Enzyme assays were performed in mucosa obtained from segments of the proximal (duodenum), mid (jejunum), and distal (ileum) small intestine. In addition, three colonic segments from proximal (caecum), mid (transverse colon), and distal (sigmoid colon) large intestine were investigated. Activities were determined in triplicate for each subject and are given as means (SEM) for three subjects. Small intestinal values are from four subjects since some data from Peters et al are included. nd=not detectable.
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The isoenzyme composition from cytochromes P-450 as well as glutathione S-transferases may also be different in large bowel mucosa. Most available data, however, come from analysing separate specimens from different subjects. Therefore, the possibility cannot be excluded that some of the differences are due solely to interindividual variations.

We have investigated the longitudinal distribution and isoenzyme composition of phase I (cytochrome P-450) and phase II drug-metabolising enzymes (uridine diphosphate-glucuronosyltransferases, glutathione S-transferases) in normal small and large intestinal mucosa from three kidney donors. Significant differences in isoenzyme composition and activity were found between the two parts of the intestine.

Methods

Tissue
Human intestines were obtained from three kidney donors. Donor 1 was a female aged 3-5 months, who died after a head injury (trauma capitis). Donor 2 was an 18 year old man who died from cerebral damage after a subdural/subarachnoid haemorrhage. Donor 3 was a 49 year old man who died after a head injury. Data on small intestinal biotransformation activities obtained from another male kidney donor (an 18 year old) are also incorporated in Figures 1 and 4. None of the donors had gastrointestinal disorders.

After removal, intestine was stored on ice during transport to the laboratory. The intestine was opened and cleaned by repeated washings with ice cold 0.9% NaCl. Tissue was cut into segments, frozen in liquid nitrogen, and stored at −80°C until use.

From segments (length 20–40 cm; area 70–200 cm²), taken at intervals of 60–120 cm, mucosa was isolated. Mucosa was homogenised and cytosolic and microsomal fractions were made as described previously.

The investigations were approved by the local ethical committee on human experimentation.

Figure 2: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of purified intestinal glutathione S-transferases. Glutathione-agarose purified glutathione S-transferases were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (10% acrylamide, w/v) and subsequently stained with Coomassie brilliant blue. Purified intestinal glutathione S-transferases from patient 1 (5–10 μg protein) are shown in panel A, slots 1–6. Purified transferases from patient 2 (4–12 μg protein) are shown in panel A, slots 7–12, and those from patient 3 (3–10 μg protein) are shown in panel B, slots 1–4. In panel B slot 5 purified hepatic glutathione S-transferases (5 μg protein) from patient 3 were applied. Preparations shown are from duodenum (A, 1 and 7; B, 1), jejunum (A, 2 and 8), ileum (A, 3 and 9; B, 2), caecum (A, 4 and 10; B, 3), transverse colon (A, 5 and 11), and sigmoid colon (A, 6 and 12; B, 4). Glutathione S-transferase Pi subunits are indicated by arrow.

Figure 3: Immunodetection of intestinal glutathione S-transferase-Pi. Human intestinal cytosol (75 μg protein) was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and subsequent Western blotting to nitrocellulose. GST-Pi was immunodetected with a monoclonal antibody against GST-Pi from human placenta. Cytosols from proximal to distal small intestine (panel A, slots 1–6, patient 1; A, 10–15, patient 2; B, 2 and 3, patient 3) and from proximal to distal large intestine (A, 7–9, patient 1; A, 16–18, patient 2; B, 4 and 5, patient 3) are shown. In panel A, slot 19 and B slot 1 purified glutathione S-transferase Pi from human placenta (0.5 μg protein) was applied to the gels. The lowest band visible in panel B slot 2 may be an additional acidic glutathione S-transferase-Pi isofrom.
ENZYME DETERMINATIONS

Cytosolic glutathione S-transferase activity with 1-chloro 2,4-dinitrobenzene as substrate was measured by the method of Habig et al.\(^6\) UDP-glucuronosyltransferase activity with 4-nitrophphenol and bilirubin as substrates was measured in microsomes and whole homogenate, respectively, in the presence of 0-0.0125% Triton X-100.\(^{18-20}\)

MISCELLANEOUS

Glutathione S-transferases were purified as described previously.\(^7\) Protein was determined by the method of Lowry et al.\(^7\) Sodium dodecyl sulphate-polyacrylamide gel electrophoresis was done according to Laemmli.\(^{22}\) After staining with Coomassie brilliant blue, gels were scanned at 600 nm with a laser densitometer (LKB 2202 Ultrascan, LKB, Bromma Sweden). Immunoblotting with monoclonal antibodies against cytochrome P-450,\(^4\) UDP-glucuronosyltransferase,\(^9\) and glutathione S-transferases\(^{12,13}\) was performed as described previously.\(^{16}\)

Results

The intestines from the kidney donors were divided in segments of approximately 20 cm. Mucosa was isolated from segments taken at the indicated locations (see figure legends). Mucosa was homogenised and subcellular fractions made.

In the cytosolic fractions specific activity of glutathione S-transferase was determined (Fig 1). Activities are highest in the proximal small intestine and show a sharp fall from small to large intestine.

The longitudinal distribution of microsomal UDP-glucuronosyltransferases with bilirubin and 4-nitrophphenol as substrates is also shown in Figure 1. In the small intestine activity of 4-nitrophphenol UDP-glucuronosyltransferase is more or less constant, whereas the conjugation of bilirubin is decreasing. In the three subjects investigated a dramatic fall in activity for 4-nitrophphenol as well as for bilirubin UDP-glucuronosyltransferase in the large intestine was observed.

Small and large intestinal glutathione S-transferases were purified by affinity chromatography on glutathione-agarose. These purified preparations are shown in Figure 2. Glutathione S-transferases from small intestine are composed of both class Alpha (25 kDa subunits) and class Pi (24 kDa subunits) isoenzymes. In purified glutathione S-transferase preparations from colonic mucosa, after Coomassie brilliant blue staining only class Pi transferases were present. With a monoclonal antibody developed against glutathione S-transferase Pi from human placenta\(^{17}\)

Figure 4: Immunodetection of intestinal UDP-glucuronosyltransferases. Intestinal microsomes from patients 2 and 3 were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (7% acrylamide; w/v) and subsequent Western blotting. The blot was incubated with a monoclonal antibody against human UDP-glucuronosyltransferases. In panel A small intestinal microsomes from patient 2 (20 μg protein) originating at distances of 0, 100, 200, 340, 460, and 610 cm from the pylorus were applied in slots 1–6, respectively. In slots 7–9 large intestinal microsomes (40 μg protein) from the caecum and transverse and sigmoid colon are shown. Slot 10 contains 4 μg hepatic microsomes from another patient. In panel B microsomes from liver (15 μg protein), duodenum (50 μg), ileum (50 μg), caecum (100 μg), and sigmoid colon (100 μg) from patient 3 are shown in the slots 1–5, respectively.

Figure 5: Immunodetection of intestinal cytochromes P-450.\(^{\ldots}\) Intestinal microsomes from patient 1 (panel A, slots 2–10), patient 2 (A, 11–19), and patient 3 (B, 1–4) were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (7% acrylamide; w/v) and Western blotting and subsequently incubated with a monoclonal antibody against cytochromes P-450.\(^{\ldots}\) Hepatic microsomes (8 μg protein) are seen in panel A slot 1, microsomes from proximal to distal small intestine (30 μg protein) are in panel A, slots 2–7 and 11–16, panel B, slots 1 and 2. Microsomes from proximal to distal large intestine (60 μg protein) were applied in panel A, slots 8–10 and 17–19, panel B, slots 3 and 4. In slot 13, for unknown reasons, little protein has moved into the gel. The 54 kDa band of cytochrome P-450, is indicated by arrow. Other cytochrome P-450 related protein bands seen have molecular masses of 52 kDa (liver and intestine) and 56 kDa (liver).
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Figure 6: Immunodetection of intestinal cytochrome P-450. Intestinal microsomes from patient 1 (panel A, slots 1–9), patient 2 (A, 10–18), and patient 3 (B, 2–5) were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (7% acrylamide, w/v) and subsequent Western blotting. Cytochrome P-450 was detected with a monoclonal antibody. Hepatic microsomes from patient 3 (4 µg protein) are seen in panel B slot 1; microsomes from proximal to distal small intestine (20 µg protein) are seen in panel A, slots 1–6 and 10–15; panel B, slots 2 and 3. Microsomes from proximal to distal large intestine (40 µg protein) were applied in panel A, slots 7–9 and 16–18, panel B, slots 4 and 5. The band representing cytochrome P-450, has a molecular mass of 52 kDa.

This isoenzyme was indeed readily shown in cytosolic fractions from both small and large intestine (Fig 3). By immunodetection, using a monoclonal antibody against class Mu glutathione S-transferases, small amounts of this isofrom were detected in all intestinal segments from two patients (not shown). In the intestine from patient 1 this isoenzyme was absent. Hepatic glutathione S-transferases from patient 3 are also shown in Figure 2. Here only class Alpha isofroms and no class Pi subunits are present.

On a Western blot, small intestinal microsomes from patients 2 and 3, incubated with a monoclonal antibody against UDP-glucuronosyltransferase, show two very close bands. In the corresponding colonic microsomes, even when twice as much protein is applied to the gel, only a very weak protein band is visible (Fig 4). Intestinal microsomes from patient 1 gave only very weak bands in the small intestine and no staining at all in the colon (not shown).

A Western blot of small intestinal microsomes, treated with a monoclonal antibody against cytochromes P-450, shows two bands of 52 and 54 kDa (Fig 5). In the proximal small intestine from patient 1, the 54 kDa band is very weak (Fig 5, panel A). In colonic microsomes the 52 kDa band is not detectable. Figure 6 shows that immunodetection of cytochrome P-450, is possible only in small intestinal, but not in colonic, microsomes from all subjects. It should be noted that here again for the large intestinal samples twice as much protein was applied to the gel.

Discussion

Longitudinal distribution of the biotransformation enzyme activities measured show a decline in activity, with a sharp fall from the small to the large intestine. For glutathione S-transferases, such a large decrease in activity in colonic mucosa was noted before in specimens obtained from patients with carcinoma of the colon and rectum and in rodents. The intestines investigated here were from patients with no intestinal disease. Thus the relatively low colonic glutathione S-transferase activities are not restricted to patients with malignancies of the large bowel.

Glutathione S-transferase subunit composition is different in small and large intestinal mucosa. The small intestine contains class Alpha, Mu, and Pi isoenzymes, whereas in the colon predominately class Pi subunits can be detected. Class Mu subunits are present in very small amounts and can hardly be seen after Coomassie brilliant blue staining of the sodium dodecyl sulphate gel shown in Figure 2. Thus in contrast to other reports, we found class Pi subunits almost exclusively, both in normal and neoplastic colonic tissue. Our results confirm the recent immunohistochemical findings of Hayes et al, giving a similar isoenzyme distribution in small and large intestine.

The presence of bilirubin UDP-glucuronosyltransferase activity in the small intestines from all subjects investigated once again confirms that bilirubin metabolism is not restricted to the liver. Moreover, specific activities of hepatic and small intestinal bilirubin UDP-glucuronosyltransferase do not differ dramatically (mean (SEM) 704 (42) and 343 (22) nmol/g tissue/h, respectively; data are from patient 3). Similar data obtained from another patient have been reported before.

Immunoblot studies with an antibody against UDP-glucuronosyltransferase show two close bands in the small intestine, whereas in colonic mucosa both bands were hardly visible. The two small intestinal bands (53 and 54 kDa) may correspond to activities for bilirubin (53 kDa) and 4-nitrophenol (54 kDa). Both activities are drastically reduced in the colon, and here the amount of UDP-glucuronosyltransferase protein may be below the detection limit for immunoblots.

For isoenzymes of the cytochrome P-450 biotransformation system a similar case exists. Isoform P-450, is detectable in the small intestine but not in the colon. Another isoenzyme, probably P-450, is present in both colonic and small intestinal mucosa. Since more large intestinal microsomes were loaded onto the gel it may be concluded from the immunoblot in Figure 5 that the levels of P-450, in the colon of all patients are lower compared with those in the small intestine.
The above experimental data on biotransformation enzyme activities in the intestines of healthy kidney donors show a constant or decreasing activity along the small intestine and a significant fall in activity in the colon. For all three enzyme systems investigated here the number of isoenzymes in the colon is reduced. This pattern of both reduced enzyme activities and isoenzyme composition undoubtedly will result in a lower level of detoxication in the colon. Activities of biotransformation enzymes in the colon may further decrease with increasing age. In addition, levels of β-glucuronidase are highest in the colon. This could result in less toxic glucuronides becoming toxic again by deconjugation. Indeed, high concentrations of mutagens have been detected in the human colon. In conclusion, the detoxicating capacity in the human colon could be at a critical low level which may contribute to the high rate of colonic cancer.

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