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CYTOCHROMES P-450 IN THE INTESTINAL MUCOSA OF MAN

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Abstract—With monoclonal antibodies against cytochrome P-450$_5$ and P-450$_{4,5,6}$, 52 and 54 kDa bands are visualized in microsomes from proximal as well as distal human small intestine. These bands most probably correspond to cytochrome P-450$_5$ and P-450$_4$, respectively. This and several other cytochrome P-450 related proteins are present in hepatic microsomes from the same patient. In both hepatic and intestinal microsomes from this patient cytochrome P-450$_8$ is hardly detectable. In contrast to the small intestine and liver, large intestinal tissue from several other patients does not contain cytochrome P-450$_5$. Here the 54 kDa isoenzyme, possibly cytochrome P-450$_4$, is most prominent. Earlier we found a higher content of total cytochrome P-450 in the proximal as compared to the distal small intestine. A similar distribution is now found with regard to aldrin epoxidase activity.

The cytochromes P-450 are a family of enzymes that catalyse the biotransformation of numerous endogenous substrates such as steroids, and xenobiotics such as food additives or drugs. The enzymes are mainly localized in the endoplasmic reticulum of cells from various organs and species [1, 2]. In man, the enzymes are most studied so far from liver, and relatively few data on intestinal cytochromes P-450 are available [3]. As compared to the liver, cytochrome P-450 content of intestinal mucosa is much lower [4–6]. One reason for this difference is the localization of the intestinal cytochromes P-450 which is mainly in villous tip cells. These are only part of the total mucosal cell population [7, 8].

In human small intestine, protection against toxic or carcinogenic compounds seems to be sufficient, since, in contrast to the large intestine, relatively few malignancies are found here [9]. In this respect we studied the cytochromes P-450, especially with regard to isoenzyme composition in liver, small and large intestine.

MATERIALS AND METHODS

Tissue. Hepatic and small intestinal tissue, from which the results are shown in Table 2 and Figs 1–3, was obtained from a kidney transplant donor who died from cerebral damage four days after a traffic accident. In the meantime, the patient was on parenteral nutrition [5]. Other small and large intestinal tissue was obtained, after surgical reactions, from patients with Crohn's disease, with large intestinal malignancies or polyposis coli. All tissue used was macroscopically normal. The tissue was cooled on ice as soon as possible after resection (within 15–60 min) and transported to the laboratory immediately. Medication of patients is not known in detail. Human specimens were obtained under protocols approved by the ethical committee for conduct of human research at our institution.

Preparation of microsomes. Intestinal mucosa was scraped off and homogenate of mucosa and liver was prepared as described before [10]. Microsomes from hepatic and intestinal tissue were prepared as follows: homogenate was centrifuged at 10,000 *g* for 20 min, subsequently the supernatant was centrifuged at 150,000 *g* for 50 min. The microsomal pellet was resuspended in homogenization buffer, frozen in liquid N_2 and stored at −80° until use.

Analysis of cytochrome P-450. Aldrin epoxidase, 7-ethoxycoumarin *O*-deethylase and aryl hydrocarbon hydroxylase assays were performed according to the methods previously described [11]. Immunoblots were made essentially as described earlier for the detection of UDP-glucuronyltransferase [12].

Monoclonal antibodies against human liver cytochrome P-450$_5$, P-450$_{4,5,6}$, P-450$_8$ and rat P-450$_{BNF}$ (β-naphthoflavone) were used in this study. Anti-P450$_5$ (denoted K03-13-7-10) was characterized and described in an earlier study [13]. Human cytochrome P-450 isoenzymes were named according to Wang *et al.* [14]. To respect the recommended nomenclature [15], P-450$_5$ should be named P450 III A3, and P-450 BNF corresponds to P450 IA1. The monoclonal antibodies have been characterized using purified fractions kindly provided by Prof. Guengerich (Nashville, TN). In consequence and to avoid any misunderstanding, Guengerich's cytochrome P-450 nomenclature has been maintained throughout the text.

RESULTS AND DISCUSSION

In Table 1, data on longitudinal distribution of total cytochrome P-450 content and of aldrin epoxidase activity in human small intestinal mucosa are shown. The data indicate that content and activity of cytochrome P-450 are higher in the proximal as compared to the distal small intestine. These results are in good agreement with earlier studies in several animal

Table 1. Longitudinal distribution of total cytochrome P-450 content* and aldrin epoxydase activity in human small intestinal microsomes

Small intestine segment number†	CO binding hemoprotein (pmol/mg protein)	Aldrin epoxidase (pmol/min/mg protein)
1	12.6 ± 0.4	n.d.
2	17.4 ± 1.6	12.0 ± 1.4
3	20.0 ± 3.0	54.0 ± 4.0
4	13.4 ± 2.0	30.0 ± 2.4
5	21.4 ± 2.0	7.4 ± 1.0
6	10.6 ± 4.4	7.8 ± 0.8
7	6.6 ± 2.4	4.6 ± 0.6
8	5.6 ± 2.2	18.6 ± 2.0
9	6.0 ± 0.8	<2
10	9.2 ± 4.4	<2

* Data are from Ref. 5.

† Segment number 1, 2, 3 . . . 10 corresponds with small intestinal mucosal microsomes, originating at distances of 0, 50, 100 . . . and 450 cm from pylorus, respectively [5].

n.d., not determined. Values are given with SD for three determinations.

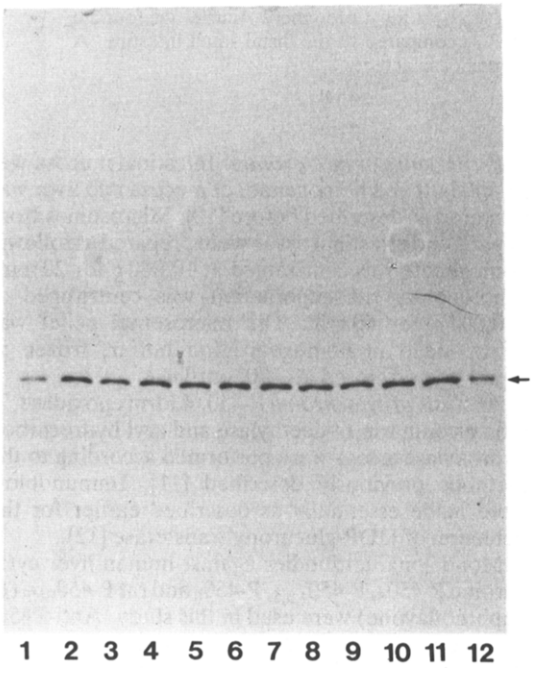

Fig. 1. Immunoblot of human small intestinal and liver microsomes, with anti-cytochrome P-450$_5$. Microsomes were separated on a SDS polyacrylamide gel (10% acrylamide, w/v) and a Western blot of this gel was incubated with 1/500 diluted anti-cytochrome P-450$_5$ ascites fluid. Lanes 1 and 12 contain identical samples of hepatic microsomes (4 μg protein) and lanes 2–11 contain small intestinal microsomes from the same patient (20 μg protein each), originating at distances of 0, 50, 100 . . . 450 cm from pylorus, respectively. The arrow indicates a 52 kDa band.

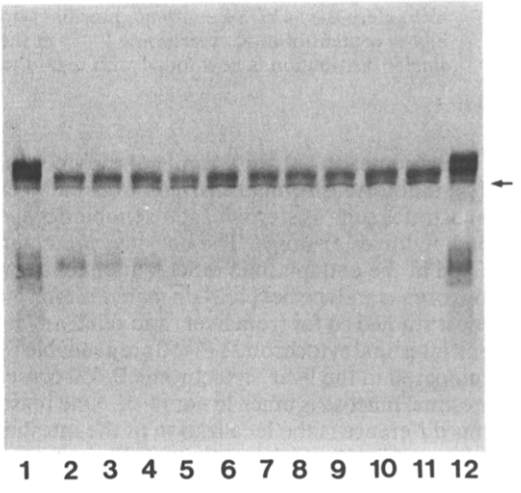

Fig. 2. Immunoblot of human small intestinal and liver microsomes, with anti-cytochrome P-450$_{4,5,6}$. A Western blot of intestinal and hepatic microsomes was prepared as described in the legend of Fig. 1. The blot was incubated with 1/50 diluted anti-cytochrome P-450$_{4,5,6}$ ascites fluid. Lanes 1 and 12 contain hepatic microsomes (7 μg protein) and lanes 2–11 contain small intestinal microsomes (30 μg protein each) identical to those of Fig. 1. The arrow indicates the 52 kDa band. Other major bands visible have molecular masses of approximately 54 kDa (intestine and liver) and 56 kDa (liver).

species where a similar distribution in small intestinal cytochrome P-450 enzymes was found [3, 8].

Jejunal cytochrome P-450 content (see Table 1, segments 2–4; 13.4–20.0 pmol/mg protein) is lower as compared to earlier reported values [4, 6]. Also the 7-ethoxycoumarin *O*-deethylase activity of 10.4 pmol/min/mg protein in microsomes from total mucosal scrapings of jejunum (segment 2) is lower as compared to corresponding values from small intestinal villous cells only [16].

Small intestinal and hepatic microsomes from the kidney donor, incubated with antibodies against human liver cytochrome P-450$_8$ did not reveal specific bands in the area of 50–58 kDa on Western blot (not shown). Aryl hydrocarbon hydroxylase activity, which is correlated to cytochrome P-450$_8$ [11, 17], is also very low in jejunal (4.4 pmol/min/mg protein) and hepatic microsomes (4.0 pmol/min/mg protein) from this patient. In normal adult hepatic tissue much higher levels are present [11, 17].

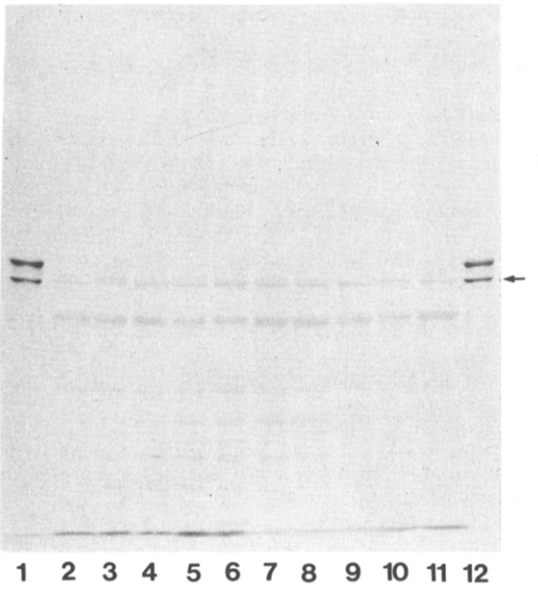

Fig. 3. Immunoblot of human small intestine and liver microsomes with anti-cytochrome P-450$_{rat\ BNF}$. A Western blot was prepared as described in the legend of Fig. 1. The blot was incubated with 1/160 diluted anti-cytochrome P-450$_{rat\ BNF}$ ascites fluid. Lanes 1 and 12 contain hepatic microsomes (15 μg protein) and lanes 2–11 contain small intestinal microsomes (60 μg protein) similar to those of Fig. 1. The arrow indicates a 54 kDa band, visible in lanes 1–12. In the hepatic microsomes (lanes 1 and 12) an additional 57 kDa band is visible.

dation levels [18], the low values obtained here, may be the result of the parenteral nutrition, received by this kidney donor.

This hypothesis may be supported by the finding that 7-ethoxycoumarin *O*-deethylase activity in small intestinal mucosa of patients receiving a semisynthetic diet was significantly lower as compared to the values on a normal diet [19].

Cytochrome P-450$_5$, the isoenzyme which in liver may reflect aldrin epoxidase activity [11], is present in relatively large amounts in human small intestine, as shown by the presence of the 52 kDa band (Fig. 1). Hepatic microsomes from the same patient showed a band of identical molecular mass which was less intensely stained. However, reduced amounts of hepatic microsomes were applied to the SDS polyacrylamide gel. Recently Watkins *et al.* [6] using our same antibody (K03-13-7-10), showed that a cytochrome P-450 protein, with molecular mass of 51 kDa and called HLp, was present in human liver and jejunum specimens from several patients. Thus in a qualitative sense our results fully agree with those of Watkins *et al.* [6].

From the immunoblot analysis (Figs 1 and 2) cytochrome P-450$_5$ appears to be a major isoenzyme in human small intestinal mucosa. This is further supported by the fact that aldrin epoxidase is the highest enzymatic activity observed in these samples.

Incubation of Western blots with anti-cytochrome P-450$_{4,5,6}$ or anti P-450$_{rat\ BNF}$, also reveals differences between hepatic and small intestinal P-450 enzyme

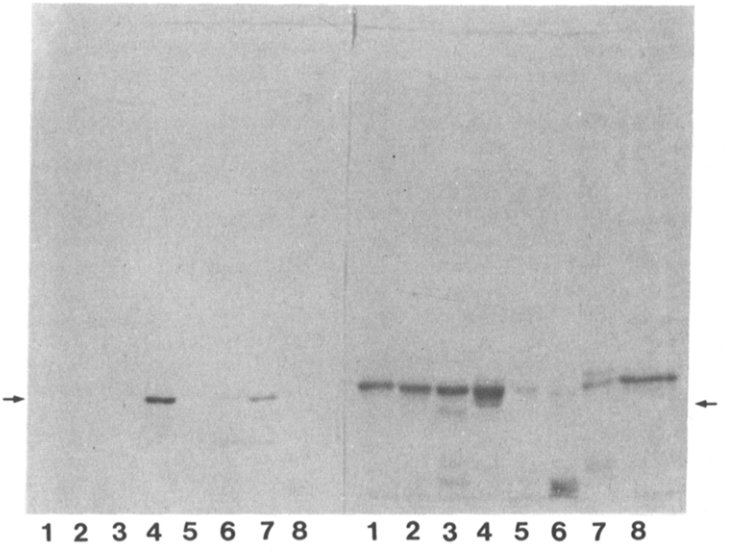

Fig. 4. Immunoblot of human small and large intestinal microsomes with anti-cytochrome P-450. Microsomes (left panel, 40 μg protein; right panel, 60 μg protein) from large intestine (slots 1–3, 5 and 8) and small intestine (slots 4, 6 and 7) from different patients, were separated on a SDS polyacrylamide gel (7% acrylamide, w/v), and subsequently a Western blot was made. The nitrocellulose paper was cut in two pieces and the left part was incubated with anti-cytochrome P-450$_5$, and the right part with anti-cytochrome P-450$_{4,5,6}$. The arrows indicate a 52 kDa band.

These lower values may be well explained by inter-individual variations or differences in preparation and subsequent stability of microsomes. However, since total parenteral nutrition is known to decrease at least hepatic but most possibly also intestinal drug oxi-

populations (Figs 2 and 3). In Fig. 2 it is shown that small intestinal microsomes contain P-450 bands in addition to P-450$_5$, with higher molecular mass (54 kDa). Hepatic microsomes from the same patient express an extra 57 kDa band not present in small

intestinal mucosa. In addition, the liver contains cytochromes P-450, which are not at all or only very little present in the corresponding small intestinal tissue. These forms react with the antibody raised against rat liver β-naphthoflavone-inducible cytochrome (Fig. 3).

Investigation of intestinal specimens from several patients also reveals differences in cytochrome P-450 enzyme populations between small and large intestine (Fig. 4). In all small intestinal preparations investigated a 52 kDa band from cytochrome $P\text{-}450_5$ related proteins is present in variable amounts. In large intestine this protein was not detectable (Fig. 4, left panel). The same preparations were tested for anti $P\text{-}450_{4,5,6}$ reactivity (right panel). The large intestinal preparations seem to contain almost exclusively cytochrome P-450 related proteins with a molecular mass of 54 kDa, whereas the composition of the cytochromes from small intestine is more complex. These findings are in excellent agreement with the result of Strobel *et al.* [20] who could detect $P\text{-}450_4$, but not $P\text{-}450_5$ in colon microsomes from one patient.

In summary, hepatic cytochromes P-450 are more complex as compared to those of the small intestine, where $P\text{-}450_5$ with a molecular mass of 52 kDa is an important isoenzyme. This form is missing in the large intestine. Here a 54 kDa isoenzyme, possibly $P\text{-}450_4$, is most prominent. Whether or not the absence of cytochrome $P\text{-}450_5$ in the human colon is related to a higher risk for carcinomas in this tissue, needs further investigation.

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