CYTOCHROME P-450 AND DRUG METABOLISM IN INTESTINAL VILLOUS AND CRYPT CELLS OF RATS: EFFECT OF DIETARY IRON

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SUMMARY: In intestinal mucosa of fed adult rats, cytochrome P-450 content and activity of benzpyrene hydroxylase and *p*-nitroanisole O-demethylase are highest in the upper small intestine and progressively decrease toward the terminal ileum. Among the mucosal cell populations, mature villous tip cells contain 6 to 10 times more cytochrome P-450 and drug-metabolizing activity per mg microsomal protein than epithelial crypt cells. On restriction of dietary iron intake for 48 hr, cytochrome P-450 content and drug-metabolizing enzyme activity of villous tip cells decrease to 42% and 13% of control values, but are restored within 24 hr by oral iron supplementation. These findings suggest that intestinal drug metabolism is localized primarily in upper villous cells of duodenal mucosa, that cytochrome P-450 is synthesized in maturing epithelial cells as they migrate to the top of the mucosal villus and that this process is critically dependent on dietary (luminal) iron.

INTRODUCTION: Mucosa of small intestine has been shown in vivo and in vitro to possess drug metabolizing activity (1,2). Wattenberg (3) has suggested that biotransformation by intestinal aryl hydrocarbon hydroxylases may represent a protective mechanism against dietary carcinogens. The intestinal monooxygenase systems most extensively investigated include benzpyrene hydroxylase (4), 7-ethoxycoumarin O-deethylase (5), and phenacetin O-dealkylase (6). Their activity per mg microsomal protein in intestinal mucosa generally is well below that in the liver (7). Many of the intestinal enzymes capable of oxidative metabolism of xenobiotics are inducible by dietary constituents such as polycyclic hydrocarbons (8) or flavones contained in standard laboratory chow (3). Moreover, significant sex and species differences in basal activity of these enzyme systems have been reported (7,9,10).

Cytochrome P-450 (5) which serves as terminal oxidase in most of these reactions, cytochrome b_5 (5), and NADPH-cytochrome c reductase (7) have been demonstrated in intestinal mucosa, but their concentration or activity is below

that in the liver (7). Moreover, technical difficulties have been encountered, particularly in rats (11), in assaying intestinal cytochrome P-450, because of the presence of a large 420 nm peak in the carbon monoxide difference spectrum of reduced microsomes. The latter is believed to result from degradation of cytochrome P-450 by a factor associated with rat intestinal microsomes (7). Recent reports that several intestinal enzyme systems catalyzing synthetic or assimilative functions are more active in mature upper villous cells than in proliferating cells of the crypt (12-14) prompted investigation of drug-metabolizing activity in different cell populations of rat small intestinal mucosa. For convenience, intestinal epithelial cells were segregated into presumably mature villous tip cells, functionally less differentiated intermediate villous cells and proliferating crypt cells (15). These cell populations obtained from rats on various dietary regimens were assayed for benzpyrene hydroxylase and *p*-nitroanisole O-demethylase activity and for cytochrome P-450 and b_5 content.

MATERIALS AND METHODS: All experiments were conducted in fed male rats (300-400 g) purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass., and certified to be "specific pathogen" (Bartonella)-free. The following diets were employed: standard rat chow diet containing 20 mg iron/100 g diet (Berkeley Diet, Feed Stuffs Processing Co., San Francisco, Calif.); semisynthetic iron-supplemented diet consisting of vitamin test casein, 27%; corn starch, 55%; hydrogenated vegetable oil, 14%; salt mixture without ferric phosphate, 3%; and vitamin mixture, 1% (Nutritional Biochemicals Corp., Cleveland, Ohio), supplemented with ferrous sulfate (40 mg iron/100 g diet); and semisynthetic low iron diet containing all above constituents except ferrous sulfate resulting in an overall iron content of less than 0.1 mg/100 g diet. Unless noted otherwise, rats were maintained on the respective diets for at least 7 days before study. They had free access to water, and weight curves on the different diets were similar. The animals were sacrificed under light ether anesthesia and exsanguinated by aortic puncture. All subsequent procedures were conducted at 4°C.

Consecutive 15 cm segments of small intestine beginning at the pylorus were excised, washed free of contents with 60 ml of ice-cold 1.15% potassium chloride (KCl) solution, split longitudinally, and opened. Villous tip, villous intermediate, and mucosal crypt cells were separated by differential scraping (15). The discriminatory effectiveness and reproducibility of this method were evaluated by determining time-dependent [^{3}H]-thymidine incorporation into these cell populations in vivo (13) and by morphologic criteria.* In cell preparations obtained 1 hr after an intraperitoneal pulse label of [^{3}H]-thymidine, isotope concentration (cpm per mg protein) in the nuclear fraction of crypt cells was at least 10 times higher than that of villous tip cells; lower villous cells contained [^{3}H]-thymidine intermediate between crypt and tip cells. Histologically, tip cell preparations contained the upper half of the villous

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height, the intermediate cell preparations the lower half of the villus, and crypt cell fractions the crypt region of the mucosa.

Individual cell preparations were homogenized in 0.1M KH_2PO_4-K_2HPO_4 buffer, pH 7.4, and sonicated for 25 s at 35 W (Sonifier cell disruptor, Heat Systems-Ultrasonics, Inc., Plainview, N.Y., Model W185D). Microsomes were prepared from 20,000 x g supernatant of homogenate by centrifugation at 105,000 x g for 60 min. The microsomal pellet was washed once with 1.15% aqueous KCl solution, then resuspended in 0.1M K-phosphate buffer to a volume containing the equivalent of 300 mg of wet mucosal tissue per ml. Benzpyrene hydroxylase activity was determined by the method of Wattenberg et al. (4) as modified by Kuntzman et al. (16) and Alvares et al. (17) and *p*-nitroanisole O-demethylase activity by the method of Netter and Seidel (18). Results were expressed as nmol product formed in 1 hr per mg of microsomal protein. Cytochrome P-450 and cytochrome b_5 content of microsomes were determined by the techniques of Omura and Sato (19,20) using an Aminco DW2 spectrophotometer. Protein was measured by the procedure of Lowry et al. (21).

RESULTS AND DISCUSSION: Preliminary experiments confirmed the reports of Wattenberg et al. (4) and Scharf et al. (5) that monooxygenase activity in microsomes obtained from whole intestinal mucosa is highest in the upper small intestine and progressively decreases toward the terminal ileum. Microsomes prepared from villous tip cells exhibited a similar activity gradient from duodenum to ileum, both for benzpyrene hydroxylase and *p*-nitroanisole O-demethylase. Moreover, villous tip cells of duodenal mucosa contained more cytochrome P-450 and b_5 than those of more distant parts of the small intestine; the respective values in nmol per mg of microsomal protein for duodenal and ileal mucosa were: cytochrome P-450, 0.110 and 0.051; cytochrome b_5, 0.109 and 0.076. For all subsequent experiments, duodenal mucosa was used.

Activity of benzpyrene hydroxylase and of *p*-nitroanisole O-demethylase and content of cytochrome P-450 were much higher in microsomes from villous tip cells than in microsomes from villous intermediate and crypt cells (Table I). Only a small difference was found for cytochrome b_5 between tip and crypt cells. In microsomes from crypt cells, *p*-nitroanisole O-demethylase activity was not measurable (Table I). Reduced microsomes from crypt cells exhibited a small absorption peak at 420 nm on exposure to carbon monoxide. Since this absorption was present also in the carbon monoxide difference spectrum of oxidized microsomes of crypt cells, it most likely was caused by carboxyhemoglobin. Arterial perfusion of the small intestine with cold isotonic saline in situ prior to pre-

TABLE I

Microsomal Hemoproteins and Drug Metabolism in Different Cell Types of Duodenal Mucosa of Rats

Cell type	Cytochrome nmol/mg protein		Activity nmol/hr/mg protein	
	P-450	b_5	Benzpyrene hydroxylase	p-Nitroanisol O-demethylase
Villous tip	0.110 ± 0.004	0.109 ± 0.003	8.41 ± 1.26	14.74 ± 1.10
Villous intermediate	0.070 ± 0.004	0.088 ± 0.007†	3.39 ± 0.10	7.62 ± 0.55
Epithelial crypt	0.011 ± 0.002	0.082 ± 0.001	1.41 ± 0.41	trace

Values represent mean ± standard error of mean of 3 individual experiments using duodenal mucosa pooled from 4 rats on standard rat chow diet. All values are significantly different from those of villous tip cells at the $p < 0.01$ level except † ($p < 0.05$).

paration of the crypt cells significantly reduced this carbon monoxide-mediated absorption in the 420 nm region. Moreover, incubation of tip cell microsomes with 105,000 x g supernatant fraction from crypt cell homogenate for 90 min at 37°C neither reduced the concentration of microsomal cytochrome P-450 nor increased absorption at 420 nm.

These observations suggest that intestinal monooxygenase activity is localized primarily in the most mature cells of the villous tip and that cytochrome P-450 is synthesized or assembled in maturing mucosal cells as they migrate from the crypt to the top of the villous structure. The cytochrome P-450 content and benzpyrene hydroxylase and *p*-nitroanisole O-demethylase activity of villous tip cells from rats fed standard chow diet were higher than from animals fed a semisynthetic iron-supplemented diet (Table II). By contrast, semisynthetic diet with or without iron supplementation moderately increased the cytochrome b_5 content (Table II). This confirms previous reports that drug metabolism in whole intestinal mucosa is affected by dietary constituents (3,8) and also suggests that dietary factors influence cytochrome P-450 synthesis in villous cells.

An important question raised by these findings is the source of the iron required for synthesis of cytochrome P-450 in the epithelial cells of the upper intestinal villus. Dietary iron is absorbed predominantly by mucosal villous cells of the duodenum (22,23), whereas parenterally administered iron is taken up largely by mucosal crypt cells (24). Apparently, iron for cytochrome P-450 synthesis is derived either from dietary sources in the intestinal lumen or from body iron that the epithelial cells carry with them in their migration from the mucosal crypt to the top of the villus. To test the first of these two possibilities, rats that had been fed semisynthetic iron-supplemented diet were given a semisynthetic low iron diet for 48 hr (less than 0.1 mg iron per 100 g diet). The cytochrome P-450 content of villous tip cells declined by 60% in 48 hr and benzpyrene hydroxylase activity also decreased (Table II). On reinstitution of the dietary iron supplement these values returned to their original

TABLE II

Effects of Diets on Microsomal Hemoproteins and Drug Metabolism in Villous Tip Cells of Duodenal Mucosa of Rats

Diet	Cytochrome nmol/mg protein		Activity nmol/hr/mg protein	
	P-450	b_5	Benzpyrene hydroxylase	*p*-Nitroanisole O-demethylase
Standard chow diet	0.110 ± 0.004	0.109 ± 0.003	8.41 ± 1.26	14.74 ± 1.10
Semisynthetic iron-supplemented diet	0.067 ± 0.005	0.129 ± 0.003	2.64 ± 0.05	trace
Semisynthetic low iron diet for 2 days	0.028 ± 0.003	0.137 ± 0.008	0.35 ± 0.06	trace
Semisynthetic low iron diet for 2 days, followed by iron supplementation for 24 hr	0.080 ± 0.006	0.139 ± 0.001	2.12 ± 0.16	trace

Values represent mean ± standard error of mean of 3-5 individual experiments using duodenal mucosa pooled from 4 rats.

All values are significantly different from those of standard chow diet, $p < 0.01$.

levels in 24 hr. Throughout these dietary manipulations, the concentration of iron in the serum remained within the control range, reflecting presumably unchanged body iron stores (25).

These findings support the concept that cytochrome P-450 is synthesized in the epithelial cells of the upper intestinal villus and suggest that this process depends largely on the supply of dietary iron from the intestinal lumen. They further indicate that restricted dietary iron intake rapidly depresses cytochrome P-450-mediated drug metabolism in the small intestine by interfering with the synthesis of cytochrome P-450. This contrasts with hepatic drug metabolism which is unaffected or stimulated by depletion of body iron stores (26).

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