Mucosal biotransformation rates in the small intestine of children

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SUMMARY Biotransformation of ingested xenobiotics is known to take place in the gastrointestinal mucosa of laboratory animals and adult humans as well as in the liver. We studied the activities of aryl hydrocarbon hydroxylase, epoxide hydrolase, and glutathione peroxidase in 242 peroral small intestinal biopsy samples of children aged eight months to 18 years: 201 with normal histology, 21 with partial villous atrophy, and 20 with severe villous atrophy. All these enzymes were detectable even in the youngest children. The aryl hydrocarbon hydroxylase activity was age dependent, while the other measured enzyme activities were not related to the age of the patients. The aryl hydrocarbon hydroxylase activity was not related to the mucosal histology, but the epoxide hydrolase and glutathione peroxidase activities were diminished in samples with severe villous atrophy as compared with normal mucosa. This suggests that small intestinal mucosa with villous atrophy may produce oxidated, reactive metabolites, but further metabolism into detoxication products is decreased. This may expose persons with mucosal atrophy to possible harmful effects of environmental xenobiotics entering the body even at low doses.

Human exposure to xenobiotics (xenos=foreign; bios=life; xenobiotics=foreign compounds), present in nature or produced by man, is one of the major present concerns. The oral exposure is of great importance as together with nutrients man ingests various additives, contaminants, and traces of other xenobiotics. Biotransformation of these chemicals into water-soluble metabolites for excretion takes place in the liver but also extrahepatic organs are capable to metabolise xenobiotics.¹ The extrahepatic biotransformation is particularly important at ports of entry where man is exposed to small doses of xenobiotics.² Ingested xenobiotics are absorbed into gastrointestinal mucosa mainly by passive diffusion.³ In enterocytes, xenobiotics are oxidised and subsequently conjugated to water soluble derivatives which are transported for renal or biliary excretion or excreted back into the intestinal lumen.^{4,5}

Aryl hydrocarbon hydroxylase is a microsomal

cytochrome P-450 dependent monooxygenase catalysing—for example, the conversion of polycyclic hydrocarbons to more polar, oxygenated compounds.⁷ Aryl hydrocarbon hydroxylase is detectable and inducible in a number of extrahepatic tissues.⁸ Intestinal aryl hydrocarbon hydroxylase activity is greatest in proximal small bowel decreasing along the intestine.⁹ Epoxides, highly toxic metabolites initially formed from polycyclic hydrocarbons by monooxygenases like aryl hydrocarbon hydroxylase, can cause mutagenesis and carcinogenesis by binding covalently to intracellular macromolecules—for example, DNA. Epoxides may be detoxified by epoxide hydrolase catalysing the conjugation of epoxides with water yielding dihydrodiols. Within the enterocyte, the highly reactive oxygenated products may also be transported from the endoplasmic reticulum to cytosol where they may be conjugated with glutathione catalysed by glutathione-S-transferases,¹⁰ or reduced by glutathione peroxidase which under physiological conditions protects tissues from oxidative damage.¹¹

The intestinal activities of 7-ethoxycoumarin O-

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deethylase and NADPH-cytochrome c reductase have been evaluated in adult humans.¹² The present knowledge about intestinal biotransformation is in other respects derived from animal studies. As, particularly, nothing is known about the development of mucosal biotransformation during childhood we decided to study intestinal activities of aryl hydrocarbon hydroxylase, epoxide hydrolase, and glutathione peroxidase in children. The specific purpose of the study was to find out the existing enzyme concentrations in small intestinal mucosa during childhood and to see whether mucosal damage, through different proportions of oxidising ν conjugating enzymes, might expose children to harmful metabolites of xenobiotics.

Methods

PATIENTS

A total of 242 children, equal numbers of both sexes, were investigated because of failure to thrive, loose stools or chronic diarrhoea, abdominal pains, or raised concentrations of serum antigliadin or antireticulin antibodies. The ages of the children varied from 8 months to 18 years. After exclusion of disease 142 children were found to be healthy; the symptoms had disappeared or they were considered constitutional or functional. Among the remaining patients, in addition to children with coeliac disease, there were children with insulin dependent diabetes mellitus, juvenile rheumatoid arthritis, cow's milk protein intolerance, and inflammatory bowel disease. Peroral intestinal biopsies were taken as a part of the patients' clinical work up and the enzyme measurements brought no additional discomfort to the children. The study was approved by the ethical committee of the Department of Paediatrics, Turku University Hospital.

BIOPSY SPECIMENS

After fasting (infants for six hours, older children over night), peroral small intestinal biopsies were taken between nine and 11 am at the ligament of Treitz with rapid technique using either a two port paediatric Crosby-Kugler capsule or a paediatric Watson capsule. When a Crosby-Kugler capsule was used one of the two specimens was taken for histology and the other for enzymatic determinations. The single specimen obtained with a Watson capsule was divided in two parts: one for histology, the other for enzyme measurements. Biopsy specimens for enzyme studies were immersed in normal saline, immediately cooled, and stored at -80°C .

HISTOLOGY

The fresh biopsy specimens were inspected with a

stereomicroscope followed by the preparation of paraffin sections and standard staining with haematoxylin and eosin. The histology was evaluated by pathology staff and, in addition, all pathological and many normal findings were confirmed by Professor JK Visakorpi. The villous structure was normal in 201 biopsies. Partial villous atrophy with stunted and broadened villi was found in 21 children and 20 biopsies showed severe villous atrophy with hyperplastic crypts. No submucosal layers were included in biopsies.

ENZYME ASSAYS

All assays were carried out independently of the clinical findings. Because of the small sample sizes, optimal assay conditions were predetermined using rat small intestine. After thawing, the biopsy specimens were weighted and homogenised in 1 ml 0.15 M potassium chloride. Protein concentrations were measured according to Lowry.¹³ Alkaline phosphatase activities were measured spectrophotometrically¹⁴ using 50 μl intestinal homogenate; the linearity and saturation kinetics are described before.¹⁴

The aryl hydrocarbon hydroxylase activity was measured with the radiochemical method of DePierre *et al.*¹⁵ 25 μl intestinal homogenate, 100 μl NADPH regenerating system, 10 μl tritiated benzo(a)pyrene, and water added to the final volume of 250 μl were incubated for 45 minutes; the reaction was stopped with 250 μl 0.5 N sodium hydroxide in 80% ethanol and unmetabolised benzo(a)pyrene was extracted by hexane and counted in a scintillation counter. The linearity of the assay has been checked in rat intestine.¹⁶ Aryl hydrocarbon hydroxylase activities were expressed as pmol/min/mg protein.

The epoxide hydrolase activity was measured with a modification of the method of Oesch¹⁷ using radioactive 4,5-benzpyrene oxide as a substrate: 25 μl intestinal homogenate, 25 μl buffer, and 2 μl 4,5-benzpyrene oxide were incubated for 20 minutes. The reaction was stopped with 50 μl 20 nM CdSO_4 and 100 μl DMSO and extracted with 1 ml ether four times. The remaining activity was counted in a scintillation counter after adding of 0.8 ml water. The linearity and saturation kinetics of the method are published elsewhere by Hietanen *et al.*¹⁸ The results were expressed as pmol/min/mg protein.

The glutathione peroxidase activity was measured according to a modification of the kinetic method of Paglia and Valentine;¹⁹ the linearity of the reaction is controlled during the course of the assay. After incubating glutathione, NADPH, glutathione reductase, and 50 μl intestinal homogenate, 100 μl cumene hydroperoxide was added as the enzyme substrate and the consumption of NADPH⁺ was

recorded at 37°C on the wave length of 340 nm. The results were expressed as nmol/min/mg protein.

All the assays used in this work are long established routines in our laboratory. The storage of the samples at -70-80°C does not significantly affect the enzyme activities.^{18,20} The coefficients of variation of the methods have been published recently.²¹

STATISTICAL METHODS

Standard descriptive statistics, correlations of two variables, Student's *t* test, and analysis of variance were used.

Results

Normal histology was found in 201 small intestinal biopsies. The mean age of these children was 6.3 (SD 5.1) years. Pathological villous structure was found in 41 biopsy specimens: the mean age of the 21 children with partial villous atrophy was 6.2 (SD 5.7) years, and the mean age of the 20 children with severe villous atrophy was 8.6 (SD 5.7) years.

NORMAL MUCOSA

Among the 201 children with normal biopsy there were 142 children who did not have any chronic disease: their symptoms were constitutional, functional, or transitory. We consider these children as healthy. The normal development of mucosal bio-transformation rates by age was studied in these children. The alkaline phosphatase activity was constant throughout childhood. The aryl hydrocarbon hydroxylase, epoxide hydrolase, and glutathione peroxidase activities were detectable in all age groups. The aryl hydrocarbon hydroxylase activity increased by age; the correlation coefficient was 0.308 ($p<0.001$). The epoxide hydrolase and glutathione peroxidase activities were unaffected by age (Table 1). There were no significant differences by sex in any of the studied enzymes. There

were no significant correlations between the bio-transformation enzymes and the alkaline phosphatase activities: the correlation coefficient between alkaline phosphatase and aryl hydrocarbon hydroxylase was 0.023, between alkaline phosphatase and epoxide hydrolase 0.041, and between alkaline phosphatase and glutathione peroxidase 0.070 suggesting that the alkaline phosphatase activity used as a reference enzyme reflecting mucosal state was independent from the other studied enzymes.

Among the children with normal mucosa there were 26 children with insulin dependent diabetes mellitus and 21 children with juvenile rheumatoid arthritis. There were no differences in the activities of alkaline phosphatase, aryl hydrocarbon hydroxylase, epoxide hydrolase, or glutathione peroxidase between these disease groups and age matched healthy children (data not shown).

PATHOLOGICAL MUCOSA

The biopsy specimens with abnormal villi tended to be lighter than the normal samples. The alkaline phosphatase activities were significantly reduced in villous atrophy: the activity in the samples with partial villous atrophy was 73% ($p<0.01$) and in the samples with severe villous atrophy 34% ($p<0.001$) of the activity in normal samples.

Partial villous atrophy did not differ significantly from normal mucosa regarding aryl hydrocarbon hydroxylase, epoxide hydrolase, or glutathione peroxidase activities. In severe villous atrophy, aryl hydrocarbon hydroxylase activity was normal but the activities of epoxide hydrolase and glutathione peroxidase were diminished as compared to normal biopsies, $p<0.05$ and $p<0.02$, respectively (Table 2).

According to the analyses of variance, the activities of alkaline phosphatase, epoxide hydrolase, and glutathione peroxidase varied significantly in relation to histology; the F-values were 31.675, 3.183, and 3.093 respectively. The differences in aryl hydro-

Table 1 Activities* of alkaline phosphatase (AP), aryl hydrocarbon hydroxylase (AHH), epoxide hydrolase (EH), and glutathione peroxidase (GSHPx) in jejunal biopsies in relation to age among healthy children

Age (yrs)	AP nmol/min/mg protein	AHH pmol/min/mg protein	EH pmol/min/mg protein	GSHPx nmol/min/mg protein
< 1 (n=8)	197 (39) [120-273]	64 (16) [32-96]	105 (31) [44-166]	27.4 (9.5) [8.8-46.0]
1-2 (n=52)	202 (13) [175-227]	81 (12) [58-104]	117 (13) [91-143]	38.8 (6.1) [26.8-50.8]
3-4 (n=24)	181 (16) [150-212]	87 (21) [46-128]	130 (16) [99-162]	66.6 (15.3) [36.6-96.6]
5-6 (n=12)	209 (28) [153-264]	90 (31) [30-151]	166 (29) [110-222]	37.1 (7.2) [23.0-51.2]
7-8 (n=12)	198 (24) [150-245]	45 (20) [5-60]	93 (27) [41-145]	25.2 (4.8) [15.8-34.6]
9-10 (n=12)	188 (21) [148-229]	102 (19) [65-140]	145 (29) [88-202]	30.9 (9.9) [11.5-50.3]
11-12 (n=7)	190 (14) [164-217]	185 (16) [153-217]	137 (60) [20-255]	46.5 (12.0) [23.0-70.0]
13-14 (n=8)	199 (23) [154-245]	49 (23) [4-93]	167 (32) [104-230]	54.9 (11.6) [32.2-77.6]
≥15 (n=7)	132 (28) [76-187]	291 (155) [0-594]	61 (20) [21-101]	26.3 (7.5) [11.6-41.0]
r	-0.02	0.31 ($p<0.001$)	0.06	-0.02

*mean (SE) 95% confidence limits within square brackets.

Table 2 Activities* of alkaline phosphatase (AP), aryl hydrocarbon hydroxylase (AHH), epoxide hydrolase (EH), and glutathione peroxidase (GSHPx) in jejunal biopsies in relation to histological findings

	Normal villi	Partial villous atrophy	Severe villous atrophy
n	201	21	20
Age, yrs	6.3 (0.4) [5.5-7.1]	6.2 (1.2) [3.8-8.6]	8.6 (1.3) [6.1-11.1]
Weight, mg	6.10 (0.32) [5.47-6.73]	4.69 (0.76) [3.20-6.18]	3.26 (1.96) [0.7-10]
AP, ¶ nmol/min/ mg protein	210 (6) [199-221]	153 (16)† [121-185]	72 (5)‡ [62-81]
AHH, ‡ pmol/min/ mg protein	102 (10) [82-123]	139 (71) [0-279]	97 (23) [51-142]
EH, ** pmol/min/ mg protein	128 (7) [114-141]	94 (17) [62-127]	79 (15)§ [50-108]
GSHPx, †† nmol/ min/mg protein	41.5 (2.1) [37.4-45.6]	45.4 (11.0) [23.8-67.0]	16.3 (3.8) [8.9-23.7]

*mean (SE) 95% confidence limits within square brackets; Student's *t* test, significances of the differences from normal mucosa: †*p*<0.01, ‡*p*<0.001, §*p*<0.05, ||*p*<0.02; Analysis of variance: ¶*p*<0.001 (F 31.675), ***p*<0.05 (F 3.183), ††*p*<0.05 (F 3.093), ‡‡*p*>0.05 (F 0.431).

carbon hydroxylase activities were insignificant also in analysis of variance, F 0.431.

Discussion

It is known from animal studies and adult human studies that the gastrointestinal mucosa has several biotransformation enzyme activities including both phase I reactions of functionalisation and phase II reactions of conjugation in the metabolism of xenobiotics and endogenous substances such as steroid hormones, bile acids, and prostaglandins.^{6,22}

Polycyclic hydrocarbons are a large group of chemical compounds which are abundant in the environment in industrialised countries. General population is also exposed to polycyclic hydrocarbons through consuming broiled and smoked food as well as through certain drugs and environmental pollution. Polycyclic hydrocarbons are potentially carcinogenic as they are biotransformed to epoxides which bind covalently to intracellular macromolecules. Coke oven workers who were exposed to polycyclic hydrocarbons including benzo(a)pyrene were shown recently to have benzo(a)pyrene diol epoxide-DNA adducts in peripheral lymphocytes and antibodies against the adducts in serum as indications of the metabolism of benzo(a)pyrene *in vivo*.^{23,24}

In unexposed laboratory animals, the levels of biotransformation enzymes are low after birth and increase gradually postnatally³ but when enzyme inducers are administered into pregnant or lactating

females, the aryl hydrocarbon hydroxylase^{25,26} and epoxide hydrolase^{27,28} activities increase significantly in weanling rats. The development of these enzymes in different human tissues during childhood has not so far been investigated. We found measurable concentrations of aryl hydrocarbon hydroxylase and epoxide hydrolase in jejunal biopsy samples in all age groups of children. This indicates that the induction of enzyme synthesis has taken place during pregnancy or very early childhood or that there exists a natural basal activity of aryl hydrocarbon hydroxylase and epoxide hydrolase in man. The intestinal mucosa of children has enzyme activities for the biotransformation of polycyclic hydrocarbon as well as for further metabolism of their oxygenated derivatives. Normal mucosa may also participate in the biotransformation of endogenous steroids and structurally related compounds.

Glutathione peroxidase is the main known site of selenium in human body and glutathione peroxidase has been found in most human tissues,¹¹ most often investigated in blood cells, plasma, and serum.²⁹ Glutathione peroxidase protects tissues from oxidative damage by catalysing the reduction of hydrogen peroxide and organic hydroperoxides thus preventing the progression of tissue destruction initiated by these oxidated compounds. Glutathione peroxidase is thought to be a cytosolic alternative to peroxisomal activities.¹¹ Our results show that glutathione peroxidase is present in the jejunal mucosa of normal children in constant quantities during the entire childhood.

We classified biopsy specimens in normal, partial villous atrophy, and severe villous atrophy by visual evaluation. The alkaline phosphatase activities proved that the visual evaluations of histology were correct as the alkaline phosphatase activity of crypts is shown to be about half of the activity of villous tips.³⁰ The aryl hydrocarbon hydroxylase activity of mucosa with villous damage was similar to normal mucosa suggesting that oxygenated derivatives are formed at normal rates. The diminished concentrations of epoxide hydrolase and glutathione peroxidase in mucosal samples with severe villous atrophy indicate that the capacity of mucosa with villous damage to metabolise formed oxygenated derivatives is decreased.

Studies on the association between serum selenium concentrations and cancer arrive at controversial results.³¹⁻³³ Maybe tissue contents of selenium and tissue activities of glutathione peroxidase are more important for the development of malignancy than serum levels. Coeliac disease has been associated with cancer, particularly with malignant gastrointestinal lymphomas, in case reports³⁴⁻³⁷ as well as in an extensive British multicentre study.³⁸ Our results

could provide an explanation for increased risk of cancer in patients with untreated or poorly treated coeliac disease: jejunal mucosa with severe villous atrophy may accumulate oxidated metabolites of xenobiotics as a consequence of intact aryl hydrocarbon hydroxylase activity but defective detoxication due to impaired epoxide hydrolase and glutathione peroxidase activities.

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