Caffeine phenotyping of cytochrome P4501A2, N-acetyltransferase, and xanthine oxidase in patients with familial adenomatous polyposis

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Abstract

Patients with familial adenomatous polyposis (FAP) and age and sex matched controls were tested for cytochrome P4501A2 (CYP1A2), N-acetyltransferase, and xanthine oxidase activities using caffeine urinary metabolites as a discriminator. FAP patients showed significant underactivity of N-acetyltransferase (which inactivates some carcinogens) and significant overactivity of CYP1A2 (which activates some carcinogens). Xanthine oxidase activity, which can generate free radicals and cause cellular damage, was significantly increased in the FAP patients. All but one of the FAP patients had undergone colectomy. A separate group of six patients was therefore assessed before and at an average time of eight weeks after colectomy. No effect on enzyme activity was seen. The differences in enzyme activities detected in this study could produce an excess of active carcinogenic metabolites in the bile of FAP patients and contribute to the high risk for intestinal cancer in FAP.

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Familial adenomatous polyposis (FAP) is a premalignant autosomal dominant condition characterised by multiple colonic adenomas and by disparate extracolonic manifestations. Among the second are foregut adenomas and cancers, which occur most commonly in the duodenum, particularly around the ampulla of Vater.¹ A similar distribution of intestinal tumours occurs in the rat model for colorectal cancer, where a carcinogen, given subcutaneously, is excreted into the bile duct after undergoing hepatic metabolism.²

In humans, cytochrome P4501A2 (CYP1A2) and hepatic N-acetyltransferase enzymes participate in the metabolism of carcinogens.³ Xanthine oxidase promotes the generation of free radicals and reactive quinones, leading to intracellular damage.⁴ The activities of these enzymes differ between subjects. This variability might contribute to differences in cancer risk.⁵ In the context of FAP, abnormal hepatic metabolism of

carcinogens might explain the bile dependent distribution of foregut tumours seen in this condition.¹ We therefore measured the activity of these enzyme systems in patients with FAP and in healthy controls.

Subjects and methods

CYP1A2, N-acetyltransferase, and xanthine oxidase activities were measured in 29 unrelated patients with FAP (average age 38.3 years, range 19-70; 16 males, 13 females) and in 54 unrelated healthy volunteers (average age 39 years, range 18-70; 27 males, 27 females). Of the FAP patients, six (21%) were smokers, while 11 (20%) of the controls were smokers. All patients were white with normal liver and kidney function. All but one of the FAP patients had undergone colectomy between 1 and 38 years before sampling. All controls had intact colons.

To assess the effect of colectomy on enzyme function, five FAP patients and one patient with ulcerative colitis (average age 24 years, range 17-46; 5 males, 1 female) received analysis of N-acetyltransferase, CYP1A2, and xanthine oxidase activity immediately before colectomy and at a mean time of eight weeks (range 6 to 12) after colectomy.

Urine samples were collected at two to six hours after the oral administration of a 300 mg tablet of caffeine (FAP patients) or after a caffeine containing beverage (controls), caffeine intake having been shown not to affect the metabolic profiles obtained.⁶ Patients were not fasted and were not subject to any dietary restrictions. Ethics committee approval was obtained for this study. Urine samples were stored at -20°C and were later analysed as a single batch.⁷

The caffeine metabolites 1-methyluracil (1-MU), 1-methylxanthine (1-MX), 5-acetylamin-6-amino-3-methyluracil (AAMU - which is the metabolite of 5-acetylamin-6-formylamin-3-methyluracil (AFMU)) were assayed in the urine samples by a modification of the method of Grant *et al.*⁸ The polar metabolites 1-MU and 1-MX were extracted in chloroform:isopropanol (50:50% vol/vol) and assayed by reverse phase high performance liquid chromatography (HPLC) at 280 nm, using a gradient elution system starting with 6% acetonitrile: 94% acetic acid (0.5%), increasing to 50% of each in 25 minutes. The metabolite AFMU was deformedylated to its more stable metabolite AAMU by the method of Tang

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Figure 1: Metabolism of caffeine. 1,7-DMX=1,7-dimethylxanthine (see text for other abbreviations).

Median enzyme activity, determined by caffeine metabolite ratios, for FAP and control groups

	FAP	Controls	p Value
N-acetyltransferase (iqr)	0.297 (0.20-0.41)	0.376 (0.26-0.57)	0.028
CYP1A2 (iqr)	13.77 (10.47-21.15)	8.0 (5.68-14.12)	0.007
Xanthine oxidase (iqr)	0.644 (0.62-0.69)	0.563 (0.52-0.65)	0.0028

p Values refer to FAP v control groups by two tailed Mann-Whitney U test; iqr=interquartile range.

et al⁹ and assayed by HPLC at 263.5 nm, using 0.5% acetic acid (pH 2) as the mobile phase.

N-acetyltransferase phenotype was determined by the ratio of AAMU/(AAMU plus 1-MU plus 1-MX) in the urine samples in all patients.¹⁰ Subjects with metabolite ratios less than 0.48 were classified as slow acetylators. The proportion of slow acetylators in each group were compared by χ^2 analysis.

CYP1A2 activity was measured by the ratio of AAMU plus 1-MX plus 1-MU/1,7 dimethyluric acid.¹⁰

Xanthine oxidase activity was calculated using 1-MU/(1-MU plus 1-MX).¹⁰ Figure 1 shows the formation of the metabolites of caffeine and the site of action of the three enzymes measured in this study.

N-acetyltransferase, CYP1A2, and xanthine oxidase activity, calculated as urinary metabolite ratios in FAP and control subjects, were

compared using the two tailed Mann-Whitney U test. Results are expressed as the median values and interquartile ranges obtained for each group of patients.

Results were also tested for correlation between length of time from colectomy and N-acetyltransferase, CYP1A2, and xanthine oxidase enzyme status using the Spearman rank correlation test.

Results

The Table shows that patients with FAP had significantly lower N-acetyltransferase activity than the controls. Of the 29 FAP patients, 25 were slow acetylators compared with 35 of 55 controls (p=0.05).

FAP patients had significantly higher CYP1A2 activity than controls.

Xanthine oxidase activity was also significantly higher in the FAP group than in controls.

There was no correlation between length of time from colectomy and enzyme status (N-acetyltransferase $r_s=0.2$, p=0.28; CYP1A2 $r_s=0.3$, p=0.1; xanthine oxidase $r_s=0.3$, p=0.1).

Figure 2 shows that there was no significant difference between before and after colectomy values for N-acetyltransferase, CYP1A2, and xanthine oxidase activity in the six patients in whom caffeine testing was performed before and after colectomy.

Discussion

We have found that postcolectomy patients with FAP differ in their N-acetyltransferase, CYP1A2, and xanthine oxidase status from control subjects with intact colons.

The relevance of this finding to pre-colectomy FAP patients remains to be determined. However, the problem of duodenal cancer is one that continues after colectomy. Indeed, duodenal cancer accounts for more deaths than any other single cause in patients who have undergone a colectomy.¹¹ Our findings should be viewed in this context.

We have previously tested the foregut mucosa of patients with FAP and of control patients for evidence of exposure to carcinogens, using the presence of DNA adducts as a

Figure 2: Lack of effect of colectomy on activity of (A) N-acetyltransferase, (B) xanthine oxidase, and (C) CYP1A2, determined by caffeine metabolite ratios, in six patients. Postcolectomy samples were taken at an average of eight weeks after surgery.

marker.¹² DNA adducts were distributed in the same bile dependent pattern as foregut tumours in the FAP patients. The concentration of DNA adducts was significantly greater in postcolectomy FAP patients than in controls. Bile from precolectomy FAP patients was obtained and was tested both in a bioassay¹³ and in vitro.¹⁴ FAP bile was found to impart a significantly greater adduct load on DNA in both of these studies than did control bile, implying that the excess of DNA adducts detected in FAP mucosa was produced by carcinogens in FAP bile rather than from faulty repair of DNA in FAP mucosa.

This study suggests that the distribution of DNA adducts and tumours in the intestinal mucosa of patients with FAP may be partly explained by defective detoxification of carcinogens by N-acetyltransferase in the liver, by increased activation of carcinogens by hepatic CYP1A2, and by tissue damage occurring as a result of hepatic xanthine oxidase overactivity.¹⁵

This study has also shown that colectomy does not affect the activity of N-acetyltransferase, CYP1A2, and xanthine oxidase in the first two postoperative months, and that there is no correlation between the length of time from colectomy and enzyme activity for all three enzyme systems tested. This implies that the findings of this study might be relevant to precolectomy patients.

The excess number of slow acetylators in patients at high risk for bowel cancer seen in this study, however, differs from the findings reported in three other studies of hepatic acetylation polymorphism in postcolectomy patients with sporadic large bowel cancer.^{16–18} Of these studies, two showed a statistically significant excess of fast acetylators in large bowel cancer patients,^{16,17} while one showed no difference between test and control groups.¹⁸ Differences between our study and the others that may explain the variation in results include our use of the 0.48 metabolite ratio value to differentiate between slow and fast acetylators, our use of caffeine as a probe drug (the other studies used sulphamethazine), and our reliance on the urinary ratio of metabolites. Indeed, if a urinary ratio is used in the study of Ilett *et al*,¹⁷ then the excess of fast acetylators seen in the group of patients with large bowel cancer compared with controls loses statistical significance.

Slow acetylators are thought to be more susceptible to bladder carcinogenesis¹⁹ because the hepatic acetylation system can detoxify the arylamine group of carcinogens. Subjects who are slow acetylators cannot detoxify these environmental carcinogens as effectively as those who are rapid acetylators.²⁰ It has been suggested that inherent differences in the metabolism of carcinogens by different target tissues might explain the apparent paradox presented by the association between slow acetylation and bladder cancer and that of fast acetylation and colorectal cancer seen by others.^{20,21} Thus, whereas N-acetylation detoxifies arylamine metabolites in the liver and in bladder mucosa to protect against

bladder cancer, O-acetylation (for which the acetylation polymorphism is also responsible)⁷ activates arylamine metabolites and may be the major pathway within colonic mucosa, thereby increasing risk for colorectal cancer.¹⁹

Although Kirilin *et al*²² failed to show a difference in acetylator phenotype in colon cytosols between those with and those without colorectal cancer, Turesky *et al*²³ showed that human colon could indeed O-acetylate the metabolites of the genotoxic heterocyclic amines. If hepatic detoxification is the more important pathway, then an association between colorectal cancer and slow acetylation would be expected. If colonic activation is paramount, then rapid acetylation would increase colorectal cancer risk.^{20,21} The lack of correlation between the length of time from colectomy and N-acetyltransferase activity and the lack of change of enzyme activity in pre and postcolectomy samples from the same patients implies that the role of colonic acetylation is not as important as hepatic acetylation. This question will only be answered by phenotyping studies of larger numbers of patients over a longer time frame, and by genotyping studies, as the genes responsible for these polymorphisms are isolated.²⁴

The same provisos also apply to the other enzyme systems participating in the metabolism of carcinogens.

CYP1A2 is one such system, activating numerous carcinogens including the heterocyclic amines and arylamines, which occur in cooked fish and meat products.²⁵ In our study, CYP1A2 activity was greatest in the FAP group.

Glutathione S-transferase mu, another carcinogen metabolising isozyme, usually acts to detoxify carcinogens. It has been found to be deficient in FAP patients²⁶ and in patients with sporadic colorectal cancer²⁷ when compared with controls. In FAP, differences in carcinogen metabolism may contribute to the different levels of severity of the condition in different patients. If these differences had an inherited basis, then they would be expressed in target tissue (such as the duodenum and colon) as well as in the liver. The effect of this would be to further diminish that subject's ability to detoxify carcinogens, leading to a more severe form of polyposis.

Genetic variation in carcinogen metabolism has been neglected in most epidemiological and rodent studies of cancer aetiology hitherto.²⁸ Investigation of the pathways of carcinogen metabolism in patients without FAP should help identify those who are at high risk for intestinal cancer.²⁹

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