Short communication

Effects of the bacterial status of rats on the changes in some liver cytochrome P450 (*EC* 1.14.14.1) apoproteins consequent to a glucosinolate-rich diet

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The aim of the present work was to investigate the influence of the intestinal microflora on the changes in hepatic cytochrome P450 apoproteins induced by dietary glucosinolates. Ten rats harbouring a conventional digestive microflora were offered either a diet containing 390 g myrosinase-free rapeseed meal/kg (*n* 5) or a control diet devoid of glucosinolates (*n* 5). A similar trial was performed using germ-free rats. After 4 weeks of exposure to the dietary regimens, animals were slaughtered and their livers removed for preparation of microsomes and analysis of cytochrome P450 (*EC* 1.14.14.1). The glucosinolate-rich diet decreased the concentration of total cytochrome P450 in conventional rats only (–34 %). The bacterial status did not modify the concentration of apoproteins CYP1A2 and CYP2B1/B2, but greatly decreased the concentration of the male constitutive isoform CYP2C11 (–53 and –45 % respectively in conventional and germ-free rats). Germ-free rats fed on the glucosinolate-rich diet had a greater concentration of CYP3A (+139 %) and a lower concentration of CYP2E1 (–32 %) than their counterparts fed on the control diet. However, these differences were absent in conventional animals. On the whole, the influence of the intestinal microflora on the changes in hepatic cytochrome P450 due to the consumption of cruciferous vegetables is very complex and obviously involves different mechanisms according to the apoprotein.

Gnotobiology: Xenobiotic metabolizing enzymes: Rapeseed

Epidemiological surveys increasingly support the hypothesis that the consumption of cruciferous vegetables is associated with a lower risk of tumour formation in the human digestive tract. Furthermore, experimental studies involving chemically-induced carcinogenesis give supporting evidence of this protective effect, mainly through changes in the xenobiotic metabolizing enzymes (for reviews, see McDanell *et al.* 1988; Nugon-Baudon & Rabot, 1994).

Cruciferous vegetables contain glucosinolates that are broken down into biologically-active derivatives, including nitriles and isothiocyanates, by plant myrosinase (thioglucoside glucohydrolase; *EC* 3.2.3.1) during preparation and chewing of vegetables (for review, see Duncan & Milne, 1989). Should the enzyme be inactivated, for example by heating, glucosinolates may be metabolized in the digestive tract by the intestinal microflora (Nugon-Baudon *et al.* 1988).

Glucosinolate derivatives are the favourite candidates to explain the protective effect of cruciferous vegetables against chemical carcinogenesis. Nevertheless, some striking discrepancies emerge from the abundant literature devoted to the relationships between cruciferous vegetables, glucosinolates or glucosinolate derivatives and the xenobiotic metabolizing enzyme (for review, see Nugon-Baudon & Rabot, 1994). Indeed, there is now strong evidence of an overall induction of transferases in the intestine and the liver, whereas results obtained on phase I xenobiotic metabolizing enzyme vary according to the species and strain of rodent, the type of cruciferous vegetable and the experimental design. The variability of responses is even greater when pure glucosinolates or glucosinolate derivatives are fed to the animals.

We previously showed that the decrease in total cytochrome P450 (*EC* 1.14.14.1) concentration as well as the

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induction of glutathione-S-transferase (*EC* 2.5.1.18) and UDP-glucuronyltransferase (*EC* 2.4.1.17) activities observed in the liver of male conventional F344 rats fed on a glucosinolate-rich but myrosinase-free diet could not be reproduced in germ-free counterparts (Nugon-Baudon *et al.* 1990; Rabot *et al.* 1993). The aim of the present work was to investigate the implications of the intestinal microflora on several hepatic cytochrome P450 apoproteins in male F344 rats.

Materials and methods

Chemicals

All chemicals were of the highest quality available from Sigma (Saint-Quentin Fallavier, France), Merck (Nogent-sur-Marne, France), Prolabo (Paris, France) and Serva (Paris, France). Hybond-C nitrocellulose (0.45 μm) was obtained from Amersham (Les Ulis, France). Peroxidase (*EC* 1.11.1.7)-conjugated rabbit anti-mouse immunoglobulins were obtained from Dako (Copenhagen, Denmark).

Animals and diets

Ten germ-free and ten conventional male F344 rats were obtained from our laboratory's breeding unit. They were aged 5–6 weeks and their mean body weight was 109 (SE 3) g at the beginning of trials. Germ-free rats were housed in a Trexler-type isolator fitted with a rapid transfer system (La Calhène, Vélizy-Villacoublay, France).

Two isoenergetic and isonitrogenous diets were used. Their composition and sterilization process have been described elsewhere (Rabot *et al.* 1993). The control diet was glucosinolate-free. The glucosinolate-rich diet (15 μmol/g) contained a dehulled and myrosinase-free rapeseed meal from the low-erucic acid, low-glucosinolate cultivar Darmor as a source of glucosinolates.

For each bacterial status, animals were randomly allocated to the control diet or to the glucosinolate-rich diet (n 5; control and glucosinolate groups). They were housed two or three per cage and given free access to their diets and to sterilized (120°, 40 min) tap water. Animal-room conditions have been described previously (Rabot *et al.* 1993).

Data and sample collection

Body-weight gain and food intake were recorded after 4 weeks of exposure to the dietary regimens. Then, the rats were killed by a sharp blow on the head and cervical dislocation. Livers were quickly removed and hepatic microsomes were immediately prepared as described by Ryan *et al.* (1978).

Hepatic enzyme assays

The concentrations of proteins and of total cytochrome P450 in liver microsomes were measured according to Lowry *et al.* (1951) and Omura & Sato (1964) respectively. The immunoblot analysis of cytochrome P450 apoproteins CYP2C11, CYP1A2, CYP2B1/B2, CYP2E1 and CYP3A (Nelson *et al.* 1996) was conducted as described by Beaune *et al.* (1985). The different antibodies raised against cytochrome P450 isoforms are detailed by de Waziers *et al.* (1990). The density of each stained band was determined by scanning using a densitometer (Hewlett Packard Scanjet II and Scan analysis; Hewlett Packard, Les Ulis, France) and was expressed as arbitrary units per mg microsomal protein. To standardize the results, mean values obtained from control rats were used as internal references (100 %; Roland *et al.* 1994). Different batches of antibodies were used for germ-free and conventional trials; thus, standardization of the results was performed separately for rats of each bacterial status.

Statistical analysis

Since different batches of antibodies were used to assay the cytochrome P450 apoproteins in germ-free and conventional trials, these were treated as different experiments. Thus, for each bacterial status, the effect of the dietary treatment on body-weight gain and the concentration and isoenzyme pattern of hepatic cytochrome P450 was analysed using a Student's t test (two-sided; $P < 0{\cdot}05$).

Results

Body-weight gain and food consumption

In conventional rats, feeding on the glucosinolate-rich diet led to a slower rate of body-weight gain ($P = 0{\cdot}005$) than that of the group fed on the control diet. After 4 weeks, the mean cumulative weight gain was 57 (SE 12) g in the glucosinolate group and 109 (SE 3) g in the control group. Similarly, overall food intakes (n 5; 4 weeks) were 1·54 and 2·38 kg in the glucosinolate and control groups respectively.

Conversely, body-weight gain in germ-free rats was slightly increased ($P = 0{\cdot}038$) when animals were fed on the glucosinolate-rich diet (mean 143 (SE 5) g) compared with those fed on the control diet (mean 130 (SE 1) g). Food intake (n 5; 4 weeks) was slightly lower in the glucosinolate group (2·24 kg) than in the control group (2·52 kg).

Total cytochrome P450

In conventional rats, the concentration of total cytochrome P450 was significantly lower ($P = 0{\cdot}036$) in the glucosinolate group (mean 0·49 (SE 0·04) nmol/mg microsomal proteins) than in the control group (mean 0·74 (SE 0·09)). No difference was observed between the two dietary groups in germ-free rats (mean 0·66 (SE 0·04)).

Cytochrome P450 apoproteins

The relative concentration of apoprotein CYP2C11 was greatly reduced in glucosinolate-fed rats compared with their control counterparts, whether the animals were conventional (−53 %; $P = 0{\cdot}018$) or germ-free (−45 %; $P = 0{\cdot}005$; Table 1). By contrast, the glucosinolate diet did not change the relative concentrations of apoproteins CYP1A2 and CYP2B1/B2, either in conventional or in

Table 1. Effect of dietary glucosinolates on the relative concentrations of five hepatic cytochrome P450 (*EC* 1.14.14.1) apoproteins in conventional and germ-free rats*

(Mean values with their standard errors for five rats per bacterial status and diet)

	Control diet †		Glucosinolate-rich diet †	
	Mean	SE	Mean	SE
Conventional rats				
CYP2C11	100[a]	9	47[b]	15
CYP1A2	100[a]	14	129[a]	17
CYP2B1/B2	100[a]	22	92[a]	15
CYP2E1	100[a]	27	81[a]	21
CYP3A	100[a]	28	62[a]	6
Germ-free rats				
CYP2C11	100[a]	11	55[b]	4
CYP1A2	100[a]	4	105[a]	4
CYP2B1/B2	100[a]	7	97[a]	9
CYP2E1	100[a]	1	68[b]	3
CYP3A	100[a]	7	239[b]	25

[a,b] Within a row, mean values with unlike superscript letters are significantly different ($P < 0{\cdot}05$, Student's *t* test).

* For details of diets, animals and procedures, see p. 232.

† For each bacterial status, mean values obtained from the glucosinolate group are expressed as percentages of the reference values (100 %) obtained from the control group.

germ-free trials. In conventional rats, the relative concentrations of apoproteins CYP2E1 and CYP3A were unchanged by the diet. In the germ-free trial, consumption of the glucosinolate-rich diet led to a 32 % decrease ($P < 0{\cdot}0001$) in the relative concentration of apoprotein CYP2E1 and to a 139 % increase ($P = 0{\cdot}005$) in apoprotein CYP3A.

Discussion

Dietary restriction or fasting of F344 rats is reported to increase the concentration of total liver cytochrome P450 (Sohn & Fiala, 1995) and to induce the hepatic apoproteins CYP2E1 and CYP2B1/B2 (Brown *et al.* 1995). It is unlikely, therefore, that the lower total cytochrome P450 or the absence of change in CYP2E1 and CYP2B1/B2 in the conventional rats can be ascribed to the lowering of their food intake and weight gain.

We have again observed that, in conventional rats, rapeseed meal depresses hepatic total cytochrome P450 concentration (Nugon-Baudon *et al.* 1990). This effect seems to depend on the type of cruciferous vegetable and/or the rat strain, since liver cytochrome P450 concentration is increased in Wistar rats fed on broccoli (Aspry & Bjeldanes, 1983) or on Brussels sprouts (Wortelboer *et al.* 1992). To our knowledge, the dramatic decrease in the P450 isoform CYP2C11 (male main constitutive form) induced by the glucosinolate-rich diet has not been reported before. None of the other apoproteins, CYP3A, CYP2E1 and CYP2B1/B2, steroid-, ethanol- and phenobarbital-inducible respectively, were altered, as observed by Wortelboer *et al*. (1992) in Wistar rats fed on Brussels sprouts. By contrast, our F344 rats fed on rapeseed meal did not display the induction of CYP1A2 reported by these authors.

The presence of a microflora seems to be a prerequisite for rapeseed meal to depress the concentration of total cytochrome P450, since this phenomenon did not occur in germ-free rats. By contrast, the glucosinolate-rich diet seems to depress CYP2C11 apoprotein independently of the presence of a microflora; a release of glucosinolate derivatives specifically active towards this apoprotein may occur in the upper part of the digestive tract; for example, by acid-hydrolysis in the stomach, or other biologically-active components in the rapeseed-meal diet may be involved. Apoproteins CYP3A and CYP2E1 were respectively induced and decreased, only in germ-free rats. Several hypotheses could explain the absence of these alterations in conventional counterparts: the intestinal microflora harboured by conventional rats may metabolize glucosinolate derivatives presumably released in the upper part of the digestive tract, thus decreasing their biological activity; alternatively, this postulated activity may be counterbalanced by other bacterial metabolites.

The modulating effect of the intestinal microflora on the changes in P450 apoproteins due to glucosinolates is obviously very complex, and probably involves different mechanisms according to the apoprotein. So far, the participation of the intestinal microflora in changes in the xenobiotic metabolizing enzymes due to glucosinolate consumption has been underestimated. Previous experiments have focused on glucosinolate derivatives resulting from acid- or myrosinase-catalysed hydrolysis, which were fed to conventional rats, rather than on feeding glucosinolates to rats harbouring a human microflora.

A major topic remaining to be addressed is the extent to which the influence of rat intestinal microflora on glucosinolate breakdown can be extrapolated to human subjects. In this respect, we have shown recently that gnotobiotic rats harbouring a human faecal flora and fed on a diet containing rapeseed meal exhibited an increase in the overall concentration of hepatic cytochrome P450 and an induction of hepatic glutathione-S-transferase and UDP-glucuronyltransferase (Roland *et al.* 1996). The influence of human intestinal microflora on the hepatic cytochrome P450 apoprotein pattern remains to be investigated.

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