GLUCOSINOLATES INTERACTIONS WITH THE HEPATIC XENOBIOTIC METABOLIZING ENZYMES (XME) - INFLUENCE OF THE INTESTINAL MICROFLORA.

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INTRODUCTION

The well-known side-effects of cruciferous vegetals (turnip, cabbage, cauliflower, rape...) are due to the presence of glucosinolates (GLS). The role of intestinal microflora in GLS toxicity in vivo has been demonstrated using rats and chickens with different bacterial status (Nugon-Baudon et al. 1988). On the other hand, several epidemiological surveys reported by the American Committee on Diet, Nutrition and Cancer (1982) and experimental studies (Stoewsand et al 1978 and 1988) seem to link GLS rich vegetals consumption with a reduction of cancer incidence at different sites in human or laboratory animals.

Recent work realized with rats fed on a rapeseed meal based-diet showed a drastic modification of the hepatic XME system : total P 450 content was depleted whereas the specific activities of two major transferases were much enhanced. Pre-induction of P 450 in those rats with phenobarbital led to an increased toxicity of the feed (very important mortality and enhanced kidneys hypertrophy and growth curve depletion) (Nugon-Baudon et al. 1990 a).

The aim of the present study was to check which isoenzymic forms of P 450 were altered by the GLS rich diet and if the modifications observed on phase I (P 450) and phase II (transferases) enzymes were related to the intestinal microflora as is the "classical" toxicity of rapeseed meal.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. Trizma-hydrochloride,DL-dithiothreitol, phenylmethylsulphonylfluoride, ovalbumine (chicken egg, grade V), Folin and Ciocalteu's reagent, sodium dithionite, glutathione (reduced form), 1-chloro-2,4-dinitrobenzene (grade I), magnesium chloride, chloramphenicol (CAP) and sodium salt of uridine 5'-diphosphoglucuronic acid (UDPGA) were from Sigma (St Louis, USA). Potassium-sodium tartrate, cupric sulphate and disodium salt of ethylenediamine-tetraacetic acid were from Merck (Damstad, RFA). ^{14}C-CAP labelled in the acetyl moiety with a specific activity of 54 mCi mmol^{-1} was from Amersham (France). Scintillation fluid (Insta-gel) was from Packard. Carbon monoxide and isoamyle acetate were from Prolabo (Paris, France).

Animals and Diets

All animals are bred in the experimental animal unit of the laboratory. Four groups of male Fischer 344 rats weighing about 100g at the beginning of trials were used. Two germ-free (GF) groups (8 animals per group) were reared in Trexler type isolators (La Calhène, Vélizy, France). GF status was ascertained twice a week as described elsewhere (Nugon-Baudon et al. 1990b). Two other conventional groups of rats (11 animals per group) were kept in animal room.

For each bacterial status, one group of rats was fed on a soyabean meal based diet (SM) and the other on a rapeseed meal based diet (RM) (Table 1), containing 37.3 µmol GLS per g of dry matter. The RM diet contained 39 % of dehulled DARMOR OO rapeseed meal which GLS composition, as analysed with a GC method, (CETIOM AFNOR) is given Table 2. Both diets were isonitrogenous and isoenergetic. Diets distributed to GF rats were packed in double vacuum bags and sterilized by irradiation at 40 kGy.

Animal and isolators rooms temperature was maintained at 21°C and 12 hours photoperiods were used.

Animals were fed ad libitum and given tap water (sterilized in the case of GF animals) to drink. The experiment lasted for 31 days.

Table 1. Composition of rats diets (g/kg of dry matter).

	Rapeseed meal diet	Soyabean meal diet
Rapeseed meal DARMOR OO	390.00	
Soyabean meal 50		276.00
Corn starch	531.40	576.00
Corn oil	20.00	20.00
Lysine HCl	0.60	
Vitamin mixture	18.00	18.00
Mineral mixture	40.00	40.00
Cellulose		70.00
Protein (N x 6.25)(% of DM)	14.50	15.00
ME (kcal/kg of DM)	3650	3690

Table 2. GLS content of DARMOR OO (µmol/g of dry matter)

Progoitrin	25.0		
Gluconapoleiferin	1.5		
Gluconapin	7.1		
Glucobrassicanapin	2.1		
epi-Progoitrin	-		
Sinalbin	-	Total alkenyls	35.7
Gluconasturtin	-	Total indols	1.6
4-hydroxy-Glucobrassicin	1.3	Total GLS	37.3
Glucobrassicin	0.3		
neo-Glucobrassicin	-		
4-methoxy-Glucobrassicin	-		

Sample Collection

Rats were knocked senseless and sacrificed by cervical dislocation. Hepatic microsomes were prepared according to Ryan et al. (1978).

Statistical Analysis

For each bacterial status, the RM group was compared to the SM group, using a Student's t test. Significance was accepted when $P<0.01$.

Enzyme Assays

Microsomal proteins concentrations were determined according to the method of Lowry et al. (1951), using ovalbumine as a standard.

Cytochrome P 450 total amount was measured by difference spectrum according to Omura and Sato (1964) and using a BECKMAN DU70 spectrophotometer.

Glutathione S-transferase (GSH-T) was assayed according to the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as a substrate and with the difference that microsomal fractions were used for the assay. Unit of activity was defined as the amount of enzymes catalysing the formation of one nanomole of product per minute.

UDP-glucuronyltransferase (UDPG-T) activity was measured according to the method of Young and Lietman (1978) using ^{14}C-CAP and with some procedure modifications already described elsewhere (Nugon-Baudon et al 1990). All counts were expressed as desintegrations per minute after correction for quenching and control counts realized with boiled enzyme (Scintillation fluid counter KONTRON SL30).

Immunoblots, P450 enzymes and Antibodies

The nomenclature of Nebert et al. (1989) was used. The origin of cytochrome P 450 enzymes and corresponding antibodies was described previously (de Wazier et al. 1990). Quantification of these P 450 iso-enzymic forms was performed by densitometry measure of immunoblots (de Wazier et al. 1990).

RESULTS

(Tables 3, 4)

As far as CV animals are concerned, the RM diet significantly depleted the total P 450 amount (-25%, $p<0.01$). This depletion was mainly observed on IIC11 P 450 (the major male constitutive form). By contrast, P 450 IA2 (induced by aromatic hydrocarbons) was slightly increased. The expression of P 450 B1/2 (constitutive and induced by phenobarbital), P 450 IIE1 (constitutive and induced by ethanol) and P 450 IIIA (constitutive and induced by steroids) was not modified.

RM diet was a excellent inducer of both transferases specific activities : +136% for glutathione-S-transferase and +272% for UDP-Glucuronyl transferase ($p<0.001$).

The association RM-GF led to very different results. The decrease of P 450 total amount observed with GF-RM rats compared to their SM counterparts is not significant in our

conditions. Furthermore,there is no inducing effect of RM on both transferases specific activities in the absence of microflora.

Table 3. Effect of the association between RM diet and bacterial status on P 450 amount and conjugative enzymes activities (expressed as % of results obtained with SM counterparts).

	Total P 450	GSH-Transferase	UDPG Transferase
CV-RM	75* ±6	236** ±18	372** ±23
GF-RM	80 ±5	103 ±7	102 ±11

Results are expressed as % (±SEM) of values obtained with SM counterparts (nmol/mg proteins for P 450 ; nmol/mn/mg proteins for GSH-T ; pmol/mn/mg proteins for UDPG-T.).
* P< 0.01 ; ** P< 0.001 compared to SM control animals..

Table 4. Effect of the association CV-RM on 5 isoenzymic P 450 forms (expressed as % of the results obtained with CV-SM animals).

	IIC11	IIB1/2	IA2	IIIA	IIE
CV-RM	34±9 n=4	103±26 n=4	161±12 n=5	0.98 n=2	126±33 n=3

Results are expressed as % (±SEM) of values obtained with SM counterparts (nmol/mg proteins for P 450).

DISCUSSION

The results concerning target organs'weight, hormonal alterations and growth curve reproduced past studies showing the major role played by intestinal microflora in GLS toxicity, and therefore have not been described here.

We reproduced the depleting effect of RM on P450, and the enhancement of specific activities of two of the major microsomal transferases when rats harbour their natural microflora (NUGON-BAUDON et al 1990). This work shows, for the first time to our knowledge, that the inducing effect of GLS on GSH and UDPG transferases is somehow mediated by the intestinal microflora as are the usual toxic side effects of rapeseed meal. As far as GF rats' hepatic total amount of P 450 is concerned, there are no significant difference there too, wathever the diet. This last point needs to be confirmed since values seem different even if they do not reach significance with the statistical procedure used.

Three of the major forms of P 450 are drastically modified by the association RM-CV. These modifications look like the

pattern observed during an isosafrol induction of CV rats Results obtained on isoenzymic P 450 forms of GF rats are being reproduced.

On the whole, rapeseed meal contains molecules which are activated into toxic coumpounds by intestinal microflora and which drastically modify the hepatic XME system. These XME play a key role in toxicity and chemical carcinogenesis. These results confirm the necessity to strictly control food additives since those may influence the quality and quantity of XME, xenobiotics metabolism and consequently their potential toxicity.

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