URINARY MERCAPTURIC ACIDS AS MARKERS FOR THE ESTIMATION OF ISOTHIOCYANATE RELEASE FROM PARENT GLUCOSINOLATES IN THE DIGESTIVE TRACT OF RATS.

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ABSTRACT

Variability in the toxicity of glucosinolate-containing diets may be related to the course of glucosinolate hydrolysis. An approach to the study of glucosinolate breakdown using urinary mercapturic acids to characterise glucosinolate breakdown *in vivo* is described. The use of this method to study the influence of diet on glucosinolate hydrolysis in rats is outlined.

INTRODUCTION

Glucosinolates are a group of thio-glucosides found in Brassica species which represent a major limitation to the use of rapeseed meal as an animal feedstuff. Glucosinolates are hydrolysed to yield toxic metabolites, such as isothiocyanates and nitriles, under the influence of plant or microbial myrosinase in the digestive tract (Nugon-Baudon *et al.* 1988). Factors influencing the extent of hydrolysis and the identity of the toxic metabolites produced are unclear. This information is critical to an understanding of glucosinolate toxicity and to allow reliable prediction of toxic effects under different circumstances. Measurement of concentrations of glucosinolates and their breakdown products in digestive fluids gives a crude picture of glucosinolate breakdown but can give misleading results. This is because instantaneous concentrations represent a balance between rates of release, degradation and absorption of metabolites. An alternative approach is to measure concentrations of excretory end-products of glucosinolate metabolites following ingestion of parent glucosinolates (Duncan, 1990). This gives an integrated measure of the release of metabolites *in vivo* over time. Isothiocyanates are excreted as their mercapturic acid derivatives in the urine which can be easily measured by HPLC. An approach to the study of isothiocyanate release *in vivo* involving quantification of mercapturic acid excretion following oral administration of sinigrin and its digestive metabolite allyl isothiocyanate (AITC) to rats is described here. The technique was used to compare isothiocyanate release in rats consuming brassica and non-brassica based diets.

EXPERIMENTAL

Sixteen Fischer 344 rats were divided into two dietary groups of eight rats. One group was offered a cauliflower-based diet and the other group a standard laboratory rat diet throughout the experiment. Cauliflower contains myrosinase and a range of glucosinolates but low concentrations of sinigrin. After a 10-day preliminary period during which rats were accustomed to the diets, isothiocyanate release was measured during 2 consecutive 6-day periods as follows. All rats were placed in metabolism cages to allow urine collection on day 1 of each period. Four rats within each dietary group were given a single oral dose of 50 µmol of sinigrin (SIN treatment) and the remaining rats received 50 µmol of AITC (ISO treatment). At the same time all rats were also dosed with 50 µmol of butyl isothiocyanate (BITC). Urine samples were collected 0, 2, 6, 24 and 48 hours after dosing and stored at -20°C pending analysis for urinary mercapturic acids by HPLC (Mennicke *et al.* 1987). This procedure was repeated, starting on day 4 of each period, except that rats previously dosed with sinigrin received AITC and *vice*

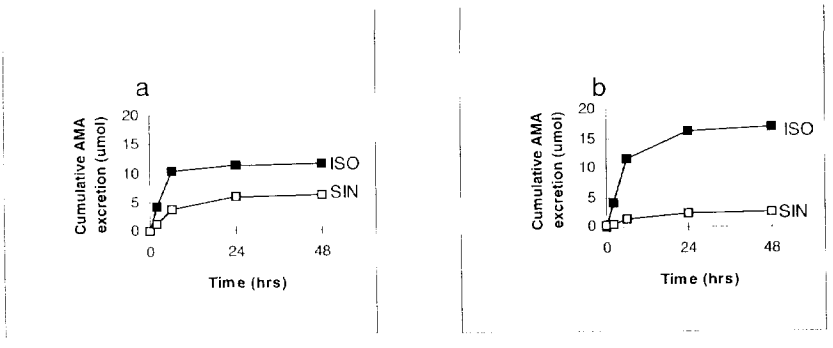
versa. The order of treatment administration (SIN,ISO or ISO,SIN) was randomised within each period.

Release of AITC in sinigrin dosed rats was calculated as follows. Total amounts of allyl mercapturic acid (AMA) and butyl mercapturic acid (BMA) excreted by ISO rats over 48 hours were used to calculate the ratio of AMA *vs.* BMA excretion. Because of its consistency (see Results), this ratio together with the total excretion of BMA in SIN rats, was used to predict the putative AMA excretion in SIN rats assuming molar conversion of sinigrin to AITC. The actual quantity of AMA excreted by SIN rats divided by the prediction based on BMA excretion gave an estimate of conversion of sinigrin to AITC *in vivo*.

RESULTS

Cumulative excretion of allyl mercapturic acid (AMA) in ISO and SIN rats fed the cauliflower and control diets are shown in Figure 1. On both diets, more AMA was excreted in ISO than SIN rats. Among ISO rats, overall recovery of AMA was greater in rats fed the control diet than in those consuming the cauliflower diet. In SIN animals considerably more AMA was excreted in rats consuming the cauliflower diet than in those offered the control diet.

Figure 1 - AMA excretion in rats fed cauliflower (a) or control (b) diets

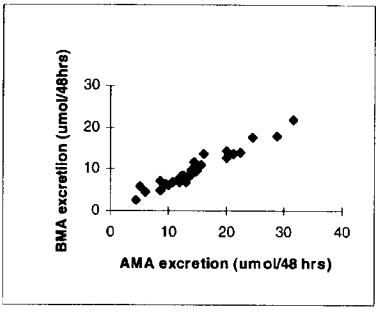


The range in the amount of AMA excreted was considerable but excretion of AMA and BMA in ISO rats was highly correlated ($r=0.971$; d.f.=30; $P<0.001$; Figure 2). The ratio of AMA:BMA excreted over 48 hrs was 1.51 and analysis of variance showed that it was not significantly influenced by period, diet or animal ($P>0.1$). This ratio was therefore used in calculations of conversion of sinigrin to AITC in SIN rats as described above. A significantly greater proportion of dosed sinigrin was converted to AITC in rats consuming the cauliflower diet than in control rats ($P<0.001$). There was no effect of animal or period (Table 1).

TABLE 1. Influence of basal diet and period on the proportional release of AITC from sinigrin in rats (SED's: diet=0.0586, period = 0.0618, diet x period = 0.0852)

		Diet		
		Control	Cauliflower	Mean
Period	I	0.114	0.474	0.294
	II	0.154	0.350	0.252
	Mean	0.134	0.412	0.273

Figure 2 - Relationship between AMA and BMA excretion over 48 hours following single oral dose of 50 μmol of AITC and BITC.



DISCUSSION

Oral administration of sinigrin and its digestive metabolite AITC followed by quantification of urinary excretion of AMA, the ultimate excretory product of AITC, provides a useful means of estimating the release of AITC from sinigrin *in vivo*. A previous experiment with sheep showed mercapturic acid excretion to vary considerably between animals (Duncan, 1990). To reduce the error associated with this variation, rats in the current experiment were concurrently dosed with BITC to allow correction for differential mercapturic acid excretion between animals. Diet had a major effect on the release of AITC from sinigrin *in vivo*. This is likely to be due to the myrosinase activity of the cauliflower diet leading to a more extensive hydrolysis of sinigrin in the digestive tract. Induction of microbial glucosinolate degradation by the presence of glucosinolates in the diet may also have had and influence on the extent of glucosinolate breakdown. A further possibility is that the course of hydrolysis was affected by the diet so that products such as nitriles, were more prominent in the digestive conditions produced by the control diet. The next stage in this work will be to quantify the excretory metabolites of other glucosinolate breakdown products to allow more definitive conclusions. The experiment has shown that the nature of the diet can have a major effect on glucosinolate hydrolysis and hence toxicity. Adoption of this approach to the study of glucosinolate breakdown could lead to better prediction of glucosinolate toxicity under different dietary circumstances.

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