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The influence of diet on drug metabolism studies of *S*-carboxymethyl-L-cysteine

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Summary

The presence of (+)-*S*-methyl-L-cysteine sulphoxide in methanolic extracts of certain vegetables has been confirmed. Urinary levels of (+)-*S*-methyl-L-cysteine sulphoxide were measured following ingestion of vegetables as part of normal dietary intake. The possibility of (+)-*S*-methyl-L-cysteine sulphoxide derived from vegetables interfering with metabolic studies of (+)-*S*-carboxymethyl-L-cysteine is discussed.

Introduction

S-Carboxymethyl-L-cysteine (SCMC) is a mucolytic agent used in the treatment of respiratory disorders. Waring (1978) investigated the metabolism of SCMC in rodents, marmosets and humans and reported that the major metabolites found in the urine were SCMC and SCMC-sulphoxide. *N*-Acetyl-SCMC and methylmercapturic acid (*N*-acetyl-*S*-methyl-L-cysteine) were reported as minor metabolites and trace quantities of *S*-methyl-L-cysteine (SMC) and its sulphoxide were also observed. A further study (Waring, 1980) reported the presence in urine of the 6 previously reported metabolites. In addition, the sulphoxides of methylmercapturic acid and *N*-acetyl-SCMC together with dicarboxymethyl sulphide were also identified. The results presented in these papers indicated that for SCMC the major metabolic routes are decarboxylation and sulphoxidation. The possibility of a polymorphic distribution of sulphoxidation capacity within the population was then studied by means of an investigation of the metabolism of SCMC in 181 human volunteers (Waring et al., 1982). These workers defined a term "sulphoxidation index" which they calculated according to the following formula:

{% administered dose excreted as
(parent compound
+non-sulphoxide metabolites)}
/{% administered dose excreted
as sulphoxide metabolites}

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The results obtained led to the conclusion that a polymorphism existed with respect to sulphoxidation within a population and that approximately 10% of the population had a reduced capacity to oxidise the sulphinyl sulphur of SCMC.

During our current studies of the analysis of the sulphoxide metabolites of SCMC (Woolfson et al., 1986, 1987; Karim et al., 1988), a literature survey revealed that *S*-methyl-L-cysteine sulphoxide (SMC-sulphoxide) was not only a metabolite of SCMC but also a constituent of a large number of vegetables (Synge and Wood, 1956; Matheson and Moir, 1976). The constituent of vegetable matter has been identified as (+)-*S*-methyl-L-cysteine sulphoxide. Since the human metabolic studies described above (Waring, 1978, 1980; Waring et al., 1982) lasted for periods of between 8 and 24 h, it must be assumed that there was some ingestion of food during the course of these studies. The possible ingestion of vegetables containing SMC-sulphoxide may therefore have taken place. Thus the levels of this compound found in the urine of the volunteers taking part in the metabolic studies may have been influenced by diet.

The present study investigates the possible influences of dietary intake on human volunteer studies of the metabolism of SCMC. The study utilises a high-performance liquid chromatographic method with electrochemical detection developed for the analysis of urinary metabolites of SCMC (Woolfson et al., 1987).

Materials and Methods

Instrumentation

The high-performance liquid chromatography (HPLC) system consisted of a Gilson Model 302 pump and manometric module (Gilson Model 803) used in conjunction with a Rheodyne Model 7125 valve equipped with a 20 μl loop. Detection was via a glassy carbon electrochemical detector (Bioanalytical Systems Model LC-4A) at +0.5 V vs Ag/AgCl. Chromatograms were recorded on a Perkin Elmer Model 56 chart recorder.

Analysis of (+)-S-methyl-L-cysteine sulphoxide in methanolic extracts of cabbage and cauliflower

Cabbage leaves (100 g) were sliced into small pieces and soaked in methanol (150 ml) for 24 h. The methanolic solution was separated from the solid material and centrifuged. 2 ml of the clear methanolic extract was diluted to 20 ml with phosphate buffer (pH 7). An aliquot (80 μl) of this buffered solution was reacted with OPT reagent (20 μl). The reaction mixture (20 μl) was chromatographed using 20% methanol in phosphate buffer (pH 7) as mobile phase. A similar procedure was adopted for the investigation of cauliflower.

Analysis of (+)-S-methyl-L-cysteine sulphoxide in boiled cabbage and cauliflower

Cabbage leaves (100 g) were sliced and boiled in water (200 ml) for 30 min. 2 ml of the boiled cabbage water was mixed with methanol (3 ml) and centrifuged. The clear liquid (2 ml) was diluted to 20 ml with phosphate buffer (pH 7). The buffered solution (40 μl) was mixed with 20% methanol/phosphate buffer (40 μl) and reacted with OPT reagent (20 μl). An aliquot of this reaction mixture (20 μl) was chromatographed using 20% methanol/phosphate buffer (pH 7) as the mobile phase.

The boiled cabbage leaves were crushed and extracted with methanol (80 ml). The methanolic extract was centrifuged and the clear liquid (1 ml) was diluted to 20 ml with phosphate buffer. The buffered solution (80 μl) was reacted with OPT reagent (20 μl). An aliquot (20 μl) of the reaction mixture was chromatographed using 20% methanol/phosphate buffer as the mobile phase. A similar experiment was carried out using cauliflower.

Analysis of S-methyl-L-cysteine sulphoxide excreted in urine samples of subjects after ingestion of food

Three subjects ingested: (a) cauliflower (80 g); (b) cauliflower (50 g) and cabbage (50 g); and (c) cauliflower (110 g) and leeks (60 g), respectively. The vegetables were cooked (boiled) and taken as part of a normal meal. Urine samples were collected prior to ingestion of the food and for four hours afterwards. The urine samples were analysed for SMC-sulphoxide content according to the

TABLE 1

Urine levels of (+)-SMC sulphoxide following ingestion of food

Subject	Amount of food ingested (g)			Amount SMC-sulphoxide recovered (mg)
	Cauliflower	Cabbage	Leek	
(a)	80	–	–	6.5
(b)	50	50	–	12.3
(c)	110	–	60	8.4

method of Woolfson et al. (1987) and the results are presented in Table 1.

Results and Discussion

Samples of cabbage leaves and sliced cauliflower were extracted with methanol. The methanolic extracts were investigated for the presence of *S*-methyl-L-cysteine sulphoxide using HPLC with ECD following derivatisation with *o*-phthalaldehyde (OPT). We have previously demonstrated that this method is capable of resolving the OPT derivatives of (+)- and (−)-*S*-methyl-L-cysteine sulphoxide (Woolfson et al., 1987). The HPLC investigation demonstrated the presence of (+)-*S*-methyl-L-cysteine sulphoxide in the methanolic extracts. No (−)-*S*-methyl-L-cysteine sulphoxide was detected. These results are consistent with previous reports (Synge and Wood, 1956; Matheson and Moir, 1976).

The second part of the investigation concerned the effects of conventional cooking methods on the (+)-*S*-methyl-L-cysteine sulphoxide content of vegetable matter. Samples of cabbage and cauliflower were boiled in water for 30 min. The boiled vegetables were crushed and extracted with methanol. (+)-*S*-methyl-L-cysteine sulphoxide was shown to be present in both the boiled water and the methanolic extracts.

Finally the urine samples of volunteers prior to and following the ingestion of cooked vegetables, as part of a meal, were investigated. Three subjects ingested: (a) 80 g of cauliflower; (b) 50 g of cauliflower and 50 g of cabbage; and (c) 110 g of cauliflower and 60 g of leeks, respectively. These vegetables had previously been reported as containing (+)-*S*-methyl-L-cysteine sulphoxide. Urine samples were collected prior to ingestion of food and then for 4 h following ingestion. No (+)-*S*-methyl-L-cysteine sulphoxide was detected in the urine samples prior to ingestion of food but was observed in the post-prandial samples. The levels of (+)-*S*-methyl-L-cysteine sulphoxide determined in the urine samples following the meal are presented in Table 1.

Thus, it has been demonstrated that (+)-*S*-methyl-L-cysteine sulphoxide may be detected in urine following the ingestion of vegetables known to contain this compound. This observation indicates that in metabolic studies involving *S*-carboxymethyl-L-cysteine it is necessary to control dietary intake since (+)-*S*-methyl-L-cysteine sulphoxide is a known metabolite of *S*-carboxymethyl-L-cysteine. For metabolic studies involving *S*-carboxymethyl-L-cysteine and lasting for periods of 24 h or longer the volunteers will require some food intake. If the food includes vegetables containing (+)-*S*-methyl-L-cysteine sulphoxide, then obviously higher urine levels of this compound will be observed than if these vegetables were not ingested. Therefore calculations of, for instance, the sulphoxidation index may be influenced by such dietary factors.

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