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ORIGINAL ARTICLE

An investigation into possible xenobiotic–endobiotic inter-relationships involving the amino acid analogue drug, *S*-carboxymethyl-L-cysteine and plasma amino acids in humans

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Abstract The amino acid derivative, *S*-carboxymethyl-L-cysteine, is an anti-oxidant agent extensively employed as adjunctive therapy in the treatment of human pulmonary conditions. A major biotransformation route of this drug, which displays considerable variation in capacity in man, involves the oxidation of the sulfide moiety to the inactive S-oxide metabolite. Previous observations have indicated that fasted plasma L-cysteine concentrations and fasted plasma L-cysteine/free inorganic sulfate ratios were correlated with the degree of sulfoxidation of this drug and that these particular parameters may be used as endobiotic biomarkers for this xenobiotic metabolism. It has been proposed also that the enzyme, cysteine dioxygenase, was responsible for the drug sulfoxidation. Further in this theme, the degree of S-oxidation of *S*-carboxymethyl-L-cysteine in 100 human volunteers was investigated with respect to it potential correlation with fasted plasma amino acid concentrations. Extensive statistical analyses showed no significant associations or relationships between the degree of drug S-oxidation and fasted plasma amino acid concentrations, especially with respect to the sulfur-containing compounds, methionine, L-cysteine, L-cysteine sulfinic acid, taurine and free inorganic sulfate, also the derived ratios of L-cysteine/L-cysteine sulfinic acid and L-cysteine/free inorganic sulfate. It was concluded that plasma amino acid levels or derived ratios cannot be employed to predict the degree of S-oxidation of *S*-carboxymethyl-L-cysteine (or vice versa) and that it is doubtful if the enzyme, cysteine dioxygenase, has any involvement in the metabolism of this drug.

Keywords Metabolomics · S-oxidation · *S*-carboxymethyl-L-cysteine · L-Cysteine/sulfate ratio · Cysteine dioxygenase

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Introduction

The amino acid analogue, *S*-carboxymethyl-L-cysteine (3-carboxymethylthio-L-alanine, carbocisteine, carbocysteine, SCMC) is employed as a anti-oxidant agent during the treatment of chronic obstructive pulmonary disease (COPD; chronic bronchitis and emphysema) and also in the management of otitis media with effusions (OME, glue ear) (Steventon and Mitchell 2006a, b). The drug undergoes extensive biotransformation as it passes through the body with sulfur oxygenation being a major pathway (Mitchell et al. 1984; Mitchell and Steventon 2007; Steventon and Mitchell 2006c). This is biologically important as the parent compound and its sulfide metabolites are presumed to act as free radical scavengers; the thioether moiety combining with harmful reactive oxygen species to form stable S-oxide metabolites (Brandolini et al. 2003). Any enzyme-mediated sulfur oxygenation of this compound would lead to therapeutically inactive species and hence an understanding of the enzyme system(s) involved in this deactivation process is of prime importance.

Owing to the similarity in chemical structure between SCMC and L-cysteine, and a preliminary investigation into the properties of 'SCMC S-oxygenase', it was intimated that cysteine dioxygenase (EC 1.13.11.20), the enzyme catalysing the first step of endogenous cysteine oxygenation to cysteine sulfinic acid, was responsible for this biotransformation reaction (Waring et al. 1986; Mitchell and Waring 1989). Two investigative accounts involving healthy volunteers and rheumatoid arthritis patients have suggested an association between the extent of SCMC S-oxygenation within an individual and their plasma L-cysteine/inorganic sulfate ratios (a presumed indicator of cysteine dioxygenase activity) (Mitchell et al. 1992; Bradley et al. 1994). Indeed, a number of scientific articles have espoused the statement that cysteine dioxygenase was responsible for the S-oxidation of SCMC, but to date there has been no published experimental evidence to support this tenuous assumption (Bradley et al. 1994; Kidd 2002; Waring and Emery 1993, 1995).

With the cooperation of 100 healthy adult volunteers, the aim of the present study was to investigate using a statistical and metabolomic approach what, if any, relationships existed between fasted plasma amino acid levels or sulfur amino acid metabolite concentrations and the extent of S-oxygenation of the xenobiotic, SCMC.

Materials and methods

Chemicals

Acetone, aqueous hydrogen peroxide (30% v/v), amino acid standards, boric acid, di-sodium hydrogen orthophosphate, dithiothreitol, 9-fluorenylmethyl chloroformate (FMOC), 2-mercaptoethanol, 1-octanesulphonic acid, ortho-phosphoric acid, ortho-phthalaldehyde, 1-pyrenyldiazomethane (PDAM), *S*-carboxymethyl-L-cysteine (SCMC), *S*-methyl-L-cysteine (SMC), sodium hydroxide, thioglycolic acid and triethylamine were purchased from Sigma-Aldrich Co. Ltd (UK). Glacial acetic acid, hydrochloric acid, sodium carbonate, sodium hydrogen carbonate and sulfuric acid were purchased from BDH Laboratory Supplies (UK). HPLC grade acetonitrile and methanol were both supplied by Rathburn Chemical Co (UK). The sulfide and sulfoxide metabolites of SMC and SCMC were chemically synthesised as previously reported (Steventon 1998).

Subjects

Healthy volunteers (100: 55 male, age 24.2 ± 8.5 year, wt. 72.4 ± 6.5 kg; 45 female, age 22.2 ± 3.7 year, wt. 56.5 ± 3.8 kg) were recruited from students and staff at the University of Birmingham, England, in accordance with University Ethics Committee regulations and guidelines and those stipulated by the World Medical Association Declaration of Helsinki 2008. Of the 45 female volunteers, 37 were taking oral contraceptives (+OC) and 8 were not taking oral contraceptives (−OC). After fasting from midnight (0000 hours), the subjects emptied their urinary bladder at 0800 hours and then venous blood (20 ml) was withdrawn to undertake both renal and liver function tests and also for the analysis of fasted plasma amino acids, sulfur amino acid metabolites and inorganic sulfate. The volunteers then ingested two capsules of SCMC (total dose 750 mg; Mucodyne®, Berk Pharmaceutical Ltd, Eastbourne, UK) and the subsequent 0–8 h urine output (0800–1600 hours) was collected. The total urine volume obtained was recorded and aliquots (2 × 20 ml) stored (−20°C) until analysis for SCMC and its metabolites. Subjects were allowed access to water from 0800–0830 hours and free access to food and drink from then onwards.

Plasma and urine analysis

Plasma amino acids and the sulfur amino acid metabolites, L-cysteine sulfinic acid (CSA) and taurine, were quantified by reverse-phase HPLC with fluorescence detection following pre-column derivatization with ortho-phthalaldehyde/2-mercaptoethanol (monobromobimane being employed for L-cysteine) as previously described in detail (Alam et al. 1998; Mansoor et al. 1992). Plasma free inorganic sulfate was measured by ion exchange chromatography with conductivity detection as reported previously (Blinn et al. 2005). Urinary SCMC and its sulfide and sulfoxide metabolites were analysed by HPLC with fluorescence detection following pre-column derivatization with FMOC and PDAM as previously reported (Steventon 1998).

Data analysis

Classical statistical analyses of the obtained results were undertaken using SPSS version 15.0 to determine the relationships between qualitative and quantitative variables. After which, a multivariate statistical analysis approach was undertaken using SIMCA version 12.0 to investigate data both in a non-supervised and in a supervised manner. For that purpose, principal component analysis (PCA); partial least square discriminant analysis (PLS-DA), orthogonal partial least square discriminant analysis (OPLS-DA) and regression analysis by means of PLS were applied. Discriminant PCA is a tool used in exploratory data analysis to identify patterns within the data and thereby expressing the data in such a way as to highlight their similarities and differences. PLS-DA and

OPL-DA are techniques that allow model building and include information of the classes enabling variables related to these classes to be highlighted. Partial least square regression methods (PLS) find linear regression models by projecting the predicted variables and the observable measured variables.

Since only two individuals (1 male and 1 female +OC) were phenotyped as poor metabolisers for the S-oxidation of SCMC, these individuals results were not used for the plasma amino acid, correlation and multivariate analysis but were included into the S-oxidation phenotyping investigation. To conduct a detailed statistical analysis of a population of $n = 2$ is meaningless.

Results

Volunteers

When the volunteers were examined for differences in age and body weight the following observations were made. There were no statistical differences observed between the age of the male and female volunteers (male 24.2 ± 8.5 years, mean ± SD; female 22.2 ± 3.7 years; $P > 0.05$ Student's t test) and no differences between the sub-groups of females either taking or not taking oral contraceptives (OC) (+OC, 22.3 ± 3.9 years, $n = 36$; −OC, 21.8 ± 2.4 years, $n = 8$; $P > 0.05$ Student's t test). As expected, males volunteers were generally heavier than female subjects (male 72.4 ± 6.5 kg; female 56.5 ± 3.8 kg; $P < 0.01$ Student's t test) and those females taking OC were heavier than those who were not taking OC (+OC, 57.1 ± 3.7 kg; −OC, 53.8 ± 2.6 kg; $P < 0.05$ Student's t test).

Plasma amino acids

The mean ± SD plasma amino acid concentrations in the volunteer population are reported in Table 1. When the levels obtained for plasma amino acids were examined for potential gender differences only L-cysteine sulphinic acid (CSA) showed any significant trend with values in females being higher than those in males (female, 0.66 (0.0–1.1) μM, median and range; male 0.54 (0.0–1.1) μM; $P < 0.01$ Mann–Whitney U test). However, when the levels in females either taking or not taking OC were compared, the cohort taking OC demonstrated significantly lower plasma values for Arg (+OC, 74.0 (48.0–89.0) μM, median and range; −OC, 84.5 (69.0–93.0) μM; $P < 0.005$ Mann–Whitney U test), Asn (+OC, 45.0 (37.0–72.0) μM; −OC, 52.5 (48.0–57.0) μM; $P < 0.01$ Mann–Whitney U test) and free inorganic sulfate (SO_4^{2-}) (+OC, 503.7 (451.7–537.5) μM; −OC, 513.9 (505.2–543.5) μM; $P < 0.05$ Mann–Whitney U test).

Table 1 Population data for fasted plasma amino acids

Amino acid	n	Mean ± SD (μM)
Ala	98	397.6 ± 32.2
Arg	98	75.0 ± 11.4
Asn	98	47.3 ± 8.2
Asp	98	5.0 ± 2.2
CSA	98	0.55 ± 0.3
Cys	98	10.2 ± 1.7
Gln	98	571.2 ± 74.8
Glu	98	36.9 ± 14.8
Gly	98	202.2 ± 40.7
His	98	80.7 ± 10.8
Leu	98	137.7 ± 23.5
Ile	98	61.6 ± 17.0
Lys	98	167.6 ± 28.2
Met	98	26.6 ± 8.5
Phe	98	54.0 ± 10.4
Ser	98	118.1 ± 17.5
Thr	98	124.8 ± 19.3
Trp	98	53.6 ± 10.7
Tyr	98	61.1 ± 9.9
Val	98	250.7 ± 41.6
Free inorganic SO_4^{2-}	98	501.9 ± 25.6
Taurine	98	59.2 ± 11.9
Cys/SO_4^{2-} ratio	98	20.2 ± 3.6
Cys/CSA ratio	98	22.0 ± 16.1

Volunteers fasted from 1200 hours (midnight). At 0800 hours, 20 ml of venous blood was withdrawn for the analysis of fasted plasma amino acids, sulfur amino acid metabolites, inorganic sulfate, renal function and liver function tests. Plasma for amino acid analysis was stored at −20°C until analysed. Blood for renal and liver function test was sent for analysis immediately after the sample was taken

S-oxidation of SCMC

The data within the frequency polygon displaying the percentage urinary S-oxide metabolites excreted in 0–8 h urine is clearly not normally distributed (Fig. 1). S-oxide metabolites related to SCMC were found in the urine of all but 2 of the 100 volunteers following drug ingestion, with values ranging from zero to 41.8% of the administered dose. Median values showed that male subjects excreted significantly more S-oxide metabolites (males 14.4%, range 0.0–41.8% vs. females 10.4%, range 0.0–25.7%, $P < 0.005$ Mann–Whitney U test) and also sulfide-related material (males 44.3%, range 12.3–81.7% vs. females 31.5%, range 15.9–60.6%, $P < 0.001$ Mann–Whitney U test), and hence had higher total drug recoveries, than the female volunteers (males 62.5%, range 21.8–96.9% vs.

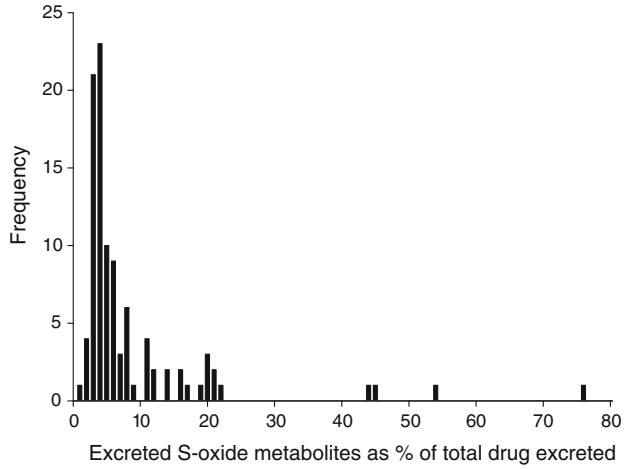

Fig. 1 Frequency distribution of urinary excreted S-oxide metabolites as % of the total drug excreted. After fasting from midnight (0000 hours), the subjects ($n = 98$) emptied their urinary bladder at 0800 hours. The volunteers then ingested two capsules of SCMC (total dose 750 mg; Mucodyne®, Berk Pharmaceutical Ltd, Eastbourne, UK) and the subsequent 0–8 h urine output (0800–1600 hours) was collected. The total urine volume obtained was recorded and aliquots (2 × 20 ml) stored (–20°C) until analysis for SCMC and its metabolites. Subjects were allowed access to water from 0800 to 0830 hours and free access to food and drink from then onwards

females 41.1%, range 17.3–75.3%, $P < 0.05$ Mann–Whitney U test). However, using median values, when the sulfoxides recovered were expressed as a percentage of the total drug-related material within the 0–8 h urine, the values obtained were quite similar (male 23.0%, female 24.8%). With respect to the variability in SCMC sulfoxide production and subsequent urinary excretion, previous studies have divided the population into three categories; 'extensive metabolisers, EM' (>14.3% recovered dose as sulfoxides), 'intermediate metabolisers, IM' (1.2–14.3%) and 'poor metabolisers, PM' (<1.2%). In the present study the proportions of the population assigned to these groups (70% EM, 28% IM, 2% PM) were not significantly different from those previously reported in the literature ($P > 0.05$ χ^2 test with Yates' correction) (Mitchell et al. 1984, Steventon et al. 2003).

Correlations

The values obtained for percentage urinary S-oxides metabolites excreted in the 0–8 h urine were examined individually for correlation with the plasma amino acids, L-cysteine sulfinic acid, taurine and inorganic sulfate levels as well as the derived ratios, L-cysteine/L-cysteine sulfinic acid and L-cysteine/inorganic sulfate. As some of these result distributions appeared non-Gaussian, both the Pearson's product moment correlation coefficient (r) and Spearman's rank difference correlation statistic (r_s) were calculated. In all cases the values of the correlation coefficients obtained were low indicating negligible relationship between the two measures. In particular, those between percentage SCMC sulfoxide metabolites and plasma L-cysteine ($r_s = -0.148$), L-cysteine sulfinic acid ($r_s = -0.071$), inorganic sulfate ($r_s = -0.274$), L-cysteine/L-cysteine sulfinic acid ratio ($r_s = 0.071$) and L-cysteine/inorganic sulfate ratio ($r_s = 0.199$) were indicative of little direct relationship and suggested effectively independent variables. As typical examples, the correlation of urinary S-oxide metabolites excreted with fasted plasma L-cysteine levels and the fasted plasma L-cysteine/inorganic sulfate ratio can be seen in Figs. 2 and 3.

Multivariate

For multivariate comparisons the population was divided into two cohorts, one possessing 'extensive metaboliser' (EM, $n = 70$) and the other 'intermediate metaboliser' (IM, $n = 28$) characteristics. The two subjects who produced no SCMC sulfoxide metabolites were omitted. The results of the PCA analysis are displayed in Fig. 4; no obvious discriminatory pattern can be seen with no type of clustering being detected. A further more detailed analysis of the data, undertaken using the supervised analysis techniques of PLS-DA and OPL-DA, again failed to uncover any obvious patterns or clustering (data not shown). As can be observed in Fig. 5, employing PLS

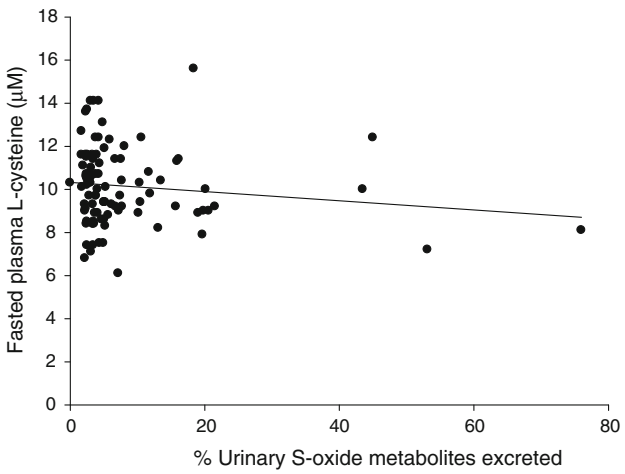

Fig. 2 Plasma L-Cys and urinary % S-oxides excreted correlation. After fasting from midnight (0000 hours), the subjects ($n = 98$) emptied their urinary bladder at 0800 hours and then venous blood (20 ml) was withdrawn for the analysis of fasted plasma L-cysteine. The volunteers then ingested two capsules of SCMC (total dose 750 mg; Mucodyne®, Berk Pharmaceutical Ltd, Eastbourne, UK) and the subsequent 0–8 h urine output (0800–1600 hours) was collected. The total urine volume obtained was recorded and aliquots (2 × 20 ml) stored (−20°C) until analysis for SCMC and its metabolites. Subjects were allowed access to water from 0800 to 0830 hours and free access to food and drink from then onwards

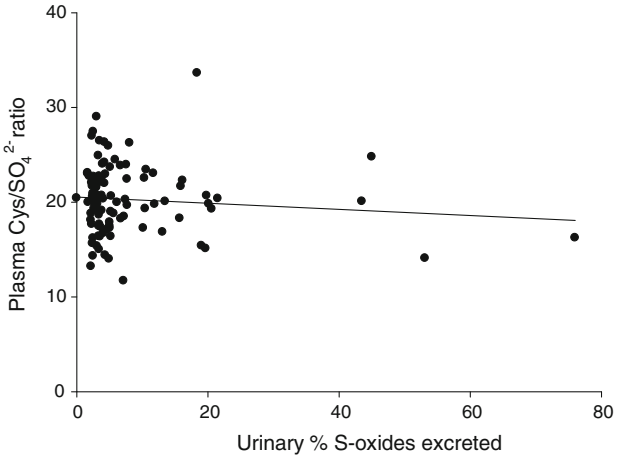

Fig. 3 Plasma L-Cys/free inorganic sulphate ratio and urinary % S-oxides excreted correlation. After fasting from midnight (0000 hours), the subjects ($n = 98$) emptied their urinary bladder at 0800 hours and then venous blood (20 ml) was withdrawn for the analysis of L-cysteine and free inorganic sulfate. The volunteers then ingested two capsules of SCMC (total dose 750 mg; Mucodyne®, Berk Pharmaceutical Ltd, Eastbourne, UK) and the subsequent 0–8 h urine output (0800–1600 hours) was collected. The total urine volume obtained was recorded and aliquots (2×20 ml) stored (−20°C) until analysis for SCMC and its metabolites. Subjects were allowed access to water from 0800 to 0830 hours and free access to food and drink from then onwards

regression analysis, the regression model obtained to predict the variable degree of SCMC sulfoxidation observed was very poor. Moreover, the predictive variables showed no significant contribution to the model (see Fig. 6).

Discussion

No significant differences were observed between the plasma amino acid levels measured in this investigation and values previously reported in the literature ($P > 0.05$, Student's t test and Mann–Whitney U test) (Blinn et al. 2005, 2006; Le Boucher et al. 1997; Cotgreave and Moldëus 1986; Fekkes et al. 1995; Mansoor et al. 1992; Wlodek et al. 2001; Ziegler et al. 1992). When coefficients of correlation were calculated to determine how close to coincidence the two data set regression lines were, low values were always obtained indicating that they were virtually independent and that little relationship existed between the amount of SCMC sulfoxidation and amino acid levels or derived ratios. When multi-variant analysis of the data was undertaken using discriminate PCA, PLS-DA, OPLS/OPLS-DA, PLS regression and PLS coefficient regression, these techniques failed to uncover any significant correlations between the variables and the regression model obtained indicated that none of these variables made a significant contribution to the model. Hence, no statistically significant relationships were detected between the EM and IM cohorts (hence the percentage SCMC dose recovered as S-oxide metabolites; the sulfoxidation capacity) and the levels of fasted plasma amino acid and metabolites measured in this investigation.

Cysteine dioxygenase is an enzyme of intermediary metabolism that is responsible for the oxidation of L-cysteine to L-cysteine sulfinic acid. It utilises molecular oxygen and adds the two oxygen atoms directly onto the sulfur centre of the cysteine molecule; the reaction is thought not to proceed via the unstable intermediate sulfenic acid (Lombardini et al. 1969). Thus the enzyme is a dioxygenase, and it is difficult to reconcile mechanistically how it could add just one oxygen atom to SCMC forming the observed sulfoxide metabolite as opposed to adding two oxygen atoms thereby forming SCMC sulfone, a metabolite that has never been reported. Any inefficiency in the enzyme's activity should be reflected in an increased level of unprocessed substrate, L-cysteine, and a consequent decreased level of formed product, L-cysteine sulfinic acid.

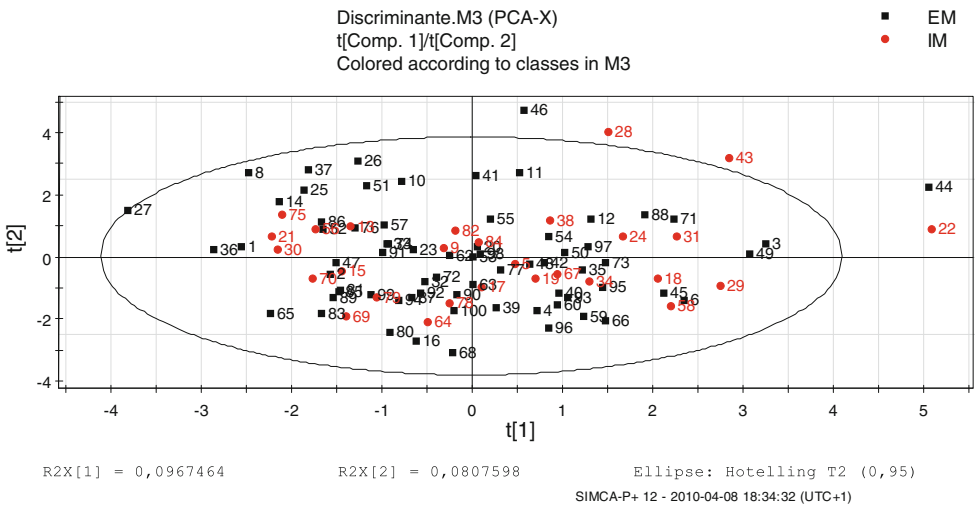

Fig. 4 Discriminate PCA of the plasma amino acids relationship to the S-oxidation phenotypes EM and PM in healthy human volunteers. Scores plot presenting the two main components (PC1 and PC2) for the matrix obtained with all data presented in Table 1 measured for each individual. 'Extensive metaboliser' (EM, $n = 70$) in *black* and 'intermediate metaboliser' (IM, $n = 28$) in *red*. The *ellipse in the plot* represents Hotelling with 95% confidence (color figure online)

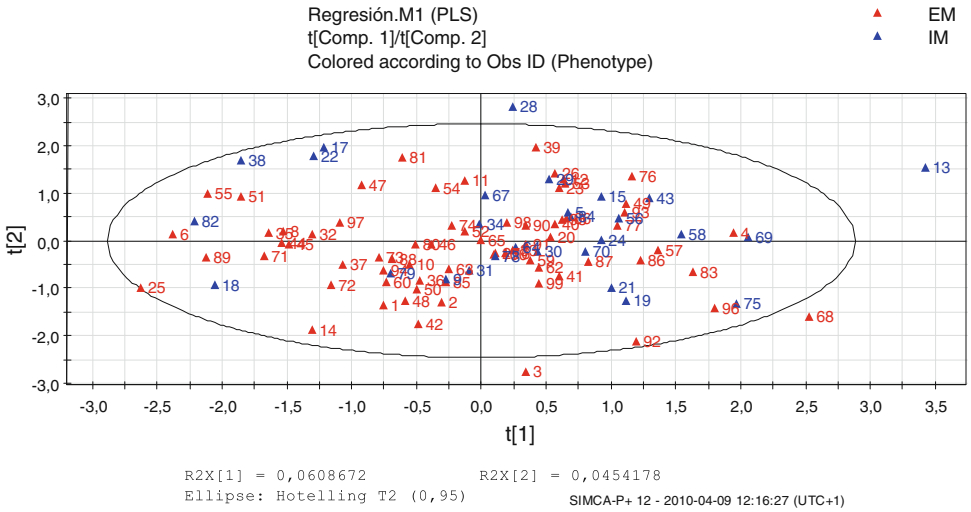

Fig. 5 PLS regression of the plasma amino acids relationship to the S-oxidation phenotypes EM and PM in healthy human volunteers. Scores plot presenting the two main components (PC1 and PC2) for the matrix obtained with all data presented in Table 1 measured for each individual. 'Extensive metaboliser' (EM, $n = 70$) in *black* and 'intermediate metaboliser' (IM, $n = 28$) in *red*. The *ellipse in the plot* represents Hotelling with 95% confidence (color figure online)

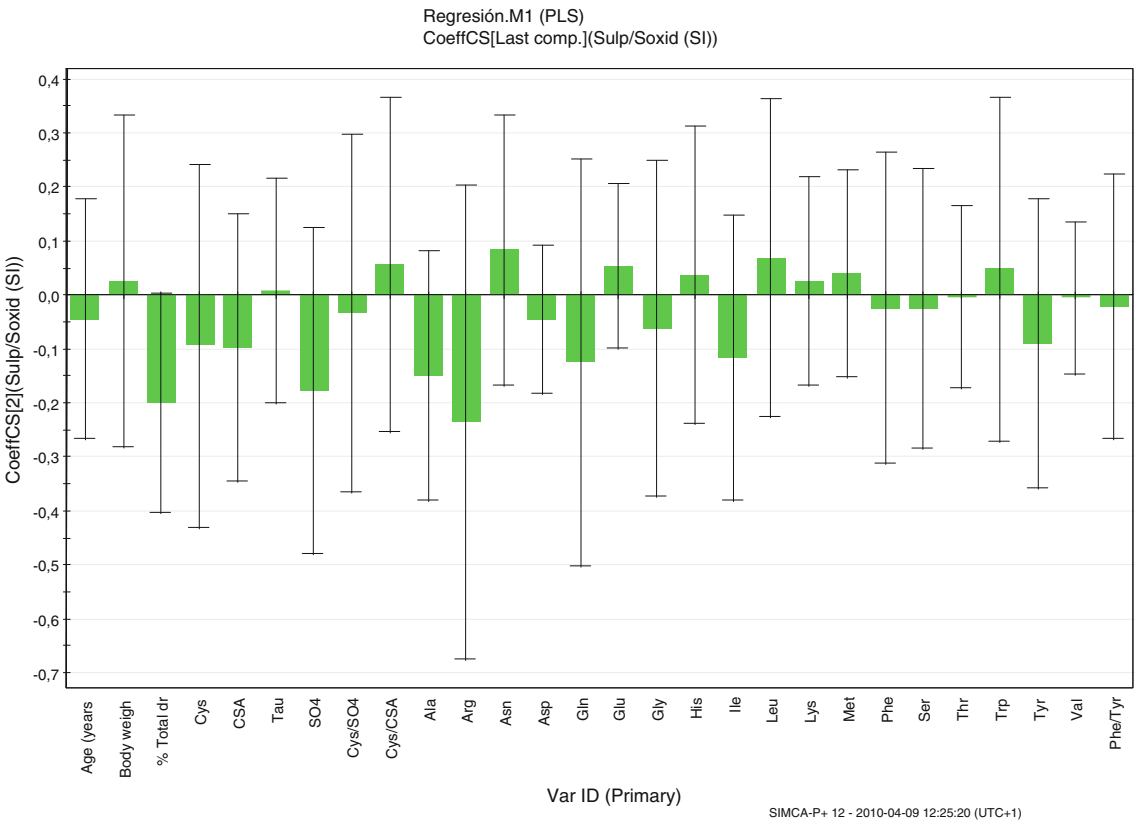

Fig. 6 The examination of the 2-dimensional regression coefficients for PLS regression of the plasma amino acids relationship to the S-oxidation phenotypes EM and PM in healthy human volunteers. The PLS regression coefficients of the two main components for the matrix obtained with all data presented in Table 1 measured for each individual

It is recognised, however, that there are other pathways for the catabolism of L-cysteine that may reduce this burden. The exhaustive oxidation product of L-cysteine sulfur, inorganic sulfate, is many metabolic steps distant from the amino acid sulfinic acid (and there are alternative routes of production) and so its levels would be less denotative of cysteine dioxygenase activity.

The lack of observed association between the degree of SCMC sulfoxidation and the plasma levels of L-cysteine, L-cysteine sulfinic acid and free inorganic sulfate as well as the L-cysteine/L-cysteine sulfinic acid and L-cysteine/inorganic sulfate ratios, strongly suggests, and virtually confirms, that these two phenomenon (SCMC S-oxidation and cysteine dioxygenase activity) are independent processes

and are neither linked nor related. The extent of SCMC S-oxidation is not an indicator of the body's ability to oxidise the sulfur moiety of L-cysteine (as previously alluded) (Bradley et al. 1994; Mitchell and Waring 1989; Mitchell et al. 1992; Waring et al. 1986) and consequently the drug cannot be used as a probe compound for this reaction process. However, interindividual variation in SCMC sulfoxidation capacity has been reconfirmed in this paper and this phenomenon, with its implications and ramifications, remains yet to be fully evaluated.

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References

Alam Z, Coombes N, Waring RH, Williams AC, Steventon GB (1998) Plasma levels of neuroexcitatory amino acids in patients with migraine or tension headache. J Neurol Sci 156:102–106

Blinn CM, Dibbs ER, Hronowski LJJ, Vokonas PS, Silbert JE (2005) Fasting serum sulfate levels before and after development of osteoarthritis in participants of the veterans administration normative aging longitudinal study do not differ from levels in participants in whom osteoarthritis did not develop. Arthritis Rheum 52:2808–2813

Blinn CM, Biggee BA, McAlindon TE, Nuite M, Silbert JE (2006) Sulphate and osteoarthritis: decrease of serum sulphate levels by an additional 3-h fast and a 3-h glucose tolerance test after an overnight fast. Ann Rheum Dis 65:1223–1225

Bradley H, Gough A, Sokhi RS, Hassell A, Waring RH, Emery P (1994) Sulfate metabolism is abnormal in patients with rheumatoid arthritis. Confirmation by in vivo biochemical findings. J Rheumatol 21:1192–1196

Brandolini L, Allegretti M, Berdini V, Cervellera MN, Mascagni P, Rinaldi M, Melillo G, Ghezzi P, Mengozzi M, Bertini R (2003) Carbocysteine lysine salt monohydrate (SCMC-LYS) is a selective scavenger of reactive oxygen intermediates (ROIs). Eur Cytokine Netw 14:20–26

Cotgreave IA, Moldëus P (1986) Methodologies for the application of monobromobimane to the simultaneous analysis of soluble and protein thiol components of biological systems. J Biochem Biophys Methods 13:231–249

Fekkes D, Van Dalen A, Edelman M, Voskuilen A (1995) Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated precolumn derivatization with *o*-phthaldialdehyde. J Chromatogr B 669:177–186

Kidd PM (2002) Autism, an extreme challenge to integrative medicine. Part 1: the knowledge base. Altern Med Rev 7:292–316

Le Boucher J, Charret C, Coudray-Lucas C, Giboudeau J, Cynober L (1997) Amino acid determination in biological fluids by automated ion-exchange chromatography: performance of Hitachi L-8500A. Clin Chem 43:1421–1428

Lombardini JB, Singer TP, Boyer PD (1969) Cysteine oxygenase. II. Studies on the mechanism of the reaction with ^{18}oxygen. J Biol Chem 244:1172–1175

Mansoor MA, Svardal AM, Ueland PM (1992) Determination of the in vivo redox status of cysteine, cysteinylglycine, homocysteine, and glutathione in human plasma. Anal Biochem 200:218–229

Mitchell SC, Steventon GB (2007) Drug metabolism and toxicity: hijacking enzymes of intermediary metabolism. Curr Topics Toxicol 3:57–63

Mitchell SC, Waring RH (1989) The deficiency of sulfoxidation of *S*-carboxymethyl-L-cysteine. Pharmacol Therap 43:237–249

Mitchell SC, Waring RH, Haley CS, Idle JR, Smith RL (1984) Genetic aspects of the polymodally distributed sulphoxidation of *S*-carboxymethyl-L-cysteine in man. Br J Clin Pharmacol 18:507–521

Mitchell SC, Waring RH, Steventon GB (1992) Variation in the S-oxidation of cysteine derivatives. In: Kalow K (ed) Pharmacogenetics of drug metabolism. Pergamon Press, Oxford, pp 367–382

Steventon GB (1998) A methodological and metabolite identification study of *S*-carboxymethyl-L-cysteine metabolism in man. Chromatographia 48:561–568

Steventon GB, Mitchell SC (2006a) Efficacy of *S*-carboxymethyl-L-cysteine for otitis media with effusion. ENT J 85:296–297

Steventon GB, Mitchell SC (2006b) The sulphoxidation of *S*-carboxymethyl-L-cysteine in COPD. Eur Respir J 27:865–866

Steventon GB, Mitchell SC (2006c) Non-classical drug metabolism enzymes: the curious case of phenylalanine 4-monooxygenase. Lett Drug Design Discov 3:405–412

Steventon GB, Waring RH, Williams AC (2003) An investigation into the inter-relationships of sulfur xeno-biotransformation pathways in Parkinson's and motor neurone diseases. Drug Metab Drug Interact 19:223–240

Waring RH, Emery P (1993) The genetic basis of responses to drugs—a rheumatological perspective. Br J Rheumatol 32:181–188

Waring RH, Emery P (1995) The genetic basis of responses to drugs. Br Med Bull 51:449–461

Waring RH, Mitchell SC, O'Gorman J, Fraser M (1986) Cytosolic sulphoxidation of *S*-carboxymethyl-L-cysteine in mammals. Biochem Pharmacol 35:2999–3002

Wlodek PJ, Iciek MB, Milkowski A, Smolenski OB (2001) Various forms of plasma cysteine and its metabolites in patients undergoing hemodialysis. Clin Chim Acta 304:9–18

Ziegler F, Le Boucher J, Coudray-Lucas C, Cynober L (1992) Plasma amino acid determinations by reversed-phase HPLC: improvement of the orthophthalaldehyde method and comparison with ionexchange chromatography. J Automat Chem 14:145–149