PHARMACOGENETICS OF THE S-OXIDATION OF S-CARBOXYMETHYL-L-CYSTEINE

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SUMMARY

The pharmacogenetics of S-carboxymethyl-L-cysteine (SCMC) have been studied in detail. When results from administration of SCMC to 200 volunteers were analysed, there was seen to be a wide interindividual variation in the percentage of sulphoxide metabolites excreted. Computer assisted analysis suggested that the population distribution observed could be most economically represented as two overlapping Gaussian distributions with the smaller mode representing poor sulphoxidisers. This phenomenon appears to be largely genetic in origin and to behave as though controlled by one autosomal recessive gene, but environmental factors may also be important. Poor sulphoxidisers seem to be overrepresented in certain patient populations with chronic diseases. These findings are discussed in terms of oxidative metabolism of sulphur-containing compounds.

I. INTRODUCTION

Although many sulphur-containing drugs are known to form sulphoxides, these metabolites have been rarely quantitated in man. From the literature it is apparent that variations between individuals do occur but their significance is often lost, being absorbed without comment into the experimental mean value with some measure of spread. However, during the last decade detailed attention has been paid to such interindividual variation with the emergence of a renewed appreciation of pharmacogenetics.

Variations in sulphoxide formation have been most extensively studied in man for the compound S-carboxymethyl-L-cysteine (SCMC). Originally synthesized in the 1930's it was introduced thirty years later as a mucolytic agent and is also employed as supportive therapy in bronchitis and cystic fibrosis. A relatively non-toxic compound, it has recently fallen out of general usage owing to apparent lack of efficacy.

II. HUMAN METABOLISM

Our interest began in 1978 when a local pediatrician enquired as to why some of his patients with cystic fibrosis responded well to SCMC whereas others did not. This observation was followed by studies on the human metabolism of SCMC which uncovered as many as nine metabolites, four of which were sulphoxides, as well as a glucuronic acid conjugate and the degradation product, inorganic sulphate /1,2/. From an investigation involving 20 medical students it was noticed that a 7-fold difference in the amount of sulphoxide metabolites present in the 0-24 hour urine existed between two of the subjects and that the distribution of the percentage total urinary recovery excreted as sulphoxides for the whole group appeared non-Gaussian /3/.

A much larger study followed in which 200 subjects ingested a 750 mg test dose of SCMC in the morning and collected their following 0-8 hour urine. Nearly a 100-fold variation was observed in this study, with the amounts of sulphoxide ranging from 0.6% to 59.1% of the total drug recovery. Mathematical modelling, using computer-generated maximum-likelihood analysis, suggested that the skew distribution of sulphoxide excretions could be represented most economically as being derived from two overlapping Gaussian distributions. A computer-derived antimode was assigned to assist in future investigations /4/.

III. POPULATION STUDIES

In the above mentioned study the total urinary sulphoxides were expressed as a ratio, the "Sulphoxidation Index" (S.I.), calculated as the percentage administered dose excreted as sulphides divided by the percentage administered dose excreted as sulphoxides, in the 0-8 hour urine sample. Using this ratio skews the data so that those individuals who produce smaller amounts of sulphoxides ("poor metabolisers"; P.M.) are more easily separated from the majority of the population ("extensive metabolisers"; E.M.). An alternative expression is the "Sulphoxidation Capacity" (S.C.), which is the percentage of the total recovery excreted as sulphoxides. It can be seen that this is related to the S.I. by the following expression, S.C. = 100/1 + S.I. Unlike the inverse S.I. scale, the larger the S.C. value, the more extensive the sulphoxidiser.

Computer analysis suggests an antimode at an S.I. value of 6.0 (S.C. = 14.3%), but it must be appreciated that since the modes overlap, classification of individuals into either mode cannot always be achieved with accuracy. It has been estimated that up to 13.6% (27 subjects) of this initial population of 200 volunteers may have been misclassified. However, the probability of misclassification is greatest around the antimode value, with P.M.'s having the greatest tendency to be placed in the wrong category /4/.

This leads to the interesting question of the incidence of poor metabolic sulphoxidation of S-carboxymethyl-L-cysteine in the general population. In the original study with 200 volunteers an incidence of 34.5% (95% confidence interval; 27.7% to 41.3%) was observed /4/. More recent, but smaller studies have given percentage values for P.M.'s ranging from 27.8% to 42.0% /5,6/. All that can be said at this stage is that the relative impairment is possessed by the smaller proportion of the population, probably about 1 in 3 individuals falling into the P.M. category.

IV OTHER ASSOCIATIONS

The sulphoxidation status appears to be a property of the individual. The day-to-day variation in sulphoxide output may fluctuate within about 10% of the mean value and is reproducible within individuals on repeat testing for up to five years /4; R.H. Waring, unpublished data/.

It has been shown that there is no association between sulphoxide output and total drug recovery, the other available pathways of SCMC metabolism, or the subject's sex, age, body weight, alcohol consumption or smoking behaviour /4/. The population distribution of SCMC sulphoxidation capacities shows no congruence with other reported polymorphisms; namely debrisoquine 4-hydroxylation /7/, N-acetylation of sulphadimidine (R.H.Waring, unpublished data), the taste threshold for phenylthiourea /8/ and the production of odorous urine after eating asparagus /6/. In addition, sulphoxidation status has no obvious associations with any HLA designation /9/.

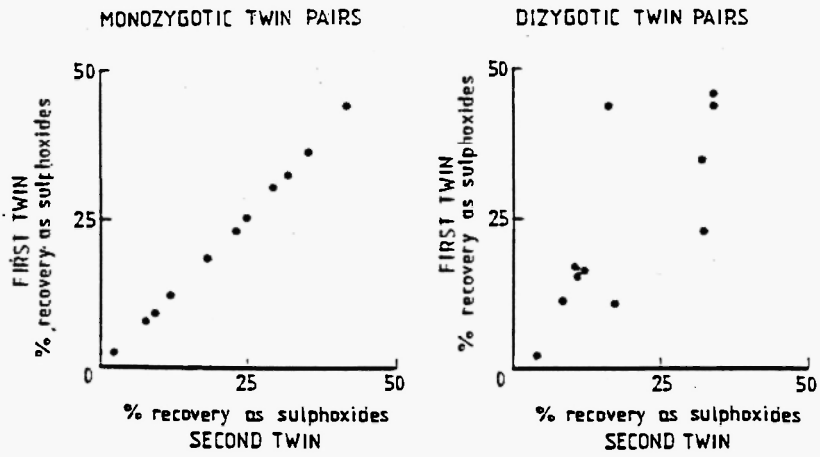

Fig. 1: Scattergrams comparing the "sulphoxidation capacity" within 11 pairs of monozygotic (identical) twins (R = + 1.0) and 11 pairs of dizygotic (non-identical) twins (R = + 0.84).
R = The coefficient of rank correlation.
$R = 1 - 6\Sigma d^2/n(n^2-1)$

V CLASSICAL GENETIC STUDIES

Studies using monozygotic and dizygotic twins have shown that whereas the identical twin pairs gave very similar values for sulphoxide output the dizygotic twin pairs did not (Figure 1). Such investigations are open to criticism but nevertheless they do suggest that heritability is an important factor. This was supported by a series of random family studies where the results obtained were compatible with a simple Mendelian model in which the P.M. trait was largely autosomal recessive in character (Figure 2) /4/.

Thus, all the results obtained so far from classical genetic studies are consistent with the idea that the sulphoxidation of S-carboxymethyl-L-cysteine is under genetic control and mediated by two alleles of major effect at one autosomal locus. The width of the modes observed in the large population study suggests that this major genetic control is superimposed upon a background of polygenic effects and environmental factors, which modulate the expression of this major gene /4/. Presumably only the techniques of molecular biology will fully elucidate this problem.

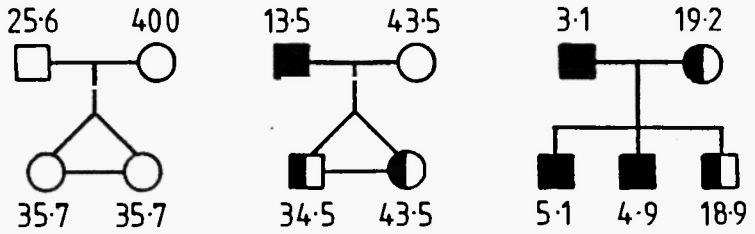

Fig. 2: The pedigrees of 3 families showing the "sulphoxidation capacity".
□ = male
○ = female
■● = homozygous impaired metabolisers (by definition, see text)
◧◐ = individuals who can be identified as heterozygotes with respect to SCMC sulphoxidation (autosomal recessive inheritance, see text).

VI. SUB-CELLULAR SITES OF SCMC SULPHOXIDATION

The formation of the sulphoxides of SCMC has been shown to occur in the cytosolic fractions of liver cells obtained from several mammalian species. The microsomal fractions, containing the cytochrome(s) P-450 and mixed function amine oxidase enzyme systems were devoid of activity /10/. Interestingly, if the sulphoxidation test is carried out overnight the percentage of the total recovery excreted as sulphoxides falls markedly, presumably reflecting the susceptibility of the cytosolic enzyme systems to diurnal variation (R.H.Waring, unpublished data). Similary, it is known that rats produce much more sulphate, from the cytosolic (and mitochondrial) S-oxidation of sulphur-containing amino acids, at night when they are more active /11/.

Observations made on patients before and after undergoing surgery have shown that resection of the lower bowel had little effect on sulphoxidation whereas liver transplantation could bring about an alteration in phenotype (Table 1). These results suggest that the liver is a major site of SCMC sulphoxidation /12/.

TABLE 1

Sulphoxidation Index (S.I.) measured in patients before and after surgery

Diagnosis & Treatment	Sulphoxidation Index Before	After
Lower Bowel Resection		
Crohn's disease	> 80	40
Ulcerative colitis	> 80	> 80
	> 80	> 80
	22	30
	16	20
	12	10
Tumour	2.6	2.5
Liver Transplant		
Primary biliary cirrhosis	> 80	10
	> 80	1.8*
	12	3.7*
	44	18
(first liver rejection)	18	8
α antitrypsin deficiency	4.5	1.9
induced liver damage	> 80	19
cryptogenic cirrhosis	48	4.7*

*Indicates apparent change of phenotype (antimode at S.I. = 6.0)

VII. CLINICAL IMPLICATIONS

It has been reported that the percentage of poor sulphoxidisers appears to be greater in populations of patients with certain chronic disease states. This increase occurs when other obvious variables (such as ethnicity, age, sex, social habits) have been taken into account.

In a population of 65 rheumatoid arthritis patients 60% were reported as P.M.'s /13/ and other studies have found an incidence of 58.6% and 70% /R.H.Waring, unpublished data; 14/. These findings have been confirmed in a prospective study before major drug dosing, eliminating possible effects of concurrent therapy /15/.

An investigation of 44 patients with primary biliary cirrhosis showed a P.M. incidence of 84% /12/. An incidence of 80% was found in patients diagnosed as having true allergic reactions invoked by food or chemical stimuli /16/ and a small study with ulcerative colitis patients showed a similar increase to 70% of P.M.'s over control groups (D.A. Clements, personal communication). Reduced sulphoxidation of SCMC has also been associated with motor neurone disease where 47% of a population produced no sulphoxide metabolites at all (73% were P.M.'s) /17/ and Parkinson's disease where some 35% of the patients showed negligible sulphoxidation capacity (63% were P.M.'s) /18/.

Although there appears to be a surfeit of associations with disease states, so that poor sulphoxidation is in danger of being viewed as a major contributing factor for ill health, it is important to realise that not all chronic disease states show an increased incidence of P.M.'s. In a study of cystic fibrosis patients the incidence of P.M.'s was 28% and the distribution similar to a healthy population (B. Salh, personal communication). Similarly the distribution of sulphoxidation capacities in general hospital patients was little different from a healthy population /5,17/. Illness itself, therefore, is not automatically associated with P.M. status.

The common factors in diseases associated with an increased incidence of P.M.'s are the presence of auto-immune processes and/or tissue destruction. This suggests some failure in the control of endogenous reactions. As SCMC is a substituted cysteine, it may be regarded as a "bridge" linking the metabolism of exogenous and endogenous compounds. A relative reduction of SCMC sulphoxidation may then reflect a more generalised impairment in cysteine and sulphur-containing amino acid metabolism with far reaching consequences including the decreased efficiency of biological sulphation. It is possible that sulphated components are involved in the expression of auto-immunity or that the sulphation of substrates plays a part in the initiation and/or continuation of pathological processes. Such a hypothesis has been discussed for primary biliary cirrhosis where it has been suggested that decreased formation of

steroid sulphate conjugates leads to the increased production of the cholestatic steroid glucuronides /19/. Alternatively, sulphoxidation may be regarded as a detoxication process, both reducing the levels of potentially harmful thiols and removing toxic species of "active" oxygen.

VIII. CONCLUSIONS

The pharmacogenetics of the sulphoxidation of SCMC are of interest both as an academic pursuit and also as a means of indirect evaluation of endogenous pathways. In the long term these studies may lead to an increased understanding of the mechanisms underlying the predisposition to chronic disease states.

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