The activation of the biguanide antimalarial proguanil co-segregates with the mephenytoin oxidation polymorphism – a panel study

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The activation of the antimalarial drug proguanil (PG) to the active metabolite cycloguanil (CG) has been evaluated in a panel of 18 subjects. These subjects had previously been screened and classified as mephenytoin poor (PMm) or extensive metabolisers (EMm) and sparteine poor (PMs) or extensive metabolisers (EMs). Five subjects had the phenotype PMm/EMs, one was PMm/PMs, six subjects were EMm/PMs and six were EMm/EMs. The PG/CG ratio in urine (8 h) was significantly higher in PMm than in EMm ($P = 0.0013$). This study shows that the P450-isozyme involved in the polymorphic oxidation of mephenytoin is of critical importance in the activation of PG to CG and this may explain the large intersubject variability in CG concentrations in man. PMm make up about 3% of Caucasians, but up to about 20% of Orientals. From the present study, it may be anticipated that the antimalarial effect of PG is absent or impaired in this phenotype. The sparteine polymorphism appeared not to influence the activation of PG to CG significantly.

Keywords proguanil mephenytoin polymorphism sparteine

Introduction

Proguanil (PG) is an arylbiguanide which was introduced as an antimalarial agent in the 1940s. This drug is relatively inactive *per se* requiring metabolism to the active dihydrotriazime metabolite, cycloguanil (CG) (Carrington *et al.*, 1951). This metabolic activation is mediated by a cytochrome P450-dependent process (Armstrong, 1973; Armstrong & Smith, 1974). CG is a potent inhibitor of the plasmodial enzyme, dihydrofolate reductase, an enzyme essential for parasite viability.

Recent studies (Helsby *et al.*, 1990b; Ward *et al.*, 1989; Watkins *et al.*, 1987) have identified large inter-subject variability in circulating plasma CG concentrations after the oral administration of PG (200 mg). Population studies (Helsby *et al.*, 1990b; Ward *et al.*, 1989) have used the ratio of PG to CG concentration in urine as an index of this variability. These studies have shown a highly skewed population distribution with respect to this ratio, with a small proportion (3–10%) producing small amounts of CG and, therefore, exhibiting a large urinary ratio.

This variability in the PG/CG ratio has been attributed to differences in the rate and extent of metabolism of PG to CG, and individuals have been classified empirically as extensive (PG/CG < 10) or poor (PG/CG > 10) metabolisers of PG (Helsby *et al.*, 1990b). Potential

sources of such variability could be environmental, pathophysiological or genetic (Alvares *et al.*, 1976; Dollery *et al.*, 1979; Vesell, 1973). Recent interest has focussed on the genetic regulation of cytochrome P450 mixed function oxidases (Alvan *et al.*, 1990). This has resulted from the observations that at least some of these isoenzymes exhibit genetic polymorphism.

Two independent polymorphisms of oxidative drug metabolism have been clearly identified. One affects the 4-hydroxylation of debrisoquine and the *N*-oxidation of sparteine (Eichelbaum *et al.*, 1979; Mahgoub *et al.*, 1977) mediated by P450IID6 (Nebert *et al.*, 1989) and the other affects the 4-hydroxylation of *S*-mephenytoin (Kupfer & Preisig, 1984) mediated by a P450 in the IIC subfamily (Nebert *et al.*, 1989). Family studies in both Caucasian and Japanese subjects have shown that PMm are homozygotes for an autosomal, recessive gene (Drøhse *et al.*, 1989; Kupfer & Preisig, 1984; Ward *et al.*, 1987). Whereas the sparteine/debrisoquine oxidation polymorphism has been shown to cosegregate with the metabolism of several clinically important drugs (Brøsen & Gram, 1989), the clinical significance of the mephenytoin oxidation polymorphism appears to be limited (Wilkinson *et al.*, 1990).

The ability of a drug to inhibit competitively the

oxidation of one of the model compounds and *vice versa* in human liver microsome experiments has been used to implicate the corresponding isoenzyme in the metabolism of the candidate drug. Such studies using PG as a substrate have shown that mephenytoin inhibits competitively the metabolism of PG to CG in human liver microsome preparations, whereas sparteine had no effect on oxidative biguanide activation (Helsby *et al.*, 1990a).

These data suggest a role for the mephenytoin hydroxylase in the activation of PG in man. The present investigation has employed the panel study approach to confirm or refute a role of the mephenytoin and sparteine/debrisoquine oxidation polymorphism in the variable activation of PG to CG.

Methods

Subject selection and phenotyping procedures

The study included 12 men and six women, aged 19 to 41 years. All were found to be healthy on clinical examination, and none had a daily alcohol or drug intake. They were selected from the participants in a population study (Drøhse *et al.*, 1989).

Each subject took one tablet of 100 mg racemic mephenytoin (Mesantoin®, Sandoz) together with one tablet of 100 mg sparteine sulphate (Depasan®, Guilini) and collected urine for the following 12 h. The urine volume was recorded and an aliquot was frozen (-20°C) until analysis.

Mephenytoin phenotyping was carried out according to Wedlund *et al.* (1984) by measurement of the peak areas of (R)- and (S)-mephenytoin in urine by gas chromatography (Sanz *et al.*, 1989) and calculation of the S/R ratio.

Ratios ≥ 0.1 could not be differentiated.

Sparteine, 2- and 5-dehydrosparteine in urine were measured by gas chromatography (Vinks *et al.*, 1982) and a metabolic ratio (MR) was calculated as: sparteine excreted/dehydrosparteines excreted.

For both sparteine and dehydrosparteines, the lower level of assay was about $0.25\ \mu\text{M}$. In samples with undetectable dehydrosparteines, the assay was used for MR calculation.

Concomitant intake of mephenytoin and sparteine and subsequent assay by gas chromatography has been shown not to influence the values of the indices used for phenotyping (Drøhse *et al.*, 1989).

There was no indication of non-compliance since urine volumes and total recoveries of sparteine plus dehydrosparteines were within the expected range (Brøsen *et al.*, 1985) in all subjects.

The subjects were classified as mephenytoin poor metabolisers (PMm: S/R > 0.9) or mephenytoin extensive metabolisers (EMm: S/R < 0.9) and sparteine poor metabolisers (PMs: MR > 20) or sparteine extensive metabolisers (EMs: MR < 20). Five patients were PMm/EMs (nos 1–5), one was PMm/PMs (no 6), six were EMm/PMs (nos 7–12) and six were EMm/EMs (nos 13–18) (see Table 1).

Subject 12, who had an intermediate S/R ratio was retested with fractional urine collections over 0–12, 12–

24 and 24–36 h. The corresponding S/R ratios were 0.85, 0.24 and < 0.1 , confirming the EMm phenotype of this subject (Sanz *et al.*, 1989).

Each subject received PG 200 mg orally (Paludrine®, ICI) late in the evening (23.00 h), and urine was collected for 8 h. Urine volume and pH were recorded and an aliquot was frozen (-20°C) until analysis. PG and CG concentrations were measured by a selective h.p.l.c. technique as described previously (Taylor *et al.*, 1987). In one subject (no 6), CG was undetectable. In this case the assay limit ($1\ \mu\text{g l}^{-1}$) was used for calculation of the PG/CG ratio.

Statistical comparisons were made using the Mann-Whitney U-test (PG/CG ratio in different phenotype groups) and the Spearman rank correlation (PG/CG ratio vs S/R ratio or MR).

The study was approved by the Regional Ethics Committee of Funen County (Odense) and each subject agreed to participate in the study on the basis of written and verbal information.

Results

Mephenytoin poor metabolisers (PMm) had significantly higher PG/CG ratios than mephenytoin extensive metabolisers (EMm), whether analysed in all patients or only in EMs (Table 1). Subject 12, who had an intermediate S/R ratio (0.63) had a high PG/CG ratio.

When data from all patients were included, there was no difference in the PG/CG ratio between PMs and EMs and there was no statistically significant relationship between MR and PG/CG ratios. On excluding the mephenytoin poor metabolisers (PMm), a difference of borderline significance was found ($P = 0.093$) with higher PG/CG ratios in EMm/PMs compared with EMm/EMs (Table 1).

Discussion

Our results confirm the hypothesis, based on *in vitro* human liver interaction studies (Helsby *et al.*, 1990a), that the oxidative activation of PG to CG cosegregates with the S-mephenytoin oxidation polymorphism. Thus, this polymorphism seems to be the major source of the pronounced interindividual variability in CG formation (Helsby *et al.*, 1990b; Ward *et al.*, 1989).

Subject 12, who had an intermediate S/R ratio, exhibited a PG/CG classifying him phenotypically as a poor metaboliser of PG (PG/CG > 10). This volunteer also participated in a recent study showing cosegregation between imipramine demethylation and S-mephenytoin oxidation (Skjelbo *et al.*, data to be published), and again, he showed intermediate values with respect to imipramine demethylation clearance. Subject 6, a mephenytoin and sparteine poor metaboliser (PMm/PMs), was the only subject with undetectable CG concentrations. This, together with the borderline significant difference between EMm/EMs and EMm/PMs, suggests a minor involvement of the P450IID6 in the PG to CG biotransformation. Confirmation would require a study

Table 1 Characteristics of subjects studied in relation to the urinary ratio of proguanil to cycloguanil

Subject	Age (years)	Sex	Body weight (kg)	Phenotype ^{a)}	Mephenytoin S/R ^{b)}	Sparteine MR ^{b)}	PG/CG ^{b)} ratio
1	19	M	75	PMm/EMs	1.06	0.21	10.1
2	28	M	72	PMm/EMs	1.27	0.24	9.69
3	22	M	72	PMm/EMs	1.12	0.41	48.2
4	21	M	74	PMm/EMs	1.21	0.70	20.4
5	30	F	55	PMm/EMs	1.01	0.27	11.1
6	24	F	58	PMm/PMs	1.12	47	48.0 ^{c)}
7	26	F	70	EMm/PMs	≤0.1	276	0.98
8	27	M	76	EMm/PMs	0.11	207	2.37
9	24	M	95	EMm/PMs	0.18	143	3.40
10	30	M	60	EMm/PMs	0.13	85	4.52
11	41	M	83	EMm/PMs	≤0.1	196	1.31
12	38	M	85	EMm/PMs	0.63	140	24.0
13	39	F	62	EMm/EMs	≤0.1	0.92	0.04
14	34	F	56	EMm/EMs	≤0.1	0.71	1.46
15	31	F	51	EMm/EMs	≤0.1	0.81	1.08
16	36	M	75	EMm/EMs	≤0.1	0.23	0.07
17	28	M	71	EMm/EMs	≤0.1	1.35	3.59
18	35	M	70	EMm/EMs	≤0.1	0.29	0.16

a: EMm: S/R < 0.9; PMm: S/R > 0.9; EMs: MR < 20; PMs: MR > 20.

b: for definitions, see text.

c: CG below detection limit (PG/CG is the minimum value possible based on the concentration of PG in the urine and the limit of assay of CG).

Statistics of group differences, Mann-Whitney U-test:

PMm (*n* = 6) vs EMm (*n* = 12) *P* = 0.0013

PMm/EMs (*n* = 5) vs EMm/EMs (*n* = 6) *P* = 0.0043

PMs (*n* = 7) vs EMs (*n* = 11) *P* = 0.724

EMm/PMs (*n* = 6) vs EMm/EMs (*n* = 6) *P* = 0.093

with larger panels of EMs and PMs who are uniform with respect to mephenytoin metabolism.

The potential clinical consequences of polymorphic PG metabolism are two-fold. Firstly, a relative inability to form the active metabolite may result in prophylactic failures with this drug and, secondly, sub-inhibitory concentrations of CG may promote the development of parasite resistance. However, the issue is more complicated. In addition to activity against the erythrocytic stages of malaria, CG is thought to exert its primary antimalarial activity at the exo-erythrocytic stages within the liver. Although the plasma CG concentrations achieved in PMm are below the erythrocytic IC₅₀ for many isolates, it is unclear if hepatic concentrations of metabolite are also sub-inhibitory.

The importance of polymorphic biguanide activation will be dependent on the incidence of the PMm phenotype within populations. This incidence is 2–3% in Caucasian populations and as high as 18–20% in Japanese subjects

(Nakamura *et al.*, 1985; Jurima *et al.*, 1985). One study undertaken in Kenya has shown that over 25% of this population exhibited a PG/CG > 10 (Watkins, personal communication). This is of added importance in view of the fact that PG has been suggested for the prophylactic treatment of young children and pregnant women in Kenya. There are, however, no published data on the PMm frequency in Kenya.

In conclusion, using the panel study approach, we can confirm the suggestion that the metabolism of PG to its active metabolite CG cosegregates with the ring-hydroxylation of S-mephenytoin. Accordingly, the metabolism of PG may exhibit a genetic polymorphism in man which could have clinical consequences.

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