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Hepatic Cytochrome P450 CYP2C Activity in Psoriasis: Studies Using Proguanil as a Probe Compound

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Retinol and proguanil are metabolised by the same family of hepatic cytochrome P450, i.e. CYP2C. We used proguanil as a probe to study CYP2C activity, and by implication retinol metabolism, in psoriasis. In vitro studies showed that retinol competitively inhibited the hepatic metabolism of proguanil to cycloguanil. Proguanil metabolism was assessed in 82 patients with chronic plaque psoriasis. Following proguanil orally (200 mg), urine was analysed for proguanil and cycloguanil. A proguanil to cycloguanil ratio <1 signified extensive metabolism and a ratio >10 poor metabolism. A wider range of ratios was observed in psoriasis than previously reported for normal subjects. The proguanil to cycloguanil ratio was assessed in 10 cases each of known severe and mild psoriasis. Low CYP2C activity was associated with severe psoriasis, poor metaboliser status occurring in 50% of the severe group, but in none of the mild cases, $p<0.01$. These findings may indicate differences in retinoid metabolism in psoriasis. *Key words: retinol; metaboliser status; disease severity*.

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Psoriasis, which affects approximately 3% of the Caucasian population (1), is characterised by dermal inflammation, epidermal hyperproliferation and changes in keratinisation. Vitamin A and its metabolites, the retinoids, are intimately involved in the normal control of epidermal differentiation and keratinisation (2). The importance of retinoids in psoriasis is highlighted by the effective treatment of some patients with synthetic retinoids, such as etretinate. However, there is considerable inter-individual variation in the response to these therapeutic agents (3).

Retinoids undergo multiple routes of metabolism, catalysed both by cytosolic and microsomal enzymes (4, 5). The hydroxylation of retinol (vitamin A) to retinoic acid is a cytochrome P450-mediated process (6, 7). In human liver microsomes the major isozyme involved appears to be CYP2C8 (8), although other isozymes may also play a role since CYP2C8 does not account for all the NADPH-dependent production of 4-hydroxyretinol (8). Within the CYP2C family, CYP2C8 shows considerable homology with CYP2C19, which exhibits a genetic polymorphism.

Proguanil (PG) is an antimalarial prodrug, which requires cytochrome P450-mediated biotransformation to the active metabolite cycloguanil (CG) (9). The metabolism of PG to CG is partly mediated by CYP3A isoforms (10) and is also known to co-segregate with the CYP2C19 genetic polymorphism (11, 12). Based on the latter observation PG cyclisation has been used as a probe for the CYP2C19 genetic polymorphism. PG is a relatively safe antimalarial prophylactic agent with few serious side-effects. Interestingly, however, long-term prophylaxis is associated with complications such as palmoplantar keratoderma, mouth ulceration and hair loss (13). These side-effects are similar to those observed in hypervitaminosis A (14). It has also been observed that chronic PG administration can exacerbate psoriasis (15). We therefore postulated that PG and vitamin A are metabolised by the same isozyme(s) of cytochrome P450.

The objectives of our studies were, firstly, to examine the distribution of CYP2C19 metaboliser status in a psoriatic population and, secondly, to investigate for a relationship between CYP2C activity, and by implication retinol metabolism, and severity of psoriasis. We initially carried out a series of investigations using human liver microsomes to determine whether CYP2C19 has a physiological role in the metabolism of vitamin A. Next we determined the urinary PG/CG ratio as an indication of CYP2C19 activity in a psoriatic population ($n=82$). This was compared with our previously reported findings in a normal population (16). Finally, we examined for a correlation between PG metaboliser phenotype (CYP2C19 activity) and disease severity.

MATERIALS AND METHODS

Ethical approval for these studies was obtained from the Royal Liverpool University Hospital ethics committee.

In vitro metabolism studies

Samples of histologically normal livers were obtained from cadaveric renal transplant donors ($n=3$). Microsomes were prepared by standard methods and incubations were carried out as described previously (12). The NADPH-dependent metabolism of PG to CG was determined in the absence and presence of retinol (vitamin A: 10.5 μM).

Population study

Eighty-two patients (aged 18–80 years) with chronic plaque psoriasis attending the Dermatology Unit, Royal Liverpool University Hospital, were recruited into this study. All were Caucasian and were receiving only topical treatment (calcipotriol, dithranol or coal tar) for their psoriasis. Each patient received a single oral dose of PG (200 mg, p.o.) and urine was collected for 8 h post dose. The urine volume was measured and an aliquot stored at -20°C until analysis by HPLC (16).

Following the determination of PG/CG ratios in the psoriatic population, two groups of patients with PG/CG ratios <1 and PG/CG ratios >10 were assessed retrospectively from the case notes. The limits <1 and >10 were selected since they reflected the extremes of the population, and the linear region of the probit plot analysis for the data was between 1 and 10. Extensive area involvement, frequent relapse history, hospital admissions, and previous use of systemic therapy were taken as evidence of severe psoriasis.

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Panel study

A prospective study was performed of a further 20 patients (aged 20–60 years), who had not taken part in the population study. These were recruited under strict criteria of disease severity. Subjects ($n=10$) with a mild form of the disease (<2% body surface area involvement) and patients ($n=10$) with a severe form of the disease (>20% body area) were assessed for their ability to metabolise PG to CG, as determined in the larger population study. Data was also collected on family history of psoriasis and age at disease onset.

Data and statistical analysis

The V_{max} and K_m values for the cyclisation of PG by human liver microsomes in the presence and absence of retinol were determined by non-weighted linear regression analysis from double reciprocal plots.

The amount (mg) of PG and CG in the urine of each patient was determined and expressed as the ratio of PG/CG. Statistical analysis of phenotype incidences was determined by the χ^2 test. Determination of non-normality was performed by the Shapiro-Wilks test. Data were analysed for bimodality by Dr A Rostami, Department of Medicine, University of Sheffield, using kernel density analysis. Significance was accepted when $p<0.05$. Difference between the severe and mild psoriatics was tested by the Mann Whitney U test.

RESULTS

In vitro hepatic microsomal studies

To investigate whether there was an interaction between PG and retinol, the in vitro metabolism of PG in the presence of retinol was determined in microsomes from three different human livers. Retinol was seen to competitively inhibit PG bioactivation to CG. Although there was some variability betweeen livers, co-incubation with retinol produced an increase in the dissociation constant (K_m), i.e. the substrate concentration at half maximum velocity of the reaction, without any change in the maximum rate (V_{max}). The K_m in the hepatic microsomes from three sources increased from 40, 59 and 148 μM in control samples to 166, 166 and 242 μM, respectively, in the presence of retinol.

CYP2C19 activity in a psoriatic population

The frequency distribution of log PG/CG ratios in the psoriatic population ($n=82$) is shown in Fig. 1. The data display a non-normal distribution ($p<0.0001$), with 91.5% of psoriatic individuals having ratios less than 10, and 8.5% of psoriatic patients exhibiting PG/CG ratios greater than 10. The range of PG/CG ratios in the psoriatic population was wide (0.02–90) and 26% of psoriatic patients had PG/CG ratios less than 1. The data were subjected to kernel density analysis and this indicated that the psoriatic population did not display any clear bimodality or trimodality. It is concluded that the psoriatic population is either a skewed unimodal distribution or has more than three modes.

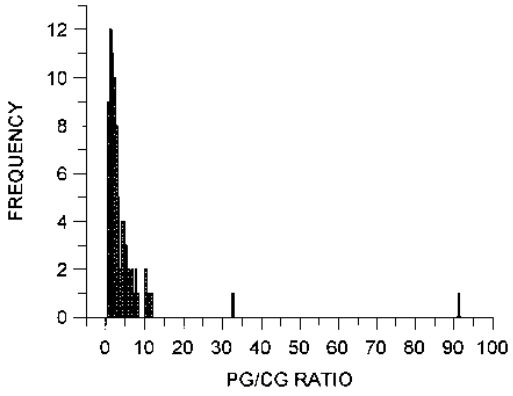

Fig. 1. The percentage frequency distribution profile of log PG/CG ratios in psoriatic patients.

To investigate whether metaboliser phenotype might be associated with severity of psoriasis, details of disease severity were obtained retrospectively from the case notes in 19 very extensive metabolisers (PG/CG<1) and 6 poor metabolisers (PG/CG>10). The case notes did not always contain a record of the % surface area of psoriasis involvement. Therefore, a record of extensive involvement, frequent relapse history, previous admissions or previous use of systemic treatment were taken as evidence of severe involvement. It was found that 5/6 poor metaboliser subjects but only 3/19 very extensive metaboliser subjects had severe psoriasis ($p<0.005$).

CYP2C19 activity in patients known to suffer mild or severe psoriasis

To assess further the influence of PG/CG metaboliser status on disease severity, a more detailed prospective study was performed, in which a panel of 20 patients were preselected on the basis of either mild (<2% body area, $n=10$) or severe (>20% body area, $n=10$) psoriasis. These subjects were then phenotyped for CYP2C19 activity. It was found that there were differences in the range of PG/CG ratios between the mild and severe groups. Patients with severe psoriasis displayed higher PG/CG ratios. The severe group had a mean PG/CG ratio of 11 (SD 7) and the mild group 4.8 (SD 2) ($p<0.05$), and the variance of the data was 10-fold higher in the severe compared with the mild psoriatic patients (49.2 compared with 4.5). The incidence of PG/CG ratios >10 was higher in the severe compared with the mild group (50% compared with 0%: $p<0.01$). There was no relationship between PG/CG phenotype and family history of psoriasis or age at onset of the disease.

DISCUSSION

Our studies have shown differences in the activity of isozyme CYP2C19 of hepatic cytochrome P450, both between normal and psoriatic populations, and between subjects with mild and severe psoriasis. Low activity of hepatic CYP2C19 was seen to be associated with severe psoriasis. In vitro studies in human liver microsomes demonstrated the ability of retinol to competitively inhibit CYP2C19-mediated metabolism of PG to CG, suggesting that both compounds are metabolised, at least in part, by the same isozyme. Hence we speculate that the observed differences in CYP2C19 activity in psoriasis might also reflect differences in retinol metabolism.

The psoriatic population exhibited a wider range of PG/CG ratios than we have previously reported for a normal population (16). In normal subjects a bimodal distribution was observed with 3% of the population with PG/CG ratios >10. In the psoriatic population, there was loss of bimodality and a significant increase in the number of individuals with a very

extensive metaboliser phenotype (PG/CG < 1). However, on retrospective review of psoriasis severity the patients with high CYP2C19 activity were found to have mild psoriasis, whereas the poor metaboliser phenotype was associated with severe psoriasis. This pattern was also observed when patients were selected prospectively on the basis of disease severity, where the incidence of the poor metaboliser phenotype (PG/CG > 10) was again significantly increased in the patients with the most severe form of the disease. We suggest that poor retinoid metabolism may be a contributing factor in severe psoriasis. However, further research is needed to explore the underlying mechanism and clinical relevance of the association. The present study has not excluded the possibility that poor ability to metabolise PG could in some way be secondary to the psoriatic process, perhaps due to the histologically observed association of fatty infiltration of the liver with severe psoriasis (17).

Our findings give support to previous studies which also implicate a role for the CYP2C family of isozymes in the metabolism of vitamin A. Leo et al. (8) demonstrated that retinol 4-hydroxylation is mediated by purified CYP2C8. Interaction between vitamin A and isozymes of the CYP2C family was also demonstrated by the studies of Martini et al. (18), where a vitamin A-deficient diet resulted in a pretranslational down-regulation of CYP2C11 in rats. This down-regulation was prevented by dietary retinoic acid supplementation. The expression of some CYP2C isozymes may therefore be partly regulated by circulating retinoid concentrations. Consequently, if CYP2C19 is intimately involved in the metabolism of the functionally inactive parent compound vitamin A (retinol) to the hormone retinoic acid, both a catalytic deficiency or an over-expression of the enzyme may be expected to result in skin abnormalities, since both inadequate or excessive retinoic acid causes serious impairment of diverse cell types in numerous organs. The activity of hepatic P450 isozymes, such as CYP2C19, could also determine inter-subject variation in response to retinoid treatment. It might be illuminating, therefore, to examine CYP2C19 activity in relation to the responsiveness of psoriasis to retinoid therapy.

In summary, we have shown evidence of an association between low hepatic CYP2C19 activity and severe psoriasis. The data presented have supported a possible physiological role for CYP2C19 with respect to retinoid metabolism; this requires confirmation and further exploration.

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