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The therapeutic response to D-penicillamine in rheumatoid arthritis: influence of glutathione S-transferase polymorphisms

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Abstract

Objectives. To investigate whether the therapeutic response of rheumatoid arthritis (RA) patients to D-penicillamine is associated with polymorphisms in genes of the glutathione S-transferase (GST) supergene family.

Methods. Disease activity in 81 patients with RA treated with D-penicillamine monotherapy was assessed using the Stoke Index, a validated index of disease activity, prior to treatment and at 6 months. GST typing was performed using a polymerase chain reaction-based approach and a logistic regression model was used to investigate any possible association between the therapeutic response to D-penicillamine and the GST genotype.

Results. A poor therapeutic response was associated with the GSTM1 null genotype [odds ratio (OR) 3.94], and in particular with the *GSTM1*0/GSTM3*A* haplotype (OR 7.63).

Conclusions. Our results suggest that GST polymorphisms may influence the response to D-penicillamine in RA, and that patients in possession of the *GSTM1*0/GSTM3*A* haplotype are significantly less likely to show a beneficial response to the drug.

KEY WORDS: D-Pencillamine, Rheumatoid arthritis, Therapeutic response, Glutathione S-transferase, Polymorphism.

Early and reliable identification of rheumatoid arthritis (RA) patients who are at risk of poor long-term outcome would be clinically useful if it allowed more aggressive treatment to be appropriately targeted. There has been considerable interest, therefore, in the identification of genetic markers of clinical outcome. Much of this work has been focused on genes of the HLA system. The HLA-DR genotype, and specifically the presence of the 'RA shared epitope', a conserved amino acid sequence (QKRAA or QRRAA) in positions 70–74 of the DRB1 molecule, has been related to disease severity [1]. It is suggested that resistance to drug therapy is a possible mechanism for this effect [2], although the influence of HLA status on the response to drugs in RA remains unclear. Thus, Ten Wolde *et al.* [3] reported that HLA typing is not helpful in predicting the therapeutic response to parenteral gold therapy. This is in contrast to the reported association of gold toxicity with HLA-DR3 [4]. There are little data on other genes that might influence the therapeutic response in RA.

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Although a wide range of drugs, with varying mechanisms of action, are available for use in the treatment of RA, selection is often problematic because of adverse reactions or lack of response. Accordingly, drug treatment in RA is often pragmatic and it is not unusual for an individual patient to be prescribed a range of different drugs during the course of the disease. The identification of markers that indicate whether or not a particular drug is likely to be beneficial would therefore have important consequences for treatment.

The enzymes of the cytochrome P450 (CYP) and glutathione S-transferase (GST) supergene families utilize a wide range of drugs and chemicals as substrates, suggesting that they may mediate individual responses to drug therapy. There are little data on the influence of these enzyme families on the metabolism of drugs used to treat RA, although a recent study demonstrated that the activity of the glucocorticoid-inducible CYP3A4 in RA patients was not altered by various anti-rheumatic agents and substrates of this isoenzyme [5]. GST enzymes catalyse the detoxification of a wide range of electrophiles by catalysing their conjugation with reduced glutathione. They are also believed to play a critical role in the detoxification of various endogenous

chemicals, including products of oxidant stress such as lipid and DNA hydroperoxides [6]. Polymorphisms have been identified in genes of the mu (GSTM1, GSTM3), theta (GSTT1) and pi (GSTP1) families [7], and there is evidence that some allelic variants are associated with differences in detoxification efficiency. In several cancers, resistance to chemotherapy has been associated with increased GST activity in tumour cells [8].

Generation of reactive oxygen species (ROS) is a marked feature of the inflammatory response in RA, and we have suggested that the ability to metabolize products of ROS may influence long-term outcome. Thus, our preliminary data indicate that polymorphisms in GST genes may have an influence on incidence and disease outcome in RA [9]. We propose that the ability to detoxify products of oxidant stress may also influence the response to certain drug therapies. In the present study, we examined the response to D-penicillamine, which has been suggested to act as an antioxidant by inhibiting lipid peroxidation [10], as well as demonstrating hydroxyl radical scavenging activity *in vitro* [11]. It has also been shown to increase levels of erythrocyte glutathione in RA patients [12]. We speculate that in RA patients with certain GST polymorphisms (e.g. GSTM1 null, GSTT1 null, GSTM3AA), there is a reduced capacity to detoxify the products of oxidative stress produced by the inflammatory process, and that increased oxidation of D-penicillamine occurs which renders it less effective. We have tested this view by investigating the association between GST polymorphisms and response to treatment as assessed by the Stoke Index [13].

Patients and methods

A study group of 81 patients (38 male, 43 female) meeting the 1987 ACR diagnostic criteria [14] for RA were selected. The mean age was 52.6 yr (S.D. 9.8) and the mean disease duration 6.1 yr (S.D. 5.8). Of this group, 48 patients (59.2%) were rheumatoid factor positive and 13 patients (16%) had rheumatoid nodules. All patients were unrelated Northern European Caucasians from North Staffordshire started on D-penicillamine monotherapy (non-steroidal anti-inflammatory drugs permitted) on clinical grounds [$\geqslant 6$ painful joints and erythrocyte sedimentation rate (ESR) >30 mm/h or C-reactive protein (CRP) >20 mg/l]. All patients were assessed prior to therapy and 6 months after starting D-penicillamine according to our standard departmental clinical protocol. Treatment commenced with 250 mg daily, increasing at 4 week intervals by increments of 125 mg until either response occurred or the maximum tolerated dose was achieved (maximum 750 mg daily). All patients were advised to take the D-penicillamine as a single morning dose prior to the intake of food and there was no concomitant oral iron therapy. Patients were reviewed monthly in our drug monitor clinics in order to identify any problems of toxicity and monitor compliance with therapy. The 81 patients studied represent those completing 6 months of D-penicillamine therapy. The original study group was 86 patients, of whom four (4.6%) dropped out because of side-effects and one (1.2%) was non-compliant. Patients were assessed using the Stoke Index, a validated composite algorithm designed to give a global measure of disease activity in RA, which is sensitive to response to drug treatment [13, 15]. The Stoke Index grades disease activity on the basis of the Ritchie articular index, proximal interphangeal joint synovitis (PIP) score, duration of morning stiffness, ESR and CRP. The algorithm consists of a 17-point scale with 1–3 representing minimal disease activity, 4–7 mild, 8–11 moderate and 12–17 severe.

In order to determine whether the D-penicillamine-treated patients represented a typical group of RA patients, we also compared the GST frequencies of the D-penicillamine-treated patients with a much larger group of RA patients untreated with this drug ($n = 194$), obtained from a study investigating the association between GST polymorphisms and disease outcome [16]. There were no significant differences between the two groups for any of the GST genotypes (data not shown).

Genotype data in the D-penicillamine-treated RA patients were also compared with those in 577 Northern European Caucasians resident in North Staffordshire. This group comprised 277 men and 300 women with a mean age of 70 yr. Patients with inflammatory pathologies such as ulcerative colitis, diabetes or asthma were excluded, although 30% of these controls suffered diseases including varicose veins, hernias, haemorrhoids, mild iron deficiency anaemia, mild hyperlipidaemia and benign ovarian cysts. The remainder suffered tension headaches (25%), benign skin papillomas (20%), benign breast lumps (5%), or cerebrovascular accidents (20%).

Outcome measures

The therapeutic response at 6 months was defined using the Stoke Index. Non-responders to therapy were defined as those patients who following 6 months treatment with D-penicillamine achieved either no change in their Stoke Index category or entered a worse category.

Genotyping

GST genotypes were identified in leucocyte DNA from whole blood collected in EDTA. The GSTM1 null, A, B and AB genotypes were identified using an amplification refractory mutation system (ARMS)-based polymerase chain reaction (PCR) approach with primer sets to intron 6/exon 7 and exon 4/exon 5. The assay identifies *GSTM1*A*/*GSTM1*B* heterozygotes and subjects with the GSTM1 A and GSTM1 B phenotypes. It does not distinguish the *GSTM1*0*/*GSTM1*A* and *GSTM1*A*/*GSTM1*A* genotypes or the equivalent GSTM1 B genotypes [17, 18]. The GSTM3 AA, AB and BB genotypes were identified by amplifying the exon 6/7 regions of *GSTM3*A* and *GSTM3*B*, and differentiating *GSTM3*A* from *GSTM3*B* by digestion

with *Mnl*1 to identify the three-base deletion in intron 6 of *GSTM3*B* [19]. The GSTP1 AA, AB and BB genotypes were identified in leucocyte DNA after PCR amplification using primers to exon 5 as described by Harries *et al.* [20]. The resulting 176 bp fragment was digested with *Alw*261 to identify the A–G transition as position 1578 in *GSTP1*B*. *GSTP1*A* was refractory to digestion whilst *GSTP1*B* gave fragments of 91 and 85 bp. The GSTT1 null and GSTT1 A expressing subjects were also identified using PCR [21].

Statistical analysis

Logistic regression was used so that any effect of the genotype on response could be corrected for age, sex, disease duration and initial Stoke Index. There was no significant difference between the responders and the non-responders for these factors individually, but they were included in the analysis in the case of a compound effect. The odds ratio (OR) was calculated from the exponential function of the regression coefficient as described by Altman [22]. Estimates of the parameters associated with the genotype are expressed in terms of OR and the associated 95% confidence interval (CI).

Results

Eighteen of the 81 patients did not respond to D-penicillamine at 6 months according to the definition given above. The characteristics of the non-responders and the responders are summarized in Table 1. There was no significant difference in age, disease duration, rheumatoid factor positivity or Stoke Index prior to treatment between the two groups. The GST genotype frequencies in the responders and non-responders are shown in Table 2. The frequencies in all the D-penicillamine-treated patients are also reported and compared to a control group of non-RA patients resident in North Staffordshire ($n = 577$). There were no significant differences between GST genotype frequencies in the D-penicillamine-treated RA group and the control non-RA group.

Using logistic regression analysis, we found that in D-penicillamine-treated patients the frequency of GSTM1 null was significantly higher in the non-responders than the responders (72.2% *vs* 47.6%: OR 3.94, 95% CI 1.07–14.45, $P = 0.039$). The frequency of the *GSTM1*0/GSTM3*A* haplotype was also significantly higher (OR 7.63, 95% CI 1.84–31.72, $P = 0.0052$) in the non-responders (72.2%) than responders (35.6%), although no significant effect of GSTM3 AA alone was seen. Furthermore, all of the non-responders with the GSTM1 null genotype were GSTM3 AA positive, whilst only 70% of the GSTM1 null responders possessed the GSTM3 AA genotype. There was no significant difference between the two groups studied in the frequency of other GST genotypes. No significant correlation between GST genotype and rheumatoid factor positivity was found.

TABLE 1. Characteristics of responders and non-responders to D-penicillamine

	Non-responders $n = 18$ (M = 7 F = 11)	Responders $n = 63$ (M = 31 F = 32)
Mean age(yr) (S.D.)	52.3 (10.5)	52.9 (9.0)
Mean disease duration (yr) (S.D.)	5.6 (4.2)	6.3 (6.2)
Mean age of onset (S.D.)	48.4 (11.8)	47.2 (9.8)
Mean initial Stoke Index (S.D.)	9.5 (2.9)	10.4 (3.4)
Mean Stoke Index at 6 months (S.D.)	8.8 (3.1)	4.3 (2.5)

Discussion

The data presented suggest that RA patients with the GSTM1 null genotype are significantly more likely than patients homo- or heterozygous for the *GSTM1*A* or *GSTM1*B* alleles to respond poorly to D-penicillamine during the first 6 months of treatment. Moreover, possession of the *GSTM1*0/GSTM3*A* haplotype further increases the likelihood of non-response compared to patients without this haplotype. The overall GST genotype distribution in the D-penicillamine-treated patients appears to be representative of the RA patient population in North Staffordshire since there were no significant differences in the genotype frequencies of these patients and a larger group of RA patients not treated with the drug.

A logistic regression model was used to correct for the effect of possible confounding factors such as age, gender and disease duration. The analysis does not exclude the possibility that the effect is mediated through another gene in linkage disequilibrium with GSTM1 and/or GSTM3, or perhaps is due to an unrelated gene encoding a drug-metabolizing enzyme which can be modulated in association with the GSTM1 null genotype. For example, the GSTM1 null genotype has been shown to be associated with high inducibility of the cytochrome P450 isoenzyme CYP1A1 [23].

In this study, we have no information on the activity of other enzyme systems, although the association of GSTM1 null with a poor response appears to be linked with the presence of the GSTM3 AA genotype. GSTM3 AA alone was not associated with response. A similar association between these genotypes was found in a study of patients with multiple basal cell carcinoma, where GSTM3 AA alone was not associated with increased risk of multiple tumours, but conferred increased risk in combination with GSTM1 null [24]. Co-ordinated expression of the mu class (i.e. GSTM1, M2, M3, M4, M5) gene cluster on chromosome 1 has also been shown in the lung where homozygotes for *GSTM1*0* express less GSTM3 than subjects with other GSTM1 genotypes [25]. Although the mechanism for this is unclear, it may be related to the finding that *GSTM3*B* is in linkage disequilibrium with *GSTM1*A* and contains a recognition motif for the YY1 transcription factor [26], which influences the expression of many genes. The YY1 recognition motif is absent in

TABLE 2. Frequency distribution of GST genotypes in D-penicillamine-treated RA patients compared with non-RA controls

Genotype	Non-responders *n* (%)	Responders *n* (%)	All patients *n* (%)	Normal controls *n* (%)
GSTM1 null	13 (72.2)	30 (47.6)	43 (53.1)	345 (58.4)
GSTM1A	3 (16.7)	24 (38)	27 (33.3)	152 (25.7)
GSTM1B	2 (11.1)	8 (12.7)	10 (12.3)	72 (12.2)
GSTM1 AB	0 (0)	1 (1.6)	1 (1.2)	22 (3.7)
GSTM3 AA	16 (88.8)	41 (73.2)	57 (77.0)	221 (74.9)
GSTM3 AB	2 (11.1)	14 (25)	16 (21.6)	59 (20)
GSTM3 BB	0 (0)	1 (1.8)	1 (1.4)	15 (5.1)
GSTP1 AA	4 (28.6)	18 (36)	22 (34.4)	136 (45.8)
GSTP1 AB	9 (64.3)	25 (50)	34 (53.1)	118 (39.7)
GSTP1 BB	1 (7.1)	7 (14)	8 (12.5)	43 (14.5)
GSTT1 null	5 (27.7)	14 (22.2)	19 (23.5)	105 (18.9)
GSTT1 A	13 (72.2)	49 (77.8)	62 (76.5)	451 (81.1)
GSTM1*0/GSTM3*A	13 (72.2)	21/59 (35.6)	34/77 (44.2)	

*GSTM3*A*, suggesting that those with either a *GSTM1*0*/*GSTM3*A* or *GSTM1*B*/*GSTM3*A* haplotype will express less GSTM3 than those with the *GSTM1*A*/*GSTM3*B* haplotype. Thus, it has been suggested that assessment of the contribution of GSTM1 genotypes as susceptibility markers for disease risk needs to take account of interactions with GSTM3 [18]. The results of this study now indicate that interactions between these mu class genes may also be important in determining the response to D-penicillamine.

The mechanism for this effect is unknown, although we have speculated that it is related to the apparent importance of the mu class GST in the detoxification of lipid hydroperoxides, alkenals, DNA hydroperoxides and other products of oxidative stress. Thus, RA patients lacking the GSTM1 enzyme and also possessing lower levels of GSTM3 will have a reduced capacity to deal with these potentially cytotoxic chemicals. Consequently, since D-penicillamine may act as an antioxidant under some circumstances [10, 11, 27], it is possible that higher levels of ROS-derived products in patients with the *GSTM1*0*/*GSTM3*A* haplotype result in increased oxidation of the drug and render it less effective. A further factor may be the effect of intracellular redox balance on the action of D-penicillamine. Synovial fluid T cells have reduced levels of intracellular glutathione, prompting the suggestion that the hyporesponsiveness of these cells results from an altered redox state. Thus, agents that replenish glutathione, such as *N*-acetylcysteine, enhance antigen-specific and mitogen-induced proliferative responses [28].

It is unknown whether the GSTs mediate these responses, although it is possible that the antioxidant capacity of glutathione-deficient synovial fluid T cells is further reduced in RA patients who are homozygous for *GSTM1*0*, leading to greater hyporesponsiveness. The inter-relationship between intracellular glutathione concentration and GST genotypes is not elucidated, but is suggested by unpublished studies from our laboratory showing an association between responsiveness to chemotherapeutic agents in epithelial ovarian cancer and GSTM1 genotypes. Thus, in women with this cancer, GSTM1 null was associated with a failure to respond to platinum-based therapy and consequently significantly poorer outcome. Resistance to platinum-based treatment has been closely associated with cellular glutathione levels, although it is not known whether our observations reflect an influence of GST allelic variants on the expression of enzymes involved with glutathione synthesis [29, 30].

Although these mechanisms are purely speculative at present, the influence of GST polymorphisms in RA appear to be important in terms of disease outcome and the response to D-penicillamine. At present, we do not feel that there is sufficient evidence to recommend pre-treatment genotyping of RA patients in routine clinical practice. More work is required to confirm these finding and to determine whether the therapeutic response to other disease-modifying anti-rheumatic drugs relates to the GST genotype.

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