PostScript

MATTERS ARISING

Will pharmacogenetics allow better prediction of methotrexate toxicity and efficacy in patients with RA?

We read with interest the paper by Ranganathan *et al* proposing that pharmacogenetics may be a useful tool to help predict methotrexate (MTX) toxicity and efficacy in rheumatoid arthritis (RA).[1]

One aspect they highlight is the potential role of drug efflux mechanisms in contributing to the lack of response to MTX in some patients. It is important to note that although they discuss the drug efflux transporter P-glycoprotein (P-gp) as being of interest, the paper they cite in support of this view actually reports an experiment in which MTX resistance was mediated by a different drug transporter, multidrug resistance protein 1 (MRP1).[2] A range of efflux transporters have been described, including P-gp, MRP, and breast cancer resistance protein (BCRP). Different drugs appear to be substrates for different efflux transporters.[3] The drug transporter that mediates MTX resistance remains somewhat controversial.

Llorente *et al* studied 16 patients with RA and found higher P-glycoprotein (P-gp) levels in patients who were defined as being refractory to disease modifying drug treatment than in treatment responders.[4] Similarly, Norris *et al* demonstrated increased P-gp expression in leukaemic cell lines resistant to methotrexate.[5] In contrast, a study using mdr1 transgenic mice (which overexpress P-gp) showed they remain susceptible to methotrexate.[6] Others have suggested that MTX may only become a substrate for P-gp when it enters cells by passive diffusion.[7]

To examine the effect of P-gp expression on MTX response we recently studied 20 patients with RA who were taking parenteral MTX at a stable dose for at least eight weeks. We compared P-gp expression on peripheral blood lymphocytes (PBLs) of patients with RA with those of 10 healthy controls.[8] The patients had established RA, with a mean (SD) age and disease duration of 57.7 (9.7) and 15.9 (12.9) years respectively. Eighteen (90%) were seropositive, and 14 (70%) had been treated with ⩾3 drugs which had failed. The median (range) MTX dose and disease activity score (DAS)28 at study entry were 17.5 mg (range 7.5–25) weekly and 4.5 (range 1.8–6.7) respectively. PBLs were separated by gradient centrifugation. P-gp expression was measured using a monoclonal antibody directed to an external epitope of P-gp (UIC2). Samples were fixed and analysed by flow cytometry. The percentage positive P-gp cells were calculated using Cellquest software. No significant difference was seen in P-gp expression between patients with RA and healthy controls. Within the RA group, response to MTX (as measured by the DAS score) was not associated with P-gp expression and there was no significant difference between responders (DAS28 <3.7) and non-responders to MTX (DAS28 >3.7)[8] (Kruskal-Wallis, p=0.27) (table 1).

Table 1 Percentage positive P-gp cells according to disease activity

	% Positive cells Mean (SD)
Control	46.5 (10.4)
MTX responders (DAS28 <3.7)	45.2 (7.3)
MTX non-responders (DAS28 >3.7)	40.0 (11.6)

No significant difference was seen between the groups (Kruskal-Wallis, p=0.27).

These results support the view that MTX is not a P-gp substrate and that P-gp expression on PBLs is not associated with MTX response in RA. As far as we know, to date there have been no published studies examining other efflux transporters and clinical response to MTX in RA. Laboratory studies, however, suggest that other transporters particularly MRP1 and MRP3[9] and BCRP[10] are primarily involved in MTX efflux.

We therefore agree with Ranganathan *et al* that drug efflux transporters may contribute to the response to MTX in RA.[1] We also agree that genetic variability in the expression of such transporters may explain part of the heterogeneity of treatment response. The evidence to date, including our own observations, does not, however, support a significant role of P-gp in mediating this. Further studies are therefore required to determine which other drug transporters are most important in RA in determining MTX resistance. Given the central role played by MTX as a disease modifying antirheumatic drug in RA, modulation of any such transporters using specific chemosensitising agents may provide a new and rational additional intervention in patients with RA.

S L Hider, C Morgan
Arthritis Research Campaign Epidemiology Unit, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK

E Bell
Department of Immunology, School of Biological Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK

I N Bruce
University of Manchester Rheumatism Research Centre, Central Manchester and Manchester Children's University Hospitals NHS Trust, Oxford Road Manchester M13 9WL, UK

Correspondence to: Dr SL Hider; sam.hider@man.ac.uk

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Authors' reply

Hider and colleagues correctly highlight the complexity surrounding the regulation of methotrexate (MTX) cellular transport. Members of both the ATP binding cassette (ABC) and solute carrier (SLC) families of transporters have been shown to include MTX among their many substrates.[1 2] Transfection of the multidrug resistance proteins MRP1 (ABCC1) and MRP2 (ABCC2) in human cells was associated with a two- to threefold lower accumulation of MTX and reduced retention of long chain polyglutamate forms of MTX.[3] Overexpression of MRP3 (ABCC3), MRP4 (ABCC4), or breast cancer resistance protein (BCRP, ABCG2), through cellular transfection or drug selection, can cause similar cellular MTX efflux and MTX resistance.[4–6]

Increased expression and function of multidrug resistance 1 (MDR1, ABCB1) messenger RNA and increased P-glycoprotein expression was also seen in a series of leukaemic sublines resistant to MTX.[7] A similar study showed that P-glycoprotein may mediate MTX resistance in cells with deficient carrier mediated MTX uptake. An MTX carrier deficient variant of murine 3T6 fibroblasts when inserted with a recombinant retrovirus expressing the human MDR1 gene showed increased survival of resistant cells.[8] The peripheral blood mononuclear cells of patients with rheumatoid arthritis who were refractory to treatment with

MTX also had higher expression of P-glycoprotein than those who responded to treatment.[9]

As highlighted in our review, there are multiple mechanisms underlying MTX transport and resistance. Some of these may be clinically significant, leading to trials of co-administration of inhibitors of transporters as a therapeutic strategy to improve the efficacy of the drug. Indeed, genetic variants in a number of components of the MTX pathway appear to contribute to the efficacy and toxicity of this agent. In the future, pharmacogenetics, together with demographic, clinical, and immunologic variables,[10] should allow better selection of patients with a high likelihood of therapeutic success and minimal toxicity.

P Ranganathan, H L McLeod
Washington University School of Medicine, Division of Rheumatology, Box 8045, St Louis 63110, USA

Correspondence to: Dr P Ranganathan; prangana@im.wustl.edu

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Scientific secretariat: Professor Guido Valesini

Email: annamaria.iagrocco@uniroma1.it

Contact: Organising secretariat: Michela Civelli, EDRA Spa, Medical Publishing and News Media, Viale Monza, 133 - 20125, Milan, Italy

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