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REVIEW ARTICLES

Methotrexate: implications of pharmacogenetics in the treatment of patients with Rheumatoid Arthritis

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ABSTRACT

Background: Methotrexate (MTX) is an anti-folate drug with anti-proliferative and anti-inflammatory effects. MTX proved to be an effective, fast-acting disease modifying anti-rheumatic drug (DMARD), being widely used for the treatment of rheumatoid arthritis (RA).

Objectives: This review aims to describe the main genetic variants identified concerning proteins that play a role in methotrexate's kinetics and efficiency profile.

Methods: A literature review was conducted since January of 2000 until December 2020, by searching the PubMed and Embase bibliographic databases, employing the following MeSH terms: methotrexate, pharmacogenetics, pharmacokinetics, and rheumatoid arthritis. The search was limited to articles in English language. Two independent reviewers screened the titles and abstracts followed by a full-text review to assess papers regarding their eligibility. A total of 48 articles matched the research criteria and were analyzed.

Results: Genetic variants of four main proteins, with different functions, have been consistently described. Reduced folate carrier 1 (RFC1), a constitutively expressed folate transport protein that has high affinity for MTX is responsible, almost exclusively, for the transport of folate and MTX into the cell. The most studied variant of the gene is the 80G>A variant, mapped within exon 2, on chromosome 21. It seems to improve RA responses to MTX, clinical efficacy with long disease remission. ABC transporters are involved in the efflux of MTX from cells. An increased expression and function of these transporters should decrease MTX concentrations in target cells, resulting in lack of therapeutic response. ABCB1 3435 C/T is a high frequency polymorphism, significantly associated with RA good responses, symptom remission and reduced adverse events, due to MTX treatment.Thymidylate synthase (TYMS) is involved in thymidine synthesis. MTX decreases TYMS activity by inhibition and decreasing the access to tetrahydrofolate (THF) cofactors. The most common genetic variant of the TYMS gene consists of a 28 bp tandem repeat, with double and triple number of repeats (2R and 3R). The 3R allele genotype was associated with decreased efficacy and increased toxicity. The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is indirectly inhibited by MTX. The most common SNPs of the MTHFR gene are C677T and A1298C. Both are associated with a decreased efficacy and an increased toxicity of MTX.

Conclusion: MTX response is affected by many gene variants; the effect of each variant separately is likely to be small. Additionally, gene-gene interaction seems to enhance the potential role of linkage disequilibrium. This shows the emerging need for a better gene characterization and to improve the knowledge about variants distribution according to ethnicity, to explain different responses to MTX at an individual level.

Keywords: Methotrexate; Rheumatoid arthritis; Pharmacogenetics.

INTRODUCTION

Methotrexate (MTX) is an anti-folate drug with anti-proliferative and anti-inflammatory effects, due to the inhibition of several key enzymes involved in folate, methionine, adenosine and *de novo* nucleotide synthesis pathways[1].

MTX enters cells via reduced folate carriers [solute carrier family (SLC)] and is transported out of cells via ATP-binding cassettes (ABCs). Intracellular activation involves MTX polyglutamation by folylpolyglutamate synthetase (FPGS). Then, polyglutamated MTX (MTX-PG) competitively inhibits dihydrofolate reductase (DHFR) activity. Other enzymes, such as methylenetetrahydrofolate reductase (MTHFR) are believed to contribute to the anti-folate effects of MTX, although not by direct inhibition[2,3]. Furthermore, MTX-PG inhibits thymidylate synthase (TYMS), 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and formyltransferase (ATIC), contributing to the intracellular accumulation of adenosine[1,2,4].

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Due to its anti-proliferative effect, MTX was initially used in high doses as a chemotherapy agent for hematological neoplastic diseases. Later, MTX proved to be the most highly effective, fast- acting disease modifying anti-rheumatic drug (DMARD), being widely used for the treatment of rheumatoid arthritis (RA)[5,6].

Even though the combined efficacy and persistence rates for MTX are superior to that of other DMARDs, considerable interpatient and intrapatient variability has been observed. Estimates indicate that up to one-third of patients fail to respond to treatment because of a lack of efficacy, and this variation limits the treatment options for certain patients[7,8].

On the other hand, the low-dose MTX used in rheumatic diseases care has a safe profile, being well tolerated by most of the patients, inclusively with less reported toxicity than other DMARDs, while maintaining a very good efficacy[7]. Nonetheless, adverse drug reactions (ADRs) are the main cause of MTX discontinuation[9], requiring close clinical and laboratorial monitoring. A pooled analysis of 21 prospective RA studies found at least one ADRs to occur in 72.9 % of patients, with gastrointestinal disorders being the most frequent (30.8 %), followed by liver (18.5 %), skin (8.9 %), neurological (5.5 %) and hematological (5.2 %) toxicity[10].

Over the past decade, numerous pharmacogenetic (PGx) studies have been undertaken to understand possible associations between genetic polymorphisms in genes encoding proteins involved in MTX carrier-mediated transport systems and/or intracellular pathways with MTX therapeutic outcome.

These PGx studies have been focusing on genes involved in MTX metabolic pathways, to identify relevant single nucleotide polymorphisms (SNPs). Potential candidate genes described are involved in influx, efflux, metabolism and effector pathways.

To date, 120 SNPs in 34 genes have already been identified, potentially contributing to treatment responses[11]. However, studies present contradictory data, which leads to equivocal interpretations. Factors that may contribute to study variance observed include relatively limited sample sizes with limited statistical power, heterogeneity between of the studied populations and lack of investigation of variants in multiple genes.

For being objective, this review will only address the most well described genetic variants of different proteins, and the effect that each of them has in the pharmacokinetics, efficiency/resistance profile and food/drug interactions related with methotrexate, in RA patients.

METHODS

A literature review was conducted since January of 2000 until December 2020, by searching the PubMed and Embase bibliographic databases. A sensitive search strategy was employed using the following medical subject headings (MeSH) terms: methotrexate, pharmacogenetics, pharmacokinetics, and rheumatoid arthritis.

The search was limited to articles in English language. Two independent reviewers (RPT, FPS) screened the titles and abstracts, followed by a full-text review to assess papers regarding their eligibility. A total of 48 articles matched the research criteria and were analyzed. The bibliography of the retrieved articles was screened for cross-references, so it would be possible to identify additional publications not indexed in the cited databases.

The considered SNPs are presented considering the function of protein they encode (Table I).

RESULTS

Cellular Transporters Genes

Reduced folate carrier 1 (*RFC1*) is a constitutively expressed folate transport protein that has high affinity for MTX and is responsible almost exclusively for the transport of folate and MTX into the cell. *RFC1* is expressed in a variety of tissues, with the highest expression in the placenta, liver, and leukocytes[1]. The high expression of *RFC1* in the placenta may contribute to the efficacy of high-dose MTX to induce abortion[12].

In a study conducted by Tazoe *et al.*, in 2015, demonstrated that subjects with RA and higher expression of *RFC1* were more likely to experience lower disease activity when medicated with MTX, which is consistent with the concept that MTX enters target cells through *RFC1*[13].

The most studied variant of this gene is the *80G > A* variant (*rs1051266*), mapped within exon 2, on chromosome 21, and encodes a substitution of histidine for the arginine at amino acid position[14]. Studies on this SNP have generated contradictory results; some studies state the *80G/A* variant is associated with improved RA responses to MTX[15,16], and improved clinical efficacy through longer disease remission times[17].

Some studies name the *80G/A* polymorphism as a clinical marker of MTX-related toxicity[18-20], while the majority of meta-analyses suggest that *80G/A* is not associated with MTX toxicity[21,22]. Nevertheless, a meta-analysis by Qiu *et al.* (that included 10 studies with 791 patients suffering from adverse RA events), reported that *RFC1 80G/A* GG genotype was linked to MTX toxicity in Europeans[23]. These contradictory data are probably due to differences in the inclusion and exclusion criteria since Qiu *et al.* focused only on the association between gene polymorphisms and the toxicity to MTX in monotherapy and did not investigate gene–gene interactions.

ABC transporters are involved in the efflux of MTX from cells. Theoretically, increased expression or func-

tion of these transporters should decrease MTX concentrations in target cells, resulting in lack of therapeutic response to MTX[4,15].

ABCB1 3435 C/T (*rs1045642*) is a high frequency polymorphism, significantly associated with RA responses, symptom remission and adverse events due to MTX treatment[23,4]. In 2016, Lee *et al*. showed no associations between *ABCB1 3435 C/T* and responses to MTX therapy. On the other hand, this polymorphism was associated with MTX toxicity in the same study[6]. Takatori *et al.*[24] found that patients with *ABCB1 3435C/C* and *3435C/T* showed higher therapeutic effects of MTX, which is inconsistent with the results of Lima *et al.*[20].

These inconsistencies concerning the association between *ABCB1 3435* polymorphisms and MTX response status are relevant, since a previous meta-analysis showed a negative association between the *ABCB1 3435C/T* polymorphism and RA susceptibility or responsiveness to MTX[6].

Regarding the *ABCC2* gene, only the studies concerning the SNP *1249G>A* (*rs2273697*) showed consistent results, namely increased MTX related toxicity[25].

MTX competes with drugs like erythromycin, estradiol and etoposide for the ABCG2 transport. Therefore, polymorphisms leading to a decrease on the *ABCG2* expression can theoretically increase MTX toxicity, although this is yet to be proven.

About 80 to 90% of the administered dose of MTX is excreted unchanged in the urine within 48 hours, mostly in the first 8 to 12 h, through ABCC2, ABCC4, ABCB1, and ABCG2 transport proteins[26].

Certain reasons can justify the lack of consistency of the mentioned studies: many of these could be considered small for genetics studies, and for that the results may not be replicable.

Intracellular pathway genes

Both MTX and MTX-PGs competitively inhibit DHFR enzyme. MTX-PGs and DHF inhibit TYMS[27]. The inhibition of DHFR and TYMS leads to impaired THF and dTMP biosynthesis and allows an accumulation of toxic inhibitory substrate DHF, resulting in the blockage of *de novo* purine synthesis, negatively affecting both DNA and RNA synthesis.

A total of nine studies have analyzed 13 different genetic variants of the *DHFR* gene, however none of the associations found for these variants have been replicated yet[28,29,30]. In addition, an observed *DHFR* gene polymorphism, *79940143T>C* (*rs1643650*), was associated with lower event-free survival likely do to the higher *DHFR* mRNA levels[31].

TYMS is involved in thymidine synthesis. MTX decreases the activity of TYMS, by inhibiting it and decreasing access to tetrahydrofolate (THF) cofactors[1]. Inhibition of TYMS decreases the number of nucleotides available for DNA synthesis, reducing its synthesis. This molecular mechanism seems to be important in the inhibition T-cell proliferation because activated T cells almost exclusively rely on the new nucleotide synthesis.

The most common genetic variant of the TYMS gene consists of a 28 bp tandem repeat (*rs34743033*), with double and triple number of repeats (2R and 3R)[32]. As the number of repeats increases, mRNA expression and enzymatic activity increases, modulating the efficacy and toxicity of MTX[33]. Studies found that 3R allele or 3R3R genotype were associated with decreased efficacy and increased toxicity[30,34,35].

The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is indirectly inhibited by MTX. The most common SNPs in the *MTHFR* gene are C677T (rs1801133) and A1298C (*rs1801131*). When C677T allele is present, enzyme activity is reduced 30% and 65% in genotypes CT and TT carriers, respectively[36]. Most of the reports showed that both genotypes were associated with an increased toxicity and decreased efficacy of MTX[30,37,38]. Still analyzing *MTHFR*, the haplotype 677T– 1298A has been associated with delayed MTX elimination, originating high incidence of toxicity and reduction of event-free survival[39,40], while the haplotype 677T– 1298C has been associated with increased remission[40].

MTX response is affected by many genes, and the effect of each of the genes separately is likely to be small. Bearing this in mind, it is difficult to consistently detect these alterations, but more importantly, a gene with a small effect is unlikely to be a helpful biomarker in precision medicine. Additionally, genes interact with one another, enhancing the potential role of linkage disequilibrium[4].

Other considerations

Given the complexity of MTX transport, cellular delivery, and action, it is likely that MTX response is dependent on multiple variables, including clinical characteristics. The odds of effective response to MTX are approximately double for male compared to female RA patients[41,42,43]. Lifestyle factors have also been investigated because they can actually be modified. Smoking is the best-established environmental risk factor for RA onset[44,45]. In a large study on patients with early RA, it was found that current smoking could predict MTX resistance, however past smokers did not differ from non-smokers in its response to MTX treatment[46].

Because MTX is a folate derivative, it interferes with folate metabolism. Therefore, folic acid supplementation prevents MTX-induced liver toxicity and gastrointestinal side effects; at the same time, meta-analysis

Table I. SNPs implicated in the methotrexate cellular pathway

Gene	Position	Variation Type	SNP Reference	Alleles	Pathway
RFC1	chr1:11796321 (GRCh38.p13)	SNP	rs1051266	80G>A	Transporter
ABCB1	chr7:87509329 (GRCh38.p13)	SNP	rs1045642	3435 C/T	Transporter
ABCC2	chr10:99804058 (GRCh38.p13)	SNP	rs2273697	1249G>A	Transporter
DHFR	chr5:80644324 (GRCh38.p13)	SNP	rs1643650	79940143T>C	Folate
TYMS	chr18:657657-657730 (GRCh38.p13)	Indel	rs34743033	28 bp VNTR	Folate
MTHFR	chr1:11794419 (GRCh38.p13)	SNP	rs1801133	C677T	Folate
	chr1:11796321 (GRCh38.p13)	SNP	rs1801131	A1298C	

Chr – Chromosome; Indel – Insertion and deletion; rs – Reference SNP; G – Guanine; A – Alanine; C – Citosine; T – Thymine; VNTR - Variable number tandem repeat.

indicates that folic acid supplementation does not significantly change the efficacy of MTX, as demonstrated by Shea *et al.*, in 2013. It is recognizable that there is still a lack of studies examining association of other dietary components with MTX efficacy, although some potentials have been considered (pe. coffee)[47].

CONCLUSION

Several studies have demonstrated associations between multiple genetic polymorphisms and MTX therapy efficacy and ADRs in RA patients. However, to date, the data are often inconsistent. In the future, precision medicine or personalized pharmacotherapy, based on selected genotypes, could provide evidence-based indications for MTX, in individual patients. So far, the use of SNPs that may influence MTX pathways, in clinical practice, is controversial.

This shows the emerging need for a better gene characterization, using new technologies. Genes and/or ethnic origin added to behavioral determinants and other disease and patient-related characteristics, should be considered to design a pharmacogenetics algorithm, that hopefully may add in therapeutical decisions in a near future[47,48].

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