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Review

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Folates and antifolates in rheumatoid arthritis

Abstract: Almost all current standard treatment of care for patients with chronic inflammatory diseases such as rheumatoid arthritis (RA) includes the folate antagonist methotrexate (MTX). Despite the proven efficacy of MTX in RA treatment, it is anticipated that further improvement of antifolate-based therapies can be achieved by integrating the continuously extending knowledge of parameters underlying drug efficacy. Herein, an overview will be presented of strategies that may assist in the future design of rationalized and personalized targeted therapies with a folate antagonist. Issues that will be discussed include (i) early detection of arthritis, (ii) genomic studies of folate/MTX pathway genes for MTX response predictions, (iii) identification of MTX resistance modalities, (iv) exploration of second/third generation of antifolates which may substitute for MTX, and (v) folate (food) supplementation.

Keywords: antifolates; folates; rheumatoid arthritis.

Abbreviations and enzyme codes: ATIC/AICARTF, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (EC 2.1.2.3); DHFR, dihydrofolate reductase (EC 1.5.1.3); FPGS, folylpolyglutamate synthetase (EC 6.3.2.17); FRα/β, folate receptor alpha/beta; GART, glycinamideribonucleotide formyltransferase (EC 2.1.2.2); GGH/FPGH, gamma-glutamyl hydrolase/folylpolyglutamate hydrolyse (EC 3.4.19.9); MTHFR, methylenetetrahydrofolate reductase (EC 1.5.1.20); MTX, methotrexate; PB, peripheral blood; PCFT/SLC46A1, proton-coupled folate transporter; PG, polyglutamates; RFC/SLC19A1, reduced folate carrier; TYMS, thymidylate synthase (EC 2.1.1.45).

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Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation of the synovial joints which leads, when left untreated, to bone and cartilage destruction along with other co-morbidities [1, 2]. Inflamed synovium has several hallmarks reminiscent of neoplastic processes including blood vessel formation (angiogenesis) and infiltration of blood-derived cells (i.e., T cells, B cells, monocytes, macrophages). Together with synovial fibroblasts, these cells are responsible for the production of multiple proinflammatory cytokines (e.g., TNFα, IL1β, and IL6) that trigger synovial proliferation [3]. Figure 1 illustrates the various types of immune cells that infiltrate RA synovial tissue as part of the pathogenesis of RA. The diversity and dynamic infiltration of immune cells in inflamed RA synovium holds challenges for therapeutic options of targeting specific cell types or neutralizing proinflammatory cytokines they produce.

Early detection of RA

Biomarkers that can predict the early onset and progression of RA are helpful in designing optimal treatment strategies. Beyond the classical IgM rheumatoid factor, recently detection of auto-antibodies against citrullinated proteins (anti-CCPs) became popular as they are highly specific and predictive for two-thirds of RA patients and

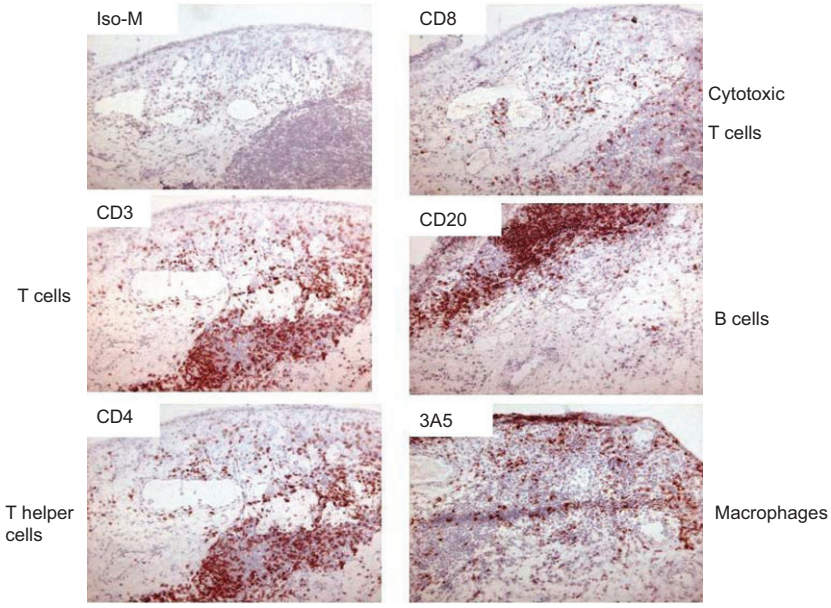

Figure 1 Immune cells in inflamed RA synovial tissue. Macrophages reside in the synovial lining layer and sublining. B cells and T cells appear in clusters in the synovial sublining. Immunohistochemical staining is performed for cell type specific cell surface markers, except for 3A5, which stains a macrophage lysosomal protein.

detectable years before the first clinical symptoms of RA emerge [4]. In addition to serum markers, non-invasive imaging techniques [5, 6] are also being introduced to visualize local joint inflammation and facilitate therapy response monitoring. As a prototypical example, recently a folate-based positron emission tomography (PET) tracer ([^{18}F]fluoro-PEG-folate) was synthesized and characterized in an arthritis model in rats, where it allowed detection of activated macrophages in inflamed joints through selective binding to folate receptor β (FRβ) on these cells [7].

Treatment of RA

Historically, upon RA diagnosis a mild treatment with non-steroidal anti-inflammatory drugs (NSAIDs) was initiated, which partly reduced joint inflammation, but had no effect on radiological disease progression. Currently, treatment strategies have a totally different design including rapid and aggressive therapy with disease modifying antirheumatic drugs (DMARDs) as single agent or combined with glucocorticoids and/or biological agents (Table 1) [1, 2]. Biological agents refer to antibodies or soluble receptors to proinflammatory cytokines. As illustrated in Table 1, methotrexate (MTX) serves as an anchor drug in RA treatment [8], both in DMARD combination schedules and in combination with biologicals.

Table 1 Current treatment modalities for RA.

NSAIDs	Non-steroidal anti-inflammatory drugs
	Sulindac, Indomethacin
DMARDs	Disease modifying anti-rheumatic drugs
	Methotrexate (MTX, anchor drug)
	Sulfasalazine (SSZ)
	Hydroxychloroquine (HCQ)
	Cyclosporine A (CsA)
Biologicals	MTX + Infliximab (chimeric MoAb to TNFα)
	MTX + Etanercept (soluble TNFα receptor)
	MTX + Adalimumab (human MoAb to TNFα)
	MTX + Anakinra (IL1-receptor antagonist)
	MTX + Rituximab (anti-CD20/B cell)
	MTX + Abatacept (CTLA4-Ig fusion protein/ co-stimulation (CD80/CD86) blocker)
	MTX + Tocilizumab (soluble IL6 receptor)

Mechanism of action of MTX and response predictions

Despite the key role of MTX in current RA therapies, the mechanism of action remains elusive [9–11]. In part, this may be associated with the fact that MTX may elicit differential effects on various types of immune cells. Conceivably, a primary effect of MTX is mediated via targeting of the folate metabolic pathway that regulates MTX/folate cellular pharmacology (Figure 2). The magnitude of an

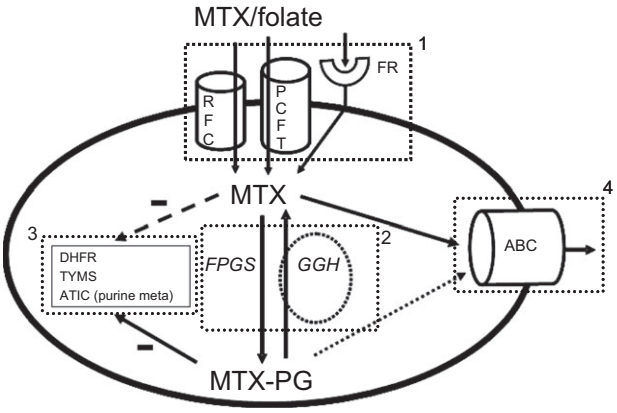

Figure 2 Determinants in the cellular pharmacology of MTX/folate. Key players in the cellular pharmacology of MTX/folate can be divided into four groups: (i) cellular uptake transporters, including the reduced folate carrier (RFC), proton-coupled folate transporter (PCFT), and folate receptors; (ii) metabolizing enzymes, including folylpolyglutamate synthetase (FPGS) and gamma-glutamyl hydrolase (GGH, compartmentalized in lissome), which facilitate the formation and breakdown of MTX/folate polyglutamates, respectively; (iii) intracellular target enzymes dihydrofolate reductase (DHFR), thymidylate synthase (TS), and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (ATIC) being involved in purine biosynthesis de novo; and, finally, (iv) MTX/folate efflux transporters belonging to the family of ATP-binding cassette proteins capable of extruding MTX/folates from cells.

effect will depend on functional activity of cellular uptake and efflux routes as well as intracellular metabolism to polyglutamate (PG) forms. MTX-PG forms have been identified to inhibit a key enzyme in purine biosynthesis de novo, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICARTF/ATIC), promotes release of the endogenous anti-inflammatory mediator adenosine [9]. MTX has also been shown to exert additional pharmacological activities [11, 12], among which generation of reactive oxygen species, induction of (activated) T cell apoptosis and NFκB inhibition, of which each contribution in therapeutic efficacy has not been established.

The expanding knowledge of the folate/MTX pathway has been exploited to build prediction models for MTX response in various stages of disease activity based on polymorphic variants of folate/MTX pathway genes [13, 14]. Additionally, analysis of MTX-PG levels in red blood cells has been evaluated as a potential response prediction marker [13, 15]. Unfortunately, the outcomes of these studies were not always consistent between various international cohorts and mostly inconclusive in predicting MTX response or toxicity [16–18]. The reason for this may be related to the fact that disease activity is not necessarily stable over time, the cross-sectional rather than prospectively controlled study design of prediction studies, the lack of information whether or not polymorphic variations actually translate into altered functional properties, and, finally, the notion that MTX-PG analysis in red blood cells remains a surrogate for actual MTX-PG accumulation in immune-competent cells. In search for additional approaches, preliminary data by Blits et al. [19] indicated that, as part of the inflammation process, expression levels of several folate/MTX pathway genes were markedly increased in peripheral blood mononuclear cells from RA patients compared with healthy controls. These parameters deserve further exploration in properly designed MTX response prediction studies.

MTX resistance modalities

Given the chronic nature of the disease, RA patients face repeated and long-term drug administration with a great likelihood of emergence of acquired drug resistance [12, 20]. For MTX, RA patients usually receive a starting dose of 7.5 mg/week, which can be escalated up to 25 mg/week approximating toxicity levels. It is conceivable that loss of MTX efficacy shares molecular mechanisms similar to those described for MTX resistance in cancer treatment. Figure 3 depicts potential modes of MTX resistance associated with poor delivery to target cells, impaired cell membrane transport, increased efflux, diminished polyglutamylation, increased catabolism, elevated levels of target enzymes, and alterations downstream of target inhibition. To date, no systematic surveys are available that disclosed which mechanism dominantly accounts for loss of MTX efficacy. Studies by Van der Heijden et al. [21], however, showed that increased expression of the drug efflux transporter ABCG2 on RA synovial tissue macrophages was implicated in reduced efficacy of the DMARDs MTX and leflunomide. As drug efflux transporters of the ATP-binding cassette transporter family are expressed in most immune cells, they could be a contributing factor in conferring drug resistance [22].

Next generation antifolates

Expanding knowledge on the molecular basis of resistance to MTX in the cancer treatment setting has prompted the development of next generation folate antagonists that may overcome acquired resistance to MTX. Over the past decade, several rationalized designed folate antagonists were synthesized harboring properties of the targeting enzyme in folate metabolism other than MTX, being better

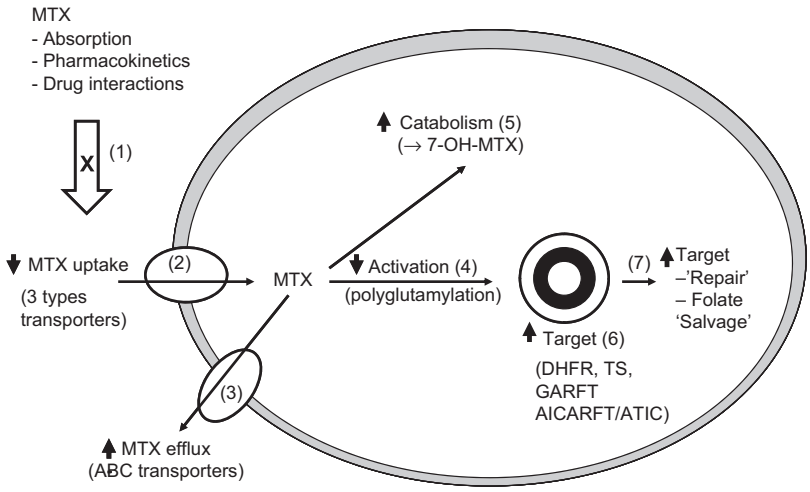

Figure 3 Molecular mechanisms of resistance of MTX. Loss of efficacy to MTX may involve multifactorial mechanisms: (i) insufficient delivery of MTX to target cells due to aberrations bioavailability, pharmacokinetics, or drug interactions; (ii) impaired cellular uptake via either RFC, PCFT, or FR; (iii) enhanced drug efflux from the cell; (iv) diminished polyglutamylation of MTX affecting its cellular retention; (v) catabolism to inactive metabolites such as 7-OH-MTX in liver; (vi) elevated levels of target enzymes; and (vii) repair/escape mechanisms downstream of target enzyme inhibition.

transported into the cell than MTX, being more efficiently metabolized to PG forms, or being independent of polyglutamylation [20, 23]. Although several next generation folate antagonists gained an established place in cancer chemotherapy, none of them has been challenged in the clinical RA setting to replace MTX, this despite proven efficacy in experimental animal models of arthritis and ex vivo assessments of potency to inhibit release of proinflammatory cytokines from blood cells of RA patients [24–26]. Recently, a series of folate antagonists were also synthesized that displayed high affinity and selective binding to folate receptors rather than other folate transporters such as reduced folate carrier (RFC) and proton-coupled folate transporter [27]. One of these prototypical compounds (i.e., BGC 945/ONX 0801; Figure 4) showed high nanomolar binding affinity to FRβ, which is increasingly expressed on activated macrophages in RA synovium [28, 29]. These rationally designed second generation folate antagonists warrant further exploration as targeted therapeutic drugs and overcoming of loss of MTX efficacy other than by expensive biological agents in an RA treatment setting.

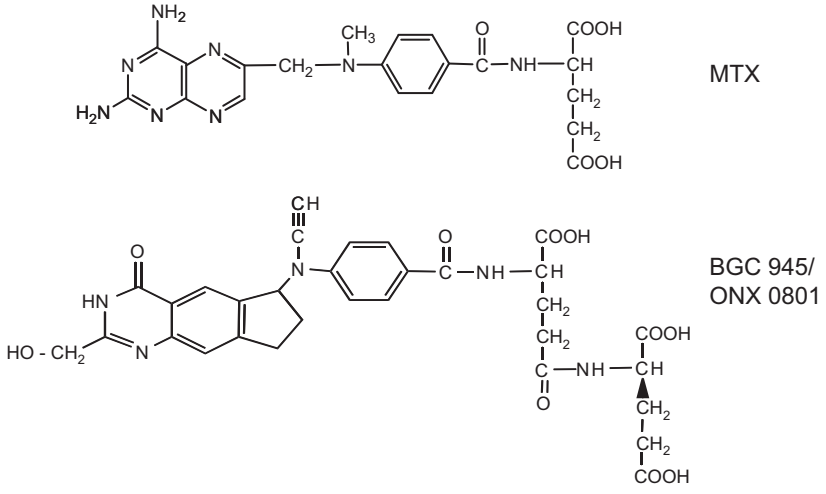

Figure 4 Folate receptor targeted antifolate. Chemical structure of BGC 945/ONX 0801, a TS-inhibitor rationally designed for selective cellular uptake via FR. Comparison with MTX reveals that BGC 945/ONX 0801 has an L-glutamate/D-glutamate side chain which abolishes substrate affinity for RFC and PCFT, whereas the 2-methoxy-4oxo moiety of the molecule introduces increased binding affinity for FR, unlike the 2,4-diamino moiety in MTX.

MTX efficacy and folate supplementation

Most RA patients on MTX-based treatment schedules receive tightly balanced co-administration of folic acid to control potential toxic side effects of MTX without compromising its efficacy [30]. Also, caution should be taken in avoiding overdosing of folic acid considering the fact that human liver has a maximal capacity to convert only approximately 1 mg of folic acid per day [31], implicating that at dosages of >1 mg/day, folic acid will appear unmetabolized in plasma leading to potential unwarranted harmful effects in folate homeostasis [32–34]. Also, national food and drug administration programs introduced folic acid food supplementations to reduce the incidence of neural tube defects. This, together with uncontrolled intake of folic acid containing multivitamin supplementations has raised plasma folate concentrations in the general population. For RA patients in the USA, this had an impact for requirements of higher dosages of MTX to achieve therapeutic efficacy [35].

Concluding remarks

Despite more than three to four decades of application of MTX in treatment of chronic inflammatory diseases such as RA, there are still many unresolved issues left related to its mechanism of action, response predictions, mechanisms involved in its loss of efficacy, and opportunities to substitute MTX with new generation of folate antagonists. These challenges should drive future research in this field to further improve clinical benefits with this class of drugs.

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