International Journal of Basic & Clinical Pharmacology
Menon A et al. Int J Basic Clin Pharmacol. 2026 Jan;15(1):195-200
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20254171

Review Article

Beyond folate antagonism: the unique pharmacological blueprint of methotrexate

Abhilash Menon*, Pradhiksha Murugesan, Pillai Pooja Tamilarasan, Sai Ahil Palanisamy, Arun Kanniyappan Parthasarathy

Department of Pharmacy Practice, JSS College of Pharmacy, Ooty, Tamil Nadu, India

Received: 24 October 2025
Revised: 01 December 2025
Accepted: 02 December 2025

***Correspondence:**
Dr. Abhilash Menon,
Email: abhilashmenon61@gmail.com

ABSTRACT

Methotrexate (MTX) possesses a uniquely complex pharmacological profile characterized by nonlinear pharmacokinetics, saturable absorption, transporter-dependent distribution, and intracellular polyglutamation, all of which contribute to substantial interindividual variability in therapeutic response and toxicity. This review summarizes current evidence on MTX absorption kinetics, dose-dependent oral bioavailability, and the role of renal and hepatic pathways in systemic clearance. Special emphasis is placed on the formation and accumulation of MTX polyglutamates (MTX-PGs), which act as long-acting intracellular metabolites that enhance therapeutic duration but may also increase the risk of adverse effects when present at higher concentrations. Genetic polymorphisms in key enzymes and transporters, including RFC1, FPGS, GGH, and members of the SLCO and ABC transporter families, significantly influence MTX disposition, efficacy, and toxicity, underscoring the expanding role of pharmacogenomics in individualized therapy. The review also highlights the relevance of routine laboratory monitoring, emerging biomarkers, and clinical dosing strategies, including split dosing and subcutaneous administration to optimize outcomes across oncology and autoimmune settings. By integrating current pharmacokinetic, pharmacodynamic, and pharmacogenomic insights, this work provides a comprehensive understanding of MTX's therapeutic behaviour and supports the need for personalized approaches to maximize efficacy while minimizing adverse effects.

Keywords: Methotrexate, Pharmacokinetics, Pharmacology, Genetic polymorphism, Antirheumatic agents

INTRODUCTION

Methotrexate (MTX) has been a key player in treating autoimmune diseases for many years, especially in conditions like rheumatoid arthritis (RA) and psoriasis.[1] Originally developed in the 1940s as an antifolate for cancer therapy, MTX was brought to prominence by Sidney Farber's groundbreaking research, which highlighted its potential in treating pediatric leukaemia.[2] By the 1980s, MTX received FDA approval for RA, further establishing its status as a versatile medication. This opened the door for its use beyond cancer treatment. Today, it is one of the most commonly prescribed disease-modifying antirheumatic drugs (DMARDs) and is often the first choice for treating RA and psoriasis due to its effectiveness, safety, and affordability. In RA, its effectiveness comes from its anti-inflammatory and immunosuppressive effects, which occur through the inhibition of dihydrofolate reductase and the enhancement of adenosine release, leading to reduced activation of immune cells. In psoriasis, MTX focuses on rapidly dividing keratinocytes and adjusts cytokine production, helping to alleviate the skin's hyperproliferative condition.[3] MTX acts as an anti-metabolite in both cancer and autoimmune disease management. It inhibits the synthesis of tetrahydrofolate (THF), disrupting the formation of DNA, RNA, and essential proteins in cancer cells.[4] For autoimmune diseases, it blocks AICAR transformylase, resulting in increased adenosine levels, which helps to counteract cytokine-driven inflammation

and provides an anti-inflammatory effect. MTX is converted by the FPGS enzyme inside cells to its active metabolites, the 5-7 polyglutamated forms, which are more effective at inhibiting DHFR than the parent drug. In the liver, MTX is transformed into its inactive form, 7-hydroxy MTX (7-OHMTX). MTX has a bioavailability ranging from 64% to 90%. Its volume of distribution (Vd) is about 0.18 L/kg, with a steady-state volume of distribution between 0.4 to 0.8 L/kg, and it is 46.5% to 54% bound to plasma proteins, mainly albumin. In low-dose therapy, the terminal half-life of MTX is approximately 3 to 10 hours, while in case of high-dose therapy, its terminal half-life is 8 to 15 hours. The drug is primarily excreted through the kidneys. The average total MTX clearance is approximately 12L/hr though the rates vary widely and it generally decreases at higher doses.[5,6] Figure 1 shows a brief description of the pharmacokinetic properties of MTX.

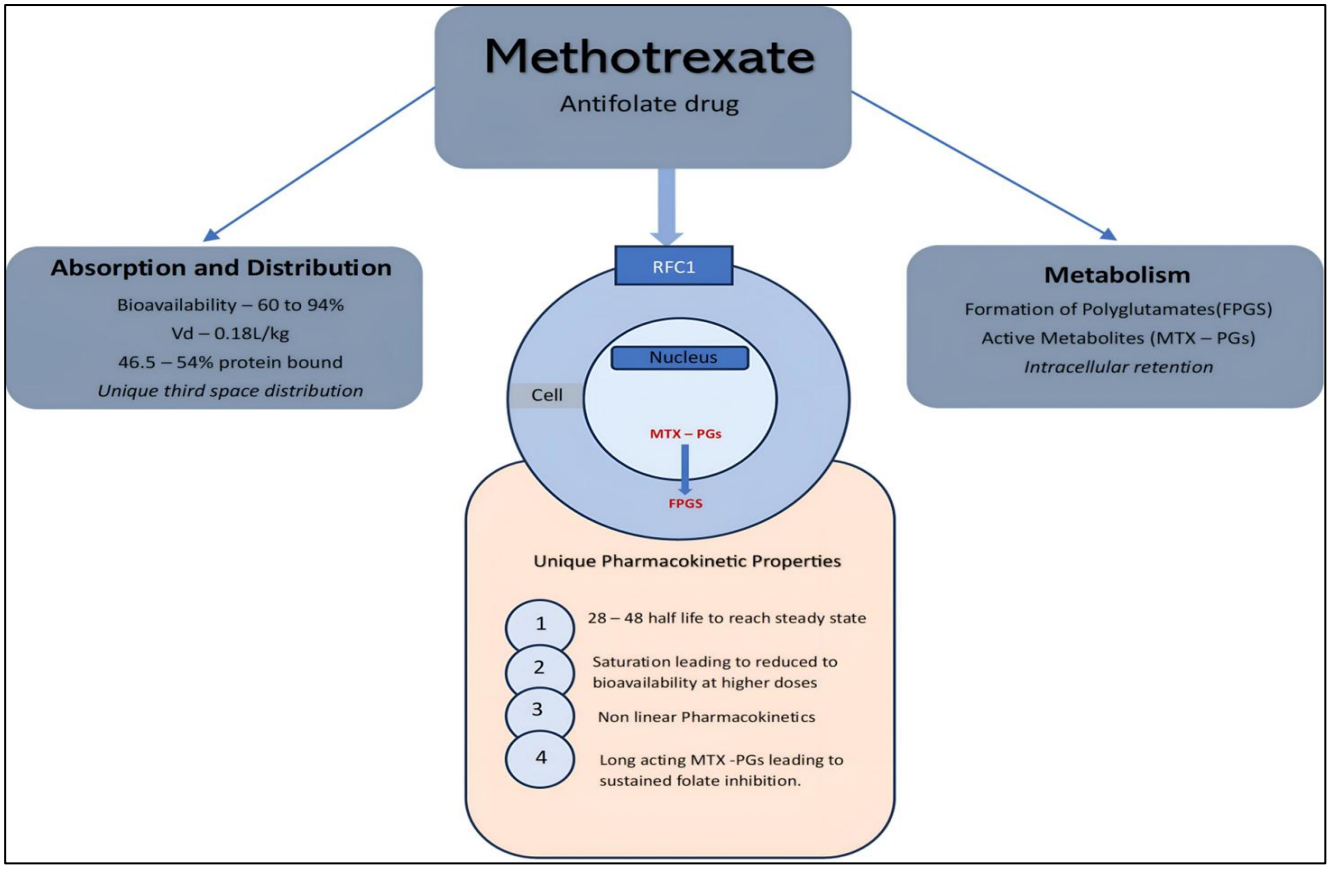

Figure 1: Overview of MTX's unique pharmacokinetics influenced by absorption, intracellular polyglutamation, and sustained retention of MTX–PGs.

Aim and objectives were to discuss the unique pharmacokinetic properties of MTX and to understand the clinical importance of its metabolite, MTX-PG and how it contributes to its unique pharmacokinetic properties.

LITERATURE SEARCH

A comprehensive literature search was conducted to gather data on the pharmacokinetics, mechanisms, and clinical applications of MTX. The search spanned multiple databases, including PubMed, Scopus, and Web of Science, from 1980 till 2024. Keywords such as "methotrexate pharmacokinetics," "methotrexate in rheumatoid arthritis," "genetic polymorphisms and methotrexate," and "antifolate therapy" were employed.

Inclusion criteria encompassed peer-reviewed articles, systematic reviews, meta-analyses, and randomized controlled trials. Articles were prioritized based on relevance, publication recency, and citation frequency. Exclusion criteria included non-English articles, editorials, and studies with insufficient methodological rigor. Grey literature, such as conference abstracts and preprints, was also reviewed for emerging insights. To ensure comprehensiveness, bibliographies of selected articles were screened for additional references. This approach ensured a robust foundation for evaluating MTX's clinical significance and identifying gaps in current knowledge.

DISCUSSIONS

When compared to other antifolate drugs like pemetrexed and pralatrexate, MTX showcases a distinct pharmacokinetic profile and clinical flexibility. While pemetrexed and pralatrexate are mainly used in oncology, MTX has a wider range of applications, including

autoimmune disorders, ectopic pregnancy, and inflammatory bowel disease.[7,8] In the realm of immunosuppressive therapy, MTX presents clear benefits over alternatives such as azathioprine and leflunomide.[9] For example, MTX's dual mechanism of action-acting as a folate antagonist and exerting anti-inflammatory effects through adenosine-creates a synergistic therapeutic impact. Moreover, MTX's long-standing use has led to well-defined dosing protocols, safety monitoring guidelines, and the availability of cost-effective generics, unlike newer immunosuppressants.[10]

Once weekly administration of oral MTX (7.5-15 mg/week) remains global standard for administration in patients with RA. BA reduces by 30% at doses >15 mg/week; switching to split-dose regimen/to subcutaneous route improves exposure with similar tolerability.[11,25] In cancers (ALL, GTD, AML, lymphomas) the dose range for intravenous MTX is from 10 to 8000 mg/m^2 in adults and in pediatric patients.[12] Lower doses are administered IM weekly and higher doses are generally given every 14 days (usually IV), but treatment is highly variable and tailored to specific protocols. In case of oral metronomic chemotherapy (OMCT), MTX is dosed twice weekly in advanced breast cancer and in head and neck cancer, and once weekly in oral squamous cell carcinoma.[13-15]

To fully comprehend the therapeutic potential of MTX, it is essential to understand its pharmacokinetic behaviour. After IV injection, the elimination of MTX from plasma is triphasic. The initial half-life is 0.75±0.11 hours. The second half-life has been reported as 2.06±0.16 hrs, 3.49±0.55 hrs, and 2.0-3.4 hours. The terminal half-life is 10.4±1.8 hours.[16] MTX is a drug with a very short terminal half-life (0.7-5.8 hrs) but the time to reach steady state concentration is 4-6 weeks. This time lag is due to the entry of MTX into the RBCs, WBCs, hepatocytes and synoviocytes.[17,18] MTX enters the RBCs via a protein named RFC1 and it undergoes polyglutamation. The polyglutamated forms of MTX viz., MTXGlu$_{1-5}$, are selectively retained within the cancer cells and eventually excreted. These long-chain MTX-PGs, with a half-life of approximately 3 days, act as a sustained intracellular drug depot, thus allowing for the once weekly administration. However, higher intracellular concentrations of long-chain MTXPGs have been associated with an increased risk of adverse effects, such as gastrointestinal intolerance and hepatotoxicity.[19] Figure 2 shows the important properties of MTX-PGs that contribute to the pharmacokinetic profile of MTX. The MTX$_{Glu}$ forms is hydrolysed by the enzyme, gamma glutamyl hydrolase (GGH) and converted back to MTX and released into the systemic circulation.[20,21]

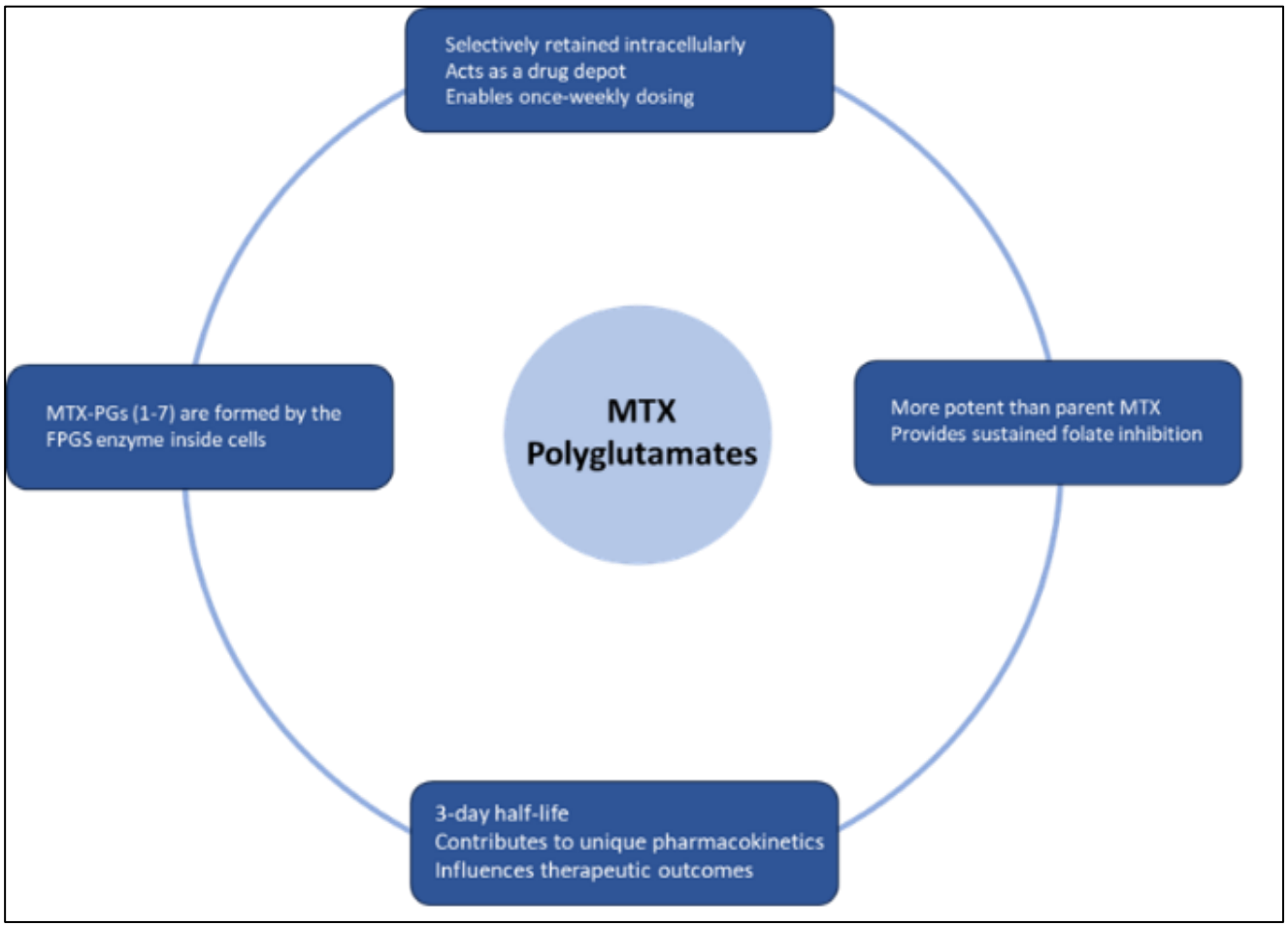

Figure 2: Key properties of MTX-PGs that contribute to the unique pharmacokinetic profile of MTX.

Altered activity of MTX-clearing transporters contributes to variability in systemic exposure; impaired clearance has been demonstrated in OAT3-deficient models.[27] Altered enzyme activity may lead to prolonged exposure to MTXPGs, increasing the risk of adverse effects. Conversely, increased conversion could reduce MTX efficacy by decreasing intracellular drug concentrations. Thus, such polymorphisms can influence both the therapeutic outcomes and toxicity profiles of MTX treatment.[22]

Genetic polymorphisms of the RFC1 protein, namely the gene variant, RFC1 c.80 A>G, plays an important role in MTX pharmacokinetics, contributing to its greater efficacy.[26] Polymorphisms in the FPGS enzyme can lead to reduced formation of MTX PGs, potentially decreasing the drug's therapeutic efficacy. Similarly, in a study involving South Indian Tamil patients with RA found that genetic polymorphisms of the enzyme FPGS, its rs1544105 GA and rs10106 AG genotypes had increased odds of experiencing MTX adverse events, with odds ratios of 1.93 and 2.11, respectively.[23]

Thus, its limitations, such as interindividual variability due to genetic polymorphisms, potential for hepatotoxicity, and lack of established therapeutic window warrant careful consideration, seen especially in RA.[24,28] Pharmacogenetic insights continue to refine dosing strategies, helping predict toxicity and therapeutic response in MTX-treated patients.[30] Studies evaluating oral MTX in RA populations further emphasize its nonlinear absorption, dose-dependent clearance, and variability in systemic exposure.[29] These features, along with other key pharmacokinetic attributes that differentiate MTX from conventional therapeutics, are summarized in the table below.

Table 1: Distinct pharmacokinetic properties of MTX, compared with usual drugs.

Pharmacokinetic characteristics	Pattern in usual drugs	Pattern in MTX
Time to reach C_{ss}	Usually, it takes 5 to 7 half-lives to attain steady state for a drug.	However, it takes 28 to 42 half-lives to attain steady state for MTX.
Distribution	Most drugs' distribution is confined to first-space fluid (intravascular fluid compartment) and at times, second-space fluids (interstitial fluid compartment).	MTX shows extensive third-space fluid accumulation as well (ascites, pleural effusion, pericardial fluid which are transcellular fluid compartments).
Metabolites	A few drugs form active metabolites in the liver by undergoing metabolism by CYP450 enzymes, UGT, etc. Most of these metabolites are not retained in the body for prolonged periods, particularly not within cells.	MTX forms MTX-PGs (1-7) as its active metabolites via FPGS enzyme. Polyglutamated forms of MTX are more potent and are retained intracellularly in the body for long periods of time contributing to sustained folate inhibition.
Bioavailability	In the case of most drugs, BA will either increase or remain constant on increasing dose. Most drugs are also absorbed from the GIT by passive diffusion.	In the case of MTX, saturation of RFC and PCFT proteins (involved in active transport) can lead to reduced fraction of the drug being absorbed. It is not well absorbed by passive diffusion. This phenomenon is also seen in Baclofen, Gabapentin and Levodopa.
Clearance	Most drugs exhibit linear pharmacokinetics, with clearance remaining constant across doses. Clearance depends on Vd and K, unaffected by dose, as elimination pathways (e.g., hepatic metabolism, renal excretion) operate within their non-saturated, linear ranges.	MTX shows non-linear pharmacokinetics. At higher doses, tubular secretion becomes saturated thereby reducing clearance making it appear dose-dependent. As mentioned before, it can distribute into third-space fluids in case of certain disease conditions like ascites and pleural effusion. High-dose MTX can also cause AKI due to precipitation of drug in renal tubules, creating a feedback loop where higher doses can further reduce clearance.

CONCLUSION

This review highlights the distinct pharmacokinetic behaviour of MTX and its clinical implications. Optimizing MTX therapy requires the integration of pharmacogenomic testing into routine practice, as genetic variations in key enzymes and transporters can influence both metabolism and therapeutic outcomes. Identifying such variations can help guide individualized dosing, particularly for patients prone to toxicity or suboptimal response.

Routine monitoring of blood counts, liver enzymes, and renal function remains essential to detect early signs of myelosuppression, hepatotoxicity, or nephrotoxicity. Emerging biomarkers such as intracellular polyglutamate

levels and adenosine concentrations may further enhance real-time assessment of treatment effectiveness. The considerable interindividual variability in MTX disposition underscores the need for additional research, especially regarding the role of transporters, metabolizing enzymes, and population-specific pharmacokinetic factors.

Overall, MTX's unique properties-including saturable absorption, nonlinear clearance, intracellular polyglutamation, and third-space distribution-highlight the importance of vigilant monitoring and personalized therapy. A deeper understanding of these characteristics will support safer, more effective, and more tailored MTX use in clinical practice.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Jolivet J, Cowan KH, Curt GA, Clendeninn NJ, Chabner BA. The pharmacology and clinical use of methotrexate. N Engl J Med. 1983;308(18):1094-104.
2. Treviño LR, Shimasaki N, Yang W, Panetta JC, Cheng C, Pei D, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. J Clin Oncol. 2009;27(35):5972-8.
3. O'Rielly DD, Rahman P. Pharmacogenetics of rheumatoid arthritis: Potential targets from susceptibility genes and present therapies. Pharmgenomics Pers Med. 2010;3:15-31.
4. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. Oncologist. 2006;11(6):694-703.
5. Weinblatt ME. Methotrexate in rheumatoid arthritis: a quarter century of development. Trans Am Clin Climatol Assoc. 2013;124:16-25.
6. Chan ES, Cronstein BN. Methotrexate-how does it really work? Nat Rev Rheumatol. 2010;6(3):175-8.
7. Hoekstra M, Haagsma CJ, Neef C, Proost JH, Kadir AA, van de Laar MA. Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis. J Rheumatol. 2004;31(4):645-8.
8. Baggott JE, Morgan SL. Methotrexate catabolism to 7-hydroxymethotrexate in rheumatoid arthritis alters drug efficacy and retention and is reduced by folic acid supplementation. Arthritis Rheum. 2009;60(8):2257-61.
9. Schmiegelow K. Advances in individual prediction of methotrexate toxicity: a review. Br J Haematol. 2009;146(5):489-503.
10. Katchamart W, Bourré-Tessier J, Donka T, Drouin J, Rohekar S, et al. Canadian recommendations for pharmacogenomics testing in rheumatology. Arthritis Care Res. 2018;70(5):687-92.
11. Alarcon GS, Tracy IC, Strand GM. Methotrexate in rheumatoid arthritis. J Rheumatol. 1989;16(19):15-7.
12. Drugs.com. Available at: https://www.drugs.com/dosage/methotrexate.html#Usual_Adult_Dose_for_Acute_Lymphoblastic_Leukemia. Accessed on 10 June 2025.
13. Patil VM, Noronha V, Joshi A, Dhumal S, Mahimkar M, Mahajan A, et al. Phase I/II Study of Palliative Triple Metronomic Chemotherapy in Platinum-Refractory/Early-Failure Oral Cancer. J Clin Oncol. 2019;37(32):3032-41.
14. Colleoni M, Orlando L, Sanna G, Rocca A, Maisonneuve P, Peruzzotti G, et al. Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer: antitumor activity and biological effects. Ann Oncol. 2006;17(2):232-8.
15. Harsh KK, Maharia SR, Nirban RK, Khatri P, Beniwal S, Kumar HS, et al. Metronomic palliative chemotherapy in locally advanced, recurrent and metastatic head-and-neck cancer: A single-arm, retrospective study. J Cancer Res Ther. 2020;16(3):559-64.
16. Bleyer WA. The clinical pharmacology of methotrexate: new applications of an old drug. Cancer. 1978;41(1):36-51.
17. Seideman P, Beck O, Eksborg S, Wennberg M. The pharmacokinetics of methotrexate and its 7-hydroxy metabolite in patients with rheumatoid arthritis. Br J Clin Pharmacol. 1993;35(4):409-12.
18. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev. 2005;57(2):163-72.
19. Yang Z, Mo J, Li W, Zhao Z, Mei S. Methotrexate polyglutamates. Expert Rev Clin Pharmacol. 2024;17(11):1025-37.
20. Song Z, Hu Y, Liu S, Jiang D, Yi Z, Benjamin MM, et al. Genetic polymorphisms in high-dose methotrexate toxicity and response in hematological malignancies: A systematic review and meta-analysis. Front Pharmacol. 2021;12:757464.
21. Dhar H, Hamdi I, Rathi B. Methotrexate Treatment of Ectopic Pregnancy: Experience at Nizwa Hospital. Oman Med J. 2011;26(2):94-8.
22. Yamamoto T, Shikano K, Nanki T. Folylpolyglutamate synthase is a major determinant of intracellular methotrexate polyglutamates in rheumatoid arthritis. Sci Rep. 2016;6:35615.
23. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of rituximab in rheumatoid arthritis. N Engl J Med. 2004;350(25):2572-81.
24. O'Dell JR, Curtis JR, Mikuls TR, Cofield SS, Bridges SL Jr, Ranganath VK, et al. Validation of methotrexate as first-line therapy in rheumatoid arthritis. Arthritis Rheum. 2007;56(4):1219-23.
25. Vermeer E, Hebing RCF, van de Meeberg MM, et al. Oral versus subcutaneous methotrexate in immune-mediated inflammatory disorders: an update. Curr Rheumatol Rep. 2023;25:276-84.

26. Lima A, Bernardes M, Azevedo R, Medeiros R, Seabra V. Pharmacogenomics of the methotrexate membrane transport pathway. Int J Mol Sci. 2015;16(6):13760-80.
27. VanWert AL, Sweet DH. Impaired clearance of methotrexate in organic anion transporter 3 knockout mice. Pharm Res. 2008;25(2):453-62.
28. Bannwarth B, Péhourcq F, Lequen L. Pharmacokinetics of methotrexate in rheumatoid arthritis. Therapie. 1997;52(2):129-32.
29. Dalrymple JM, Stamp LK, O'Donnell JL, Chapman PT, Zhang M, Barclay ML. Pharmacokinetics of oral methotrexate in rheumatoid arthritis. Arthritis Rheum. 2008;58(11):3299-308.
30. Torres RP, Santos FP, Branco JC. Methotrexate: Implications of pharmacogenetics in rheumatoid arthritis. ARP Rheumatol. 2022;1(3):225-9.

Cite this article as: Menon A, Murugesan P, Tamilarasan PP, Palanisamy SA, Parthasarathy AK. Beyond folate antagonism: the unique pharmacological blueprint of methotrexate. Int J Basic Clin Pharmacol 2026;15:195-200.