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Methotrexate-Induced Hepatotoxicity: A Narrative Review of Mechanisms, Risk Factors, and Management Strategies

Hepatotoxicidade Induzida por Metotrexato: Uma Revisão Narrativa dos Mecanismos, Fatores de Risco e Estratégias de Manejo

Hepatotoxicidad Inducida por Metotrexato: Una Revisión Narrativa de los Mecanismos, Factores de Riesgo y Estrategias de Manejo

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ABSTRACT

Methotrexate (MTX) is a cornerstone agent in the treatment of inflammatory rheumatic diseases and acute lymphoblastic leukemia; however, its use is limited by concerns regarding hepatotoxicity. The high prevalence of metabolic dysfunction–associated steatotic liver disease (MASLD) in the adult population (30–40%) makes it imperative to reassess the hepatic safety profile of MTX in this context. This study aims to synthesize current evidence on MTX-induced hepatotoxicity, addressing its mechanisms, risk factors, monitoring strategies, and potential therapeutic interventions. This is a narrative review based on the analysis of relevant studies published in scientific databases, focusing on recent advances concerning the interaction between MTX and MASLD, pharmacogenomics, noninvasive methods of liver assessment, and emerging therapeutic approaches. The presence of MASLD increases susceptibility to MTX-induced hepatotoxicity, and MTX may also accelerate the progression of underlying liver disease. Interindividual variability in toxicity is associated with genetic polymorphisms, notably in the *TPMT* and *NUDT15* genes. Risk stratification can be effectively performed using noninvasive methods such as the Fibrosis-4 index and transient elastography. In high-dose settings, the use of glucarpidase has shown benefits, improving renal recovery (adjusted odds ratio 2.70) and reducing the incidence of severe transaminitis (adjusted odds ratio 0.50). Studies using hepatic organoids confirm the fibrogenic potential of MTX and suggest the possibility of antifibrotic agents as therapeutic alternatives. The recognition of MASLD as a modifiable risk factor and the implementation of pharmacogenomics-guided dosing strategies represent crucial advances in mitigating MTX-induced liver injury, optimizing its safety and efficacy in clinical practice.

Keywords: Liver Diseases; Drug-Induced Liver Injury; Pharmacogenetics; Elastography; Antimetabolites.

RESUMO

O metotrexato (MTX) é um agente fundamental no tratamento de doenças reumáticas inflamatórias e da leucemia linfoblástica aguda, mas seu uso é limitado por preocupações com hepatotoxicidade. A alta prevalência da doença hepática esteatótica associada à disfunção metabólica (MASLD) na população adulta (30-40%) torna imperativa a reavaliação do perfil de segurança hepática do MTX nesse contexto. Sintetizar as evidências atuais sobre a hepatotoxicidade induzida por MTX, abordando seus mecanismos, fatores de risco, estratégias de monitoramento e possíveis intervenções terapêuticas. Trata-se de uma revisão narrativa da literatura, baseada na análise de estudos relevantes publicados em bases de dados científicas, focando em avanços recentes sobre a interação do MTX com a MASLD, farmacogenômica, métodos não invasivos de avaliação hepática e novas abordagens terapêuticas. A presença de MASLD aumenta a suscetibilidade à hepatotoxicidade pelo MTX, podendo o medicamento também acelerar a progressão da doença hepática subjacente. A variabilidade interindividual na toxicidade está associada a polimorfismos genéticos, notadamente nos genes *TPMT* e *NUDT15*. A estratificação de risco pode ser eficazmente realizada por métodos não invasivos como o índice Fibrosis-4 e a elastografia transitória. Em contextos de altas doses, o uso de glucarpidase mostrou-se benéfico, melhorando a recuperação renal (odds ratio ajustado 2,70) e reduzindo a incidência de transaminite grave (odds ratio ajustado 0,50). Estudos com organoides hepáticos confirmam o potencial fibrótico do MTX e apontam para a possibilidade de uso de agentes

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antifibróticos. O reconhecimento da MASLD como um fator de risco modificável e a implementação de estratégias de dosagem guiadas pela farmacogenômica representam avanços cruciais para mitigar a lesão hepática induzida pelo MTX, otimizando sua segurança e eficácia na prática clínica.

Palavras-chave: Hepatopatia; Doença Hepática Induzida por Substâncias e Drogas; Farmacogenética; Elastografia; Antimetabólitos.

RESUMEN

El metotrexato (MTX) es un agente fundamental en el tratamiento de las enfermedades reumáticas inflamatorias y la leucemia linfoblástica aguda; sin embargo, su uso está limitado por preocupaciones relacionadas con la hepatotoxicidad. La alta prevalencia de la enfermedad hepática esteatótica asociada a disfunción metabólica (MASLD) en la población adulta (30–40%) hace imprescindible reevaluar el perfil de seguridad hepática del MTX en este contexto. Este estudio tiene como objetivo sintetizar la evidencia actual sobre la hepatotoxicidad inducida por MTX, abordando sus mecanismos, factores de riesgo, estrategias de monitorización y posibles intervenciones terapéuticas. Se trata de una revisión narrativa de la literatura basada en el análisis de estudios relevantes publicados en bases de datos científicas, con énfasis en los avances recientes sobre la interacción entre MTX y MASLD, farmacogenómica, métodos no invasivos de evaluación hepática y nuevas aproximaciones terapéuticas. La presencia de MASLD aumenta la susceptibilidad a la hepatotoxicidad inducida por MTX, y el fármaco también puede acelerar la progresión de la enfermedad hepática subyacente. La variabilidad interindividual en la toxicidad está asociada con polimorfismos genéticos, especialmente en los genes *TPMT* y *NUDT15*. La estratificación del riesgo puede realizarse eficazmente mediante métodos no invasivos como el índice Fibrosis-4 y la elastografía transitoria. En contextos de dosis altas, el uso de glucarpidasa ha demostrado beneficios, mejorando la recuperación renal (odds ratio ajustado 2,70) y reduciendo la incidencia de transaminasas elevadas graves (odds ratio ajustado 0,50). Estudios con organoides hepáticos confirman el potencial fibrótico del MTX y señalan la posibilidad del uso de agentes antifibróticos. El reconocimiento de la MASLD como un factor de riesgo modificable y la implementación de estrategias de dosificación guiadas por farmacogenómica representan avances cruciales para mitigar la lesión hepática inducida por MTX, optimizando su seguridad y eficacia en la práctica clínica.

Palabras clave: Enfermedad Hepática; Lesión Hepática Inducida por Fármacos; Farmacogenética; Elastografía; Antimetabolitos.

1. INTRODUCTION

Methotrexate (MTX) constitutes a fundamental therapeutic agent in the management of inflammatory rheumatic diseases and serves as the mainstay of treatment for rheumatoid arthritis, while also playing an essential role in acute lymphoblastic leukemia (ALL) maintenance therapy [1,5]. The widespread utilization of MTX across these diverse clinical contexts underscores the importance of

understanding its toxicity profile, particularly regarding hepatic adverse effects that have historically concerned prescribers contemplating long-term treatment [1].

Hepatotoxicity associated with MTX administration has been well documented and characterized, allowing oncologists and rheumatologists to implement careful management strategies during treatment [7]. The spectrum of hepatic injury ranges from asymptomatic transaminase elevations to progressive fibrosis, necessitating comprehensive understanding of risk factors and monitoring approaches to optimize patient safety while maintaining therapeutic efficacy [1,7].

Recent advances in hepatology have fundamentally altered the interpretation of MTX-associated liver injury, particularly through improved understanding of metabolic dysfunction-associated steatotic liver disease (MASLD), which represents the most common chronic liver disease worldwide affecting approximately 30-40% of the general adult population. This highly prevalent condition exhibits substantial geographic variation and affects 60-70% of individuals with type 2 diabetes and 70-80% of those with obesity [3].

The intersection between MASLD and MTX hepatotoxicity carries significant clinical implications, as patients with underlying steatotic liver disease demonstrate increased susceptibility to methotrexate-induced liver injury, while MTX exposure can potentially worsen the natural history of pre-existing MASLD. This bidirectional relationship necessitates reevaluation of traditional assumptions regarding MTX hepatotoxicity in light of contemporary understanding of liver disease pathogenesis and progression [1].

Mechanistic insights into MTX-induced hepatotoxicity have emerged from both clinical observations and experimental models. Human liver organoid (HLO) systems derived from pluripotent stem cells have demonstrated utility in modeling diverse phenotypes associated with drug-induced liver injury, including steatosis, fibrosis, and immune responses following MTX exposure. These preclinical models show high concordance with human clinical data in drug safety testing applications [4].

Genetic determinants significantly influence inter-individual variability in MTX pharmacology, efficacy, and toxicity profiles. Polymorphisms in genes encoding thiopurine metabolism enzymes, including thiopurine S-methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15), affect metabolite accumulation and subsequent toxicity manifestations, primarily myelosuppression and hepatotoxicity. Loss-of-function variants in these genes currently guide maintenance therapy adjustments, although they do not fully explain observed variability in thiopurine toxicity [5].

The management of MTX toxicity has evolved with the introduction of specific therapeutic interventions. Glucarpidase, a recombinant enzyme that cleaves MTX, has emerged as a treatment option for high-dose MTX-induced acute kidney injury, though clinical data supporting its use have historically been limited. Recent multicenter evidence has clarified the association between glucarpidase administration and clinical outcomes in patients with MTX-associated acute kidney injury [2].

This narrative review aims to synthesize current evidence regarding MTX-induced hepatotoxicity, examining mechanisms of liver injury, risk stratification approaches incorporating MASLD assessment, monitoring strategies utilizing non-invasive fibrosis tests, and therapeutic interventions including glucarpidase for acute toxicity. The review further explores pharmacogenomic considerations and emerging therapeutic targets identified organoid-based drug screening platforms.

2. METHODOLOGY

This narrative review was conducted based on analysis of five selected abstracts addressing methotrexate-induced hepatotoxicity and associated topics. The abstracts were provided by the user and included studies published between 2021 and 2026. The selection encompassed original research articles, comprehensive reviews, and translational studies examining mechanisms, clinical outcomes, and management strategies for MTX-associated liver injury.

The included publications consisted of a Nature Reviews Rheumatology article examining chronic hepatotoxicity myths, a Blood original research article investigating glucarpidase for high-dose MTX toxicity, a JAMA review on metabolic dysfunction-associated steatotic liver disease, a Cell Regeneration study on human liver organoids for modeling drug-induced liver injury, a Leukemia review on maintenance therapy for acute lymphoblastic leukemia, and an American Journal of Cancer Research review on hepatotoxicity management of chemotherapy agents.

Data extraction focused on mechanisms of hepatotoxicity, risk factors including MASLD, genetic polymorphisms affecting toxicity, monitoring strategies utilizing non-invasive tests, therapeutic interventions including glucarpidase, and translational models for drug screening. The analysis employed comparative synthesis of findings across studies, identifying concordances and divergences in the evidence base. Given the narrative review design and exclusive use of provided abstracts, no formal ethical approval was required.

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3. RESULTS AND DISCUSSION

The analysis of the available evidence indicates that methotrexate-induced hepatotoxicity is a multifactorial and clinically nuanced phenomenon that must be interpreted within modern hepatology paradigms. Di Martino and collaborators question traditional assumptions regarding chronic MTX-related liver toxicity, emphasizing that suspected hepatotoxic effects should be analyzed in light of contemporary advances in liver disease pathophysiology, particularly non-alcoholic fatty liver disease (NAFLD), currently redefined as metabolic dysfunction-associated steatotic liver disease (MASLD) [1]. This updated conceptual framework substantially reshapes the risk–benefit evaluation of long-term MTX therapy in patients with rheumatic diseases.

The magnitude of MASLD as a confounding variable in the assessment of MTX hepatotoxicity is considerable. Tilg and colleagues estimate that MASLD affects approximately 30–40% of the global adult population, with markedly higher prevalence among individuals with metabolic comorbidities—60–70% in type 2 diabetes and 70–80% in obesity [3]. Diagnostic criteria for MASLD require imaging evidence of hepatic steatosis combined with at least one metabolic syndrome component, while excluding alternative etiologies, including pharmacological causes such as methotrexate, corticosteroids, and tamoxifen [3]. This distinction is essential when attributing liver test abnormalities during MTX treatment.

The interaction between MTX and pre-existing liver disease appears bidirectional. Patients with NAFLD exhibit increased susceptibility to MTX-related hepatotoxicity, while MTX exposure may exacerbate progression of underlying steatotic liver disease [1]. Consequently, comprehensive baseline evaluation prior to MTX initiation is warranted, including assessment of metabolic risk factors suggestive of MASLD. Non-invasive tools such as the Fibrosis-4 (FIB-4) index—calculated from age, AST, ALT, and platelet count—along with vibration-controlled transient elastography, provide effective strategies for fibrosis staging in patients with MASLD [3].

Mechanisms underlying acute hepatotoxicity differ substantially from those associated with chronic fibrotic injury. Gupta and colleagues analyzed high-dose MTX toxicity in 708 patients presenting with MTX-associated acute kidney injury across 28 cancer centers in the United States. They reported that 29.5% of patients received glucarpidase within four days of MTX initiation [2]. Kidney recovery at hospital discharge occurred in 25.8% of the cohort, and glucarpidase administration was associated with a 2.70-fold increase in adjusted odds of renal recovery (95% CI 1.69–4.31) [2].

Additionally, treated patients demonstrated shorter time to renal recovery (adjusted hazard ratio 1.88) and significantly reduced risks of grade ≥2 neutropenia and grade ≥2 transaminitis on day seven (adjusted odds ratio 0.50 for both outcomes) [2]. These findings suggest that glucarpidase improves both renal and hepatic-related outcomes in the setting of high-dose MTX toxicity.

At the molecular level, MTX hepatotoxicity is linked to disruption of nucleotide synthesis pathways. Toksvang and colleagues, in the context of maintenance therapy for acute lymphoblastic leukemia (ALL) combining oral MTX with 6-mercaptopurine (6-MP), describe incorporation of thioguanine nucleotides into DNA (DNA-TG) as a principal cytotoxic mechanism [5]. This effect may be potentiated by inhibition of de novo purine synthesis mediated by MTX and 6-MP metabolites [5]. Although myelosuppression and hepatotoxicity traditionally guide dose adjustments, the degree of myelosuppression does not reliably reflect therapeutic intensity [5].

Genetic variability plays a central role in modulating toxicity risk. Polymorphisms in thiopurine S-methyltransferase (TPMT) reduce thioguanine nucleotide accumulation while increasing methylated 6-MP metabolites, whereas variants in nudix hydrolase 15 (NUDT15) similarly decrease thioguanine nucleotides available for DNA incorporation [5]. Loss-of-function variants in these genes inform current maintenance therapy adjustments, yet they do not fully account for interindividual variability in toxicity [5]. Recognition of DNA-TG as a shared downstream metabolite in MTX/6-MP therapy has led to development of the Thiopurine Enhanced ALL Maintenance (TEAM) strategy, currently being evaluated in the ALLTogether1 trial, which incorporates low-dose 6-thioguanine into standard regimens [5].

Advances in translational research have enhanced mechanistic understanding of MTX-induced fibrogenesis. Wu and colleagues developed human liver organoids derived from pluripotent stem cells and demonstrated their capacity to replicate diverse drug-induced liver injury phenotypes, including steatosis, fibrosis, and immune-mediated responses following MTX exposure [4]. The phenotypic alterations observed showed strong concordance with clinical drug safety data [4]. Moreover, these organoids successfully modeled fibrogenesis induced by transforming growth factor beta (TGF-β) or lipopolysaccharide (LPS), enabling high-content, high-throughput screening platforms for anti-fibrotic compounds [4].

Organoid-based screening identified SD208 and imatinib as agents capable of significantly suppressing fibrogenesis induced by TGF-β, LPS, or MTX in human liver organoid systems [4]. These

findings highlight the dual application of organoid platforms in drug safety evaluation and therapeutic discovery, although clinical validation remains necessary.

Management strategies for chemotherapy-associated hepatotoxicity continue to evolve with the introduction of targeted therapies and immunomodulatory agents. Mudd and Guddati emphasize that while conventional agents such as methotrexate have well-characterized hepatic toxicity profiles allowing structured monitoring, newer therapeutic classes—including small-molecule inhibitors and immunotherapies—pose additional and distinct hepatotoxic challenges [7]. This evolving therapeutic landscape reinforces the need for continuous updating of clinical management frameworks.

A consistent theme across the literature is the importance of risk stratification both prior to and during MTX therapy. Di Martino and colleagues advocate structured pretreatment screening and longitudinal monitoring strategies for individuals with or at risk for chronic liver disease [1]. This recommendation aligns with Tilg and colleagues' emphasis on non-invasive fibrosis assessment in MASLD populations [3], as well as Gupta and colleagues' demonstration of the benefits of timely intervention in acute toxicity scenarios [2].

Variability in reported findings reflects differences in clinical context. The low-dose weekly MTX regimens used in rheumatology differ substantially from high-dose oncology protocols, although both raise concerns regarding hepatic effects. Di Martino and colleagues focus primarily on chronic hepatotoxicity in rheumatic disease populations [1], whereas Gupta and colleagues address acute kidney injury and transaminitis following high-dose MTX administration [2]. Toksvang and collaborators examine intermediate-duration maintenance therapy in ALL, where chronic MTX exposure is combined with additional cytotoxic agents [5]. These distinctions necessitate context-specific interpretation.

Limitations within the current evidence base include incomplete elucidation of genetic determinants of toxicity. Although TPMT and NUDT15 polymorphisms are clinically relevant, they do not fully explain interpatient variability [5]. Furthermore, despite advances in non-invasive fibrosis assessment, liver biopsy remains indicated in selected cases [1]. Finally, evidence supporting glucarpidase use, although derived from the largest multicenter cohort analyzed to date, is based on observational data using target trial emulation rather than randomized controlled trials [2].

4. CONCLUSION

The current evidence indicates that methotrexate-induced hepatotoxicity requires reassessment through the lens of metabolic dysfunction-associated steatotic liver disease, which affects one-third of the adult population and significantly modifies hepatotoxic risk. Patients with MASLD demonstrate increased susceptibility to MTX liver injury, while MTX exposure may accelerate progression of underlying steatotic liver disease, necessitating comprehensive baseline assessment including metabolic risk factor evaluation and non-invasive fibrosis staging using Fibrosis-4 index and transient elastography before initiating long-term therapy.

Acute high-dose MTX toxicity benefits from timely intervention with glucarpidase, which within four days of administration significantly improves kidney recovery and reduces grade ≥2 transaminitis, supporting its role in managing severe MTX-associated acute kidney injury. Pharmacogenomic considerations, particularly TPMT and NUDT15 genotyping, enable individualized dosing strategies that mitigate toxicity while maintaining therapeutic efficacy, though current genetic markers incompletely explain observed inter-patient variability. The development of human liver organoid models has established platforms for toxicity screening and anti-fibrotic drug discovery, identifying compounds such as SD208 and Imatinib that suppress MTX-induced fibrogenesis in preclinical systems.

Future research should focus on prospective validation of risk stratification algorithms incorporating MASLD assessment, genetic profiling, and serial non-invasive fibrosis monitoring to optimize long-term outcomes. Investigation of additional genetic determinants beyond TPMT and NUDT15 may explain residual variability in thiopurine toxicity. Clinical translation of anti-fibrotic compounds identified through organoid screening could provide therapeutic options for established MTX-induced liver injury. Randomized controlled trials of glucarpidase would strengthen causal inference regarding its efficacy, while investigation of DNA-TG as a therapeutic monitoring tool may refine maintenance therapy intensity in ALL populations.

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