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Methotrexate Toxicity: Molecular Mechanisms and Management

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Methotrexate (MTX) is the most widely used drug in cancer chemotherapy and is considered to be the first-line drug for the treatment of a number of rheumatic and non-rheumatic disorders. The pulmonary toxicity, hepatotoxicity of MTX are two of its major side effects. Other toxicities such as endocrinological toxicity, GI toxicity, cutaneous toxicity, hematological toxicity, fatal malfunction or loss, and malignancy can also occur, but at a significantly lower rate of prevalence. This review aims to provide a comprehensive understanding of the molecular mechanisms of methotrexate toxic effects and Lastly, we discussed the management of this toxicity.

Keywords: Methotrexate; Methotrexate toxicity; Rheumatoid Arthritis; Organ toxicity; Mechanism of methotrexate toxicity; Management of methotrexate-induced toxicity; hepatotoxicity; pulmonary toxicity.

1. INTRODUCTION

1.1 Overview of Methotrexate

4-amino-10-methylfolic acid, also referred to as methotrexate (MTX), acts as a folic acid analogue and antagonist. It is generally employed for the therapy of a spectrum of neoplastic and non-neoplastic pathologies [1]. Initially, MTX was utilised as an anti-tumour agent. Currently, it is one of the principal

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disease-modifying anti-rheumatic drugs (DMARDS) indicated for conditions, such as psoriatic skin disorders, rheumatoid arthritis (RA) and juvenile idiopathic arthritis. Additionally, it is a useful agent for the management of inflammatory bowel disorders, multiple sclerosis, vasculitic conditions and connective tissue pathologies, such as systemic lupus erythematosus, amongst others. In addition, its valuable anti-inflammatory and immunomodulatory features are important components of transplantations [1–3].

The treatment of viral mediated arthritis has received increasing attention due to MTX [4]. The multiple viruses associated with inflammatory joint pathologies encompass Old World alphaviruses, parvovirus B19, hepatitis B (HBV) and C (HVC), respectively, and human immunodeficiency virus (HIV) [5]. From a clinical perspective, ongoing viral-induced joint inflammation can mimic RA, and be present for periods of months to years [6]. In view of the comparable disease processes underlying RA and viral arthritides, MTX may of value in the treatment of the latter. However, an important factor that should not be disregarded is the possible risk of compromising patients' immune surveillance to avert viral reactivation [7]. The World Health Organisation (WHO) deem MTX a vital medication. Undeniably, it is one of the most significant pharmaceutical advancements as it identified indications that differ substantially from its initial purpose [8,9].

Numerous advantageous biological compounds have entered the clinical arena as therapeutic agents for autoimmune inflammatory pathologies, e.g. RA. However, MTX is well-established as highly efficacious; it is a commonly used treatment which is often used as a benchmark against which to compare the performance of de novo DMARDs [1]. As a single agent, MTX can be employed as the initial drug of choice in individuals who have not previously received DMARDs [10]. Additionally, it can be utilised as an anchor agent in individuals who have had an insufficient reaction to MTX, together with additional characteristic or biological DMARDS in order to enhance disease control [11,12]. In the case of inflammatory diseases, MTX is administered in less frequent and lower doses. To compare, treating malignancy entails 5g per week doses, whereas for RA, the most effectual clinical results are observed after 10-25mg once per week doses of MTX. This represents the most typical indication for low-dose MTX usage [13]. The essential pathways that underlie the beneficial effect of larger doses of MTX on neoplastic pathologies are well-known. In its capacity as an antagonist to folic acid, MTX inhibits the function of enzymes reliant on folate, and therefore the manufacture of purine and pyrimidine essential for the generation of nuclear material that is rapidly replicated within tumour cell lines [14].

However, there is less clarity regarding the effect of the mechanisms involved in low doses of MTX in inflammatory disorders. Whilst MTX is recognised as being very cost-effective with strong efficacy/toxicity ratios, toxicity nevertheless remains an issue. Attention has been drawn to the possible adverse incidents associated with MTX as they reflect the main reason for its cessation [15-16]. Although it is widely utilised in the treatment of a range of autoimmune and inflammatory disorders, there is still drug toxicity associated with low dose MTX. The most frequently arising adverse events associated with MTX are principally related to the digestive tract, e.g. nausea, vomiting, oral inflammation, anorexia and liver damage [17]. Typically, toxicity rather than ineffectiveness is the primary cause of MTX treatment withdrawal [17]. Thus, periodic, meticulous, and suitable patient monitoring is critical and evidently substantially decreases the risks related to MTX usage [18]. The pathways underlying the toxic consequences of MTX have not been fully delineated. Some effects, such as diminished cell counts, digestive issues and oral inflammation are related to folic acid lack; complementary folic acid or folinic acid may resolve such manifestations [19]. Adverse events that are unconnected to its effect on folic acid encompass the presence of nodules, lung fibrosis, apathy, exhaustion and kidney dysfunction [14]. A more in-depth comprehension of MTX's molecular mechanisms may aid in clarifying numerous toxicities related to MTX [14].

2. HEPATOTOXICITY

2.1 Molecular Pathways Underlying Hepatotoxicity Related to MTX Administration

There are two routes through which MTX can gain access to liver cells. These are via the folate transporter, i.e. solute carrier family 19, member 1, and via reduced folate carrier 1 [20]. Within the liver cells, MTX is converted into MXT polyglutamates (MTX-PGs), a process catalysed

by the enzyme, folylpolyglutamate synthetase (FPGS). Glutamate is concurrently eradicated from MTX-PGs via gamma-glutamyl hydrolase activity, which reverts them back to MTX [21].

Adenosine triphosphate (ATP)-binding cassette transporter A1 eliminates the glutamate-free MTX from the hepatocytes [21]. MTX-PGs stay present within the target cells for a considerable time period, during which they trigger a spectrum of disease-inducing pathways linked to inflammatory processes, oxidative stress, fibrosis and programmed cell death within the cell. Hence, the exploration of MTX-generated toxicities necessitates monitoring the level of MTX-PG [22]. Basic research has emphasized the likelihood that MTX may precipitate oxidative stress within hepatic tissue. In the liver tissue, the inception of intracellular oxidative stress suggests the MTX-PGs metabolite. As previously discussed, there has been empirical and clinical verification of the elevation of serum transaminases (AST and ALT) following the administration of MTX [23-26]. Moreover, as indicated by previous research, its MTX- PG metabolites induced lipid peroxidation signifies that the elevation of hepatotoxicity's marker enzymes can be connected to MTX-induced hepatocellular degeneration. The breakdown products from MTX-PGS initiate lipid peroxidation which liberates ROS, NO and additional free radicals; these lead to cytoplasmic antioxidant downregulation, which includes both enzymes and non-enzymatic compounds, such as SOD, CAT, GSH and GPx [24-27].

The reduction in intracellular antioxidants may reflect their excessive engagement with the surplus generation of free radicals arising from intracellular MTX-PGs. The latter lead to the breakdown of liver cell membranes, with the consequent liberation of cytoplasmic components into the extracellular matrix. This may be the immediate reason for raised serum titres of AST and ALT associated with MTX. ARE, i.e. a cis-acting enhancer sequence, and whose locus is within the gene promotor region responsible for detoxification enzyme decoding, functions to diminish oxidative stress within the cell, mediating transcriptional stimulation in genes that govern antioxidant operations [28]. The redox equilibrium within the cell is moderated by ARE-linked genes, i.e. Nrf2, HO-1 and NQO1 [29-31]. It has been demonstrated that ARE is restricted by intracellular ROS which are derived from MTX-PGs; these are additionally responsible for the downregulation of oxidant resistant mediators, e.g. Nrf2, HO-1 and NQO1 [32-35]. These studies also highlight MTX's oxidative stress-inducing potential in the tissue of the liver. NF-κB, JNK and JAK (Janus kinase)-STAT-3 signalling cascades may also be initiated during the inflammatory response [36].Intracellular ROS are triggered by MTX-PG which, in turn, activates transcription factors, e.g. NF-kB and Nrf-2. A pro- inflammatory response is instigated via their nuclear translocation and the liberation of multiple enzymes and cytokines related to the inflammatory process, such as COX-2, NOS, TNF-α, IL-1β, 6 and 12 [37-38].

Typically, advancement to hepatic fibrosis is induced by continual liver inflammation [39]. Following damage to the liver, injured hepatocytes discharge diverse pro-inflammatory markers, which convert inactive HSCs to an active state or generate phenotypes akin to myofibroblasts (MFBs). The triggered HSCs give rise to the marked production of the extracellular matrix that generates liver fibrosis [40-41]. Furthermore, the accrual of MTX-PG within the liver tissue stimulates MTHFR, which, in turn, precipitates raised titres of intracellular and serum homocysteine [42-43].There is an association between the accumulation of intracellular homocysteine levels generated by MTX-PGs and the progression of hepatic oxidative stress, and inflammatory and fibrotic processes [44-45]. Oxidative stress is a major pathogenetic mechanism for the latter [46] qHSCs are stimulated within the peri-sinusoidal space as a consequence of the MTX-PG-induced ROS synthesis and subsequent oxidative stress; a stimulated MFBs-like phenotype is thus exhibited. This gives rise to the heightened extracellular matrix manufacture and liver tissue fibrosis. The ATIC enzyme, which converts AICAR to formyl AICAR, is inhibited by MTX-PG. Additionally, MTX-PG obstructs DHFR, which is a catalyst for the decrease of dihydrofolate to tetrahydrofolate. It constrains thymidylate synthetase, which causes thymidine residues to form. Thus, the accrual of adenosine within the cell is a function of the MTX-PGs. This then activates the fibrotic process via collagen manufacture amplification and matrix- degrading matrix metalloproteinase (MMP) downregulation (Fig. 1).

The demise of liver cells reflects the diminution of liver folate concentrations and the manufacture of nuclear material within the cells as a result of the activities of MTX-PGs [47-48].

Programmed cell death of liver cells is a recognised outcome associated with DILI [49]. Caspase 3 expression is amplified by MTX; this initiates the intrahepatocytic apoptotic pathway, thus leading to cellular demise [50]. The equilibrium of pro- (bcl2) and anti- (bax) apoptotic indicators is altered by MTX-induced proinflammatory indicators [51].

The mitochondria operate as the source of ROS and their target concurrently. MTX has been demonstrated to stimulate hepatic steatosis, a process which arises via the mitochondrial respiratory chain and mitigation of the oxidation of fatty acids. Surplus ROS within the cell can alter the membrane potential of the mitochondria (ΔΨm) [52]. Following administration of MTX in liver tissue, both ROS-mediated mitochondrial toxicity and ΔΨm dispersion were observed [53]. The changes in bax and bcl-2 equilibrium together with ΔΨm attrition initiated by MTX-PG can lead to the liberation of cytochrome c from the mitochondria into the cytoplasmic matrix. Here, apoptosomes are generated from cytochrome c, which synthesise caspase 3; this enzyme triggers programmed liver cell death. The ways in which MTX can induce liver toxicity are illustrated in Fig. 2.

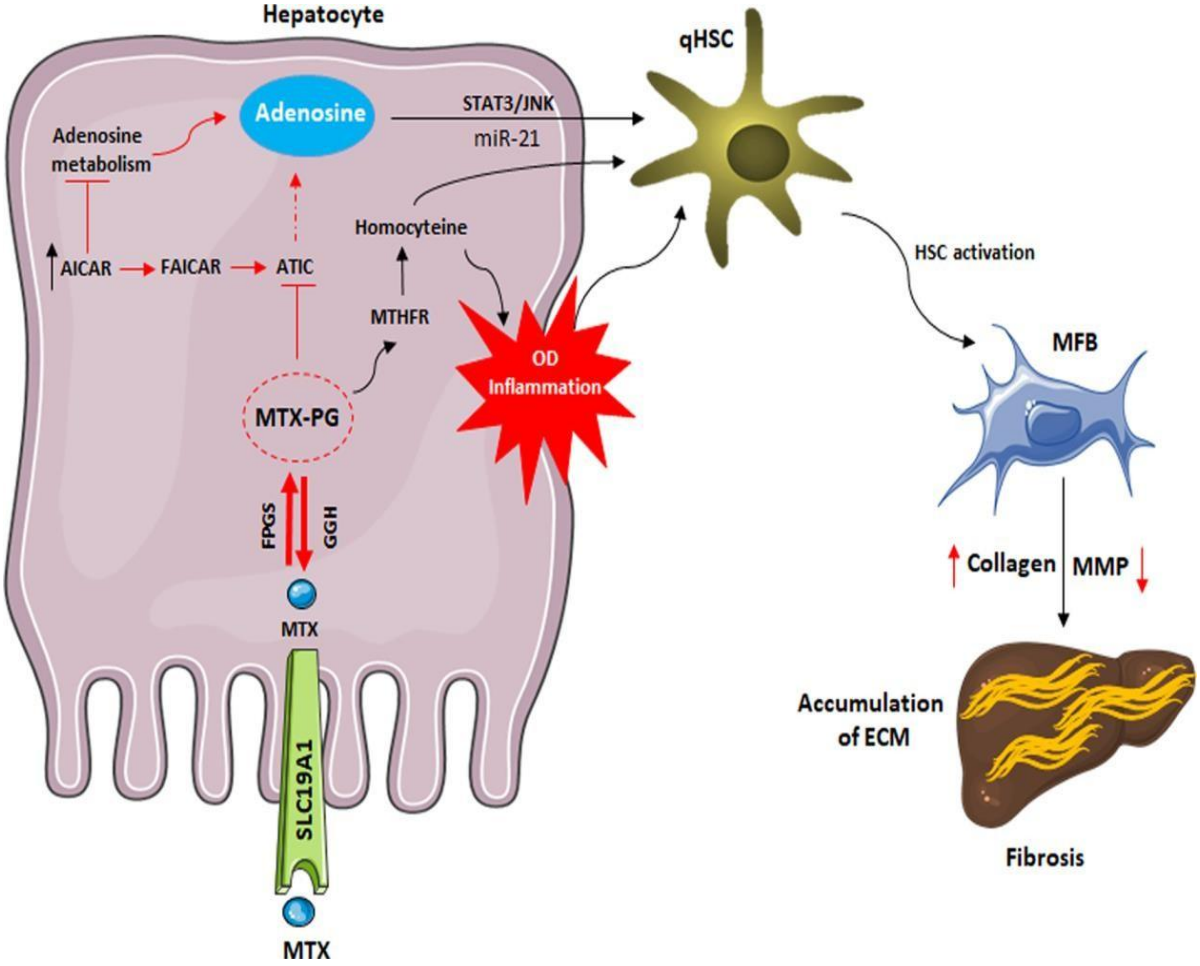

Fig. 1. Potential pathways underlying liver fibrosis associated with methotrexate (MTX). ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase; FAICAR, 5-formamidoimidazole-4-carboxamide ribonucleotide; MTX-PG, MTX- polyglutamates; FPGS, folylpolyglutamate synthetase; GGH, gamma-glutamyl hydrolase; SLC19A1, solute Carrier Family 19 Member 1; qHSC, quiescent HSC; MFB, myofibroblast; MMP, matrix metalloproteinases; ECM, extracellular matrix; MTHFR, Methylenetetrahydro folate reductase; OD, oxidative stress; STAT3, signal transducer and activator of transcription 3; JNK, c-Jun N-terminal kinases.Adopted from [42]

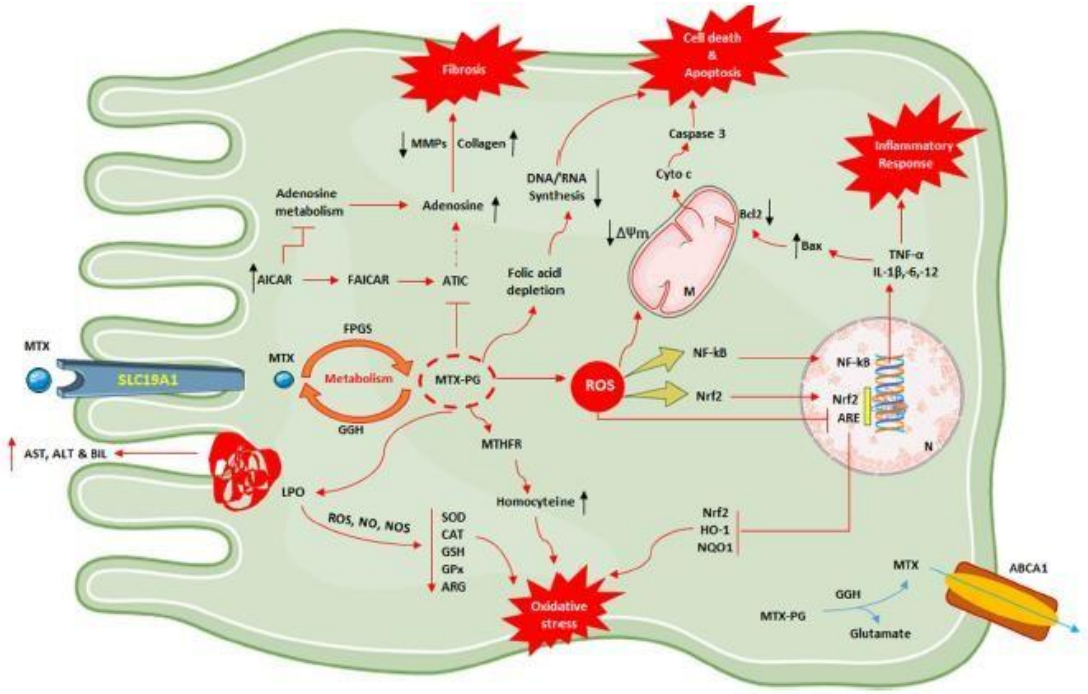

Fig. 2. Pathways underlying liver toxicity associated with methotrexate (MTX). SLC19A1, solute Carrier Family 19 Member 1; ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase; FAICAR, 5-formamidoimidazole-4-carboxamide ribonucleotide; MTX-PG, MTX- polyglutamates; FPGS, folylpolyglutamate synthetase; GGH, gamma- glutamyl hydrolase; qHSC, quiescent HSC; MFB, myofibroblast; MMP, matrix metalloproteinases; ECM, extracellular matrix; LPO, lipid peroxidation; ROS, reactive oxygen species; NO, nitric oxide; NOS, nitric oxide synthase; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; GPx, glutathione peroxidase; ARG, arginase; MTHFR, methylenetetrahydro folate reductase; TNF-α tumour necrosis factor-α; NF-κB, nuclear factor kappa B; IL, interleukin; ΔΨm, mitochondrial membrane potential; M, mitochondria; N, nucleus; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, hemoxygenase-1; NQO1, NAD(P)H dehydrogenase (quinone) 1; ARE, antioxidant response element; ABCA1, adenosine triphosphate (ATP)-binding cassette transporter A1, AST, aspartate transaminases; ALT, alanine transaminases; BIL, bilirubin .Adopted from [42].

3. PULMONARY TOXICITY

3.1 Molecular Pathways Underlying Pulmonary Toxicity Related to MTX Administration

It was documented that approximately 25% of RA patients undergoing MTX therapy displayed a range of symptoms including wheezing, coughing, exertional dyspnoea, and other pulmonary indications [54]. Side effects could occur in patients from as early as four weeks following the commencement of the treatment. They are considered an idiosyncratic immune response rather than a dose-related toxic occurrence impacting the lung [55]. Additionally, RA patients who had undergone MTX treatment presented a greater risk of developing lung diseases than other DMARDs [56]. Damage to the lungs associated with MTX could additionally manifest as fibrosis, interstitial pneumonitis or widespread alveolar injury [54]. Therefore, it is critical that recognition is rapid before interstitial pneumonitis worsens to severe pulmonary fibrosis. The pathological mechanism could be separated into the following three categories: (i)inflammatory; (ii) infections; (iii) lymphoproliferative [57]. However, the precise mechanism is complex and the full picture lacks clarity.

Elevated levels of IL-8 in BAL fluid and the build-up neutrophils cause MTX-induced pneumonitis [58]. IL-8 is a primary neutrophil chemotactic element that results in a neutrophilic influx in the lung [59, 60]. The mRNA expression of IL-8 and

the secretion of IL- 8 and other cytokines are increased by MTX. Therefore, IL-8 may be integral in pneumonitis out of MTX-induced lung damage [61]. In airway epithelial (A549) cells, the discharge of cytokines such as IL-8, MCP-1 and G-CSF is provoked by MTX-induced pneumonitis [62]. Moreover, in patients with hypersensitivity pneumonitis and sarcoidosis, the discharged IL-8 levels were elevated in their BAL fluid [62]. Pro-inflammatory cytokines, e.g. IL-1β and TNF- α, stimulate the liberation of IL-8; p38 phosphorylation governs the production of IL-8 [61].

There are three main classes of MAPK, i.e. (i) extracellular signal-regulated kinase (ERK); (ii) c-Jun NH2-terminal kinase (JNK); and (iii) p38 kinase pathways [63]. The latter are stimulated by inflammatory cytokines responsible for initiating asthmatic episodes or autoimmune pathology [64]. A MAPK cascade consists of a MAPK kinase kinase (MAPKKK)–MAPK kinase pathway; this is linked by a variety of methods between receptors and transcription targets, upstream and downstream, respectively [64]. In these MAPK signalling cascades, MAPKK is controlled by the phosphorylation of serine/threonine residues on MAPKKK, and this kinase prompts the serial activation of MAPK [65]. Finally, MAPKKK–MAPKK–MAPK pathways phosphorylate diverse substrates including transcription factors. The p38 and JNK pathways in the main MAPK pathways are related to chronic inflammation [64].

It was shown that MTX modulated the p38 MAPK signalling cascade (which is the TAK1–MKK3/MKK6–p38 MAPK–MAPKAPK2–HSP27 module [58]. This signalling pathway caused the discharge of IL-1β and IL-8 [66]. IL-1 causes endogenic TAK1 action [67]. TAK1 (an MAPKKK) is a ubiquitin-reliant kinase of MAPKK that intermingles with the controlling proteins TAB1, TAB2 and TAB3 [68]. The autophosphorylation at Thr-184, Thr-187 and Ser-192 was facilitated by the TAK1/TAB1 complex [65]. TAK1 triggers either MKK4 or MKK3/6, both of which are MAPKK phosphorylate, p38 MAPK members [69]. MAPKAPK2 is activated by the p38 MAPK, which then initiates HSP27's phosphorylation [70]. Moreover, the secretions of IL-1 and IL-8 are elevated by HSP27 [66]. These cytokines respond to TAK1, which is upstream of the p38 MAPK [71]. As presented in Fig.1, the pulmonary inflammatory reaction is the consequence of recycling the IL-1β–TAK1–MKK3/MKK6–p38 MAPK– MAPKAPK2–HSP27-IL-8 module as illustrated in Fig. 3.

It is contended that pro- and anti-inflammatory cytokines' modulation stimulate the pulmonary inflammatory response (Kim et al.) [66,72,73]. MTX's immunoregulatory contribution facilitates a harmony between pro- and anti-inflammatory cytokines. Previous research has demonstrated that IL-1β and IL-8 expression heightened the impact of MTX, whereas conversely, IL-4, IL-6, IL-12, TNF-α, macrophage inflammatory protein-1α (MIP-1α), and macrophage inflammatory protein-1β (MIP-1β) expression reduced it in a dosage-determined way [66]. Pro-inflammatory cytokines are actors in IL-1β, TNF-α, IFN-γ, IL-2, IL-6, IL-8, IL- 12, MIP-1α and MIP-1β, and Regulated on Activation, Normal T Expressed, and Secreted (RANTES). Conversely, anti-inflammatory cytokines, i.e. IL-4, IL-10, TGF-β, TNF-α, IL-1 and IL-8 are believed to be of import in inflammatory lung disease and in idiopathic pulmonary fibrosis [73]. Potentially, MTX could stimulate a pulmonary inflammatory reaction through the discharge of IL-1β and IL-8 but not TNF-α in pro-inflammatory cytokines in bronchial epithelial cells. IL-4 may also be significant in the MTX-triggered pulmonary inflammatory reaction. Conversely, cytokines including TNF-α, IFN-γ, and GM-CSF in T lymphocytes constrain secretion in RA therapy, with MTX acting as an agent of chemotherapy [74]. Hence, this forms a fresh molecular foundation of the therapeutic impacts according to the constraint of molecular targets including p38, and transcription factor HSP27, and impeding the discharge of the IL-1 and IL-8 pro-inflammatory cytokines.

4. NEUROTOXICITY

It has been documented that MTX is associated with notable lethargy in some individuals [75], which may reflect the actions of intracerebral adenosine. It is widely acknowledged that adenosine has neuromodulatory properties, and effects of its build-up in the CNS include headaches, nausea, and drowsiness [76]. Adenosine is implicated in the governance of the awake status and somnolence via its operation on the A1 receptors located in the perifornical lateral hypothalamus. This action may be the underlying mechanism of the drowsiness described by some individuals after taking MTX [75]. In paediatric patients, high-dose MTX has been documented to produced marked somnolence and unconsciousness [76] . Some studies have demonstrated that the non-selective adenosine receptor antagonist,

theophylline, is able to offset the toxic effects of MTX on the CNS in this population [76].

Another potential mechanism underlying the adverse cerebral manifestations of MTX may be raised titres of homocysteine and its breakdown products, e.g. the amino acid neurotransmitters homocysteic acid and cysteine sulphinic acid; the latter can cause excitotoxic neuronal demise [77]. Another potential outcome of neurotoxicity linked to MTX is damage to the biopterin metabolism, resulting in reduced monoamine neurotransmitters synthesis [78]. It is accepted that MTX treatment stimulates the development of subcutaneous nodules, which are a build- up of multinucleated giant cells that originated from mononuclear cells [79]. One study employed an invitro model of giant cell formation to examine nodulosis caused by MTX [80]. The researchers established that the production of multinucleated giant cells is improved by MTX and also by adenosine A1 receptor occupancy. A particular adenosine A1 receptor antagonist counteracts the impact of MTX. The evolution of multiple nodules associated with MTX may therefore arise via the action of adenosine on the adenosine A1 receptors [79].

4.1 Renal Toxicity

It is well-established that renal impairment can be induced by low-dose MTX administration [81]. However, there is a lack of clarity regarding the mechanism underlying kidney injury caused by MTX. Further reports state that kidney injury is a possible consequence of high dose MTX. Urine with a low pH may give rise to precipitation of MTX, together with its principal breakdown product, 7-OH-MTX. This may be an element responsible for intratubular blockage and kidney impairment [82]. Although this process may arise with higher dose regimens of MTX, it is unusual with ongoing treatment with a low dose. Abnormal kidney function associated with MTX may occur via serum adenosine levels and the consequent triggering of A1 receptors within the kidney substance, thus diminishing kidney perfusion and leading to impaired performance [83]. A murine model which simulated kidney impairment associated with low-dose MTX indicated that long-term MTX delivery led to an accrual of MTX within the kidney tissue, together with notable glomerular and tubular injury as a result of heightened oxidative stress [84]. The method of elimination of MTX is predominantly renal. If this route is compromised, and MTX accumulates within the plasma, this may result in adverse effects from the MTX levels, of which bone marrow suppression is a frequent manifestation [85]. Thus, in the presence of a glomerular filtration rate < 30 mL/min, the prescription of MTX, even at a low dose, is ill-advised [86].

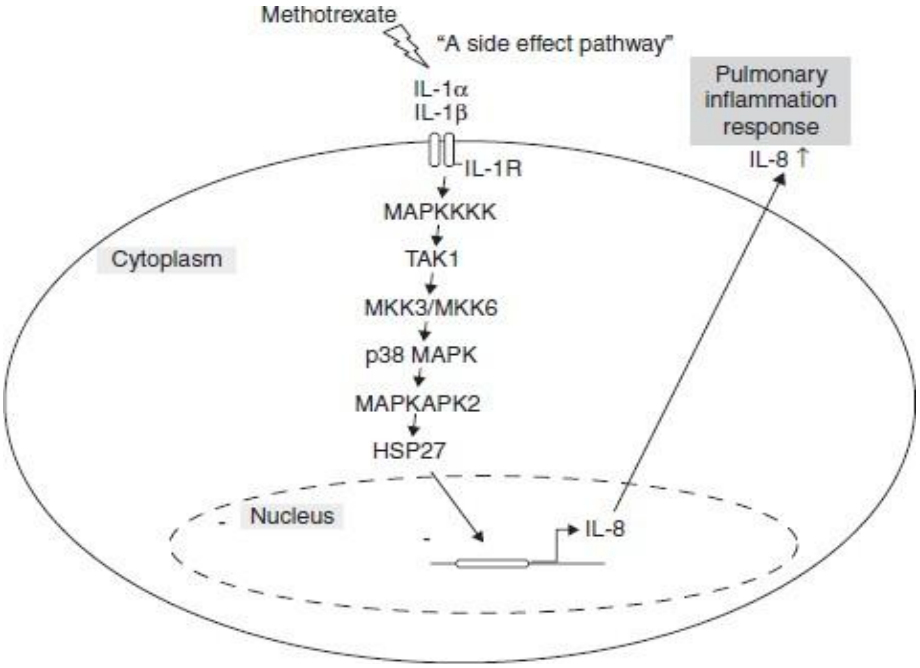

Fig. 3. A side-effect of MTX in MAPK signalling pathway. Adopted from [59]

4.2 Other Toxicities

Other toxicities such as endocrinological toxicity, GI toxicity, cutaneous toxicity, haematological toxicity, fatal malfunction or loss, and malignancy can also occur, but at a significantly lower rate of prevalence. Consequently, it is challenging to establish sufficient evidence that low dose MTX toxicity is the cause. Hence, there is greater scope for research on MTX toxicity. The lack of precise mechanism of MTX-induced organ toxicity is an issue, and offers more scope to focus on studies related to MTX toxicity [16].

4.3 Management of Toxicity

Patterns of MTX-induced toxicity may be determined by the means and dose administered. Patients may experience diverse signs and symptoms, and could potentially need hospitalisation and intensive care monitored by physicians and nurses. Typically, the preferred primary approach is to discontinue the MTX administrations as well as the concomitant drugs and monitoring blood parameters, renal parameters, and liver parameters as the initial toxicity developed. The treatment approach should be determined by the clinical symptoms and signs. Usually, these patients can be treated with the following three standard approaches: (i) maintenance of the level of MTX serum; (ii) maintenance of body hydration; (iii) promotion of MTX excretion [87].

4.4 Leucovorin (Folinic Acid) Rescue Therapy

Folinic acid is a leading antidote to MTX toxicity. The function of the folinic acid is essentially to re-establish the diminished intercellular folate. The level of MTX serum determines the administration of folinic acid. This may necessitate a comparatively higher folinic acid concentration on higher MTX concentration. Folinic acid may be inadequate in exceedingly high MTX concentration (even 10-fold higher), particularly in circumstances of renal injury. The level of MTX serum should be measured every 24 h until it reaches 0.2 μmol/L. In cases of MTX single oral overdose ingestion (<1000 mg or <5 mg/kg in children) with good hydration and normal kidney function, high dose folinic acid administration may not be required. This is determined by the clinical status and MTX serum level. Conversely, in cases of MTX under-dosing (intake daily rather than weekly dose) with the important clinical characteristic of MTX toxicity, 10mg/m2/i.v/orally administered every 6 hours with daily monitoring of blood counts, renal function, and other examinations to determine the presence of toxicity may be required. Many studies have confirmed that a lack of an initial increase in the leucovorin dose has resulted in a number of deaths [88-89].

4.5 Body Hydration Maintenance

In order to decrease MTX toxicity, it is critical to maintain body hydration and establish aimed diuresis, as this will remove excess MTX from the body. The output of urine must be sustained at either 600ml over 6h or 200ml over 2h (maintaining an approximate urine output of 2L/m2/day is essential). It is important to sustain fluid intake at approximately 3L/m2/day until the level of MTX serum reaches 0.2 μmol/L. The key to averting renal toxicity and excess fluid is to meticulously monitor and maintain both fluid input and output [88-93].

4.6 Enhance Excretion of MTX

In a low pH, MTX and its breakdown products, 2,4-diamino-N (10)-methylpteroic acid and 7-OH-MTX, have low solubility. An increase of urine pH from 6.0 to 7.0 correlated with a solubility increase in MTX and its metabolites from 5 to 8 folds. Moreover, sodium bicarbonate administration of 40-50 mEq per litre of IV fluid could potentially avert the creation and deposition of intratubular MTX crystal and improve MTX renal excretion [88-91].

4.7 Managing Delayed MTX Excretion

Renal clearance glomerular, tubular reabsorption, and secretion is pivotal in MTX excretion. MTX-induced renal toxicity primarily occurred through the formation of crystal/deposition and direct tubular kidney impairment. During renal impairment, recommendations are for delayed MTX renal clearance and serum MTX concentrations exceeding 1 μmol/L, glucarpidase (carboxypeptidase enzyme), which has received USFDA approval. Glucarpidase is an amalgamation of 390 amino acids having 83 kDa and created from *Escherichia coli*. This functions by quickly metabolizing the plasma MTX into the following two inactive metabolites: (i) Glutamate; (ii) DAMPA. Glucarpidase comes into play when the level of MTX serum is or exceeds 10 μmol/L

and 100% increase in serum creatinine. Following glucarpidase administration, an MTX serum level of 97% or higher is used for reduction within 15 min, but has minimal to no impact on intracellular MTX concentration, resulting in folinic acid being the preferred drug for the treatment of MTX toxicities. Glucarpidase is available in vials containing 100 units/vial. Each vial must be reconstituted with 1 ml sodium chloride (0.9%) and injected within 5 min via IV bolus injection. It is necessary to administer folinic acid prior to or after the 2 h of glucarpidase administration so as to avert the drug-drug interaction and to prevent diminishing levels of folate [88- 89].

4.8 Supportive Care

Treating low dose MTX toxicity requires supportive care. This could entail daily doses of folic acid, oral or systemic corticosteroids, G-CSF, IV fluids, empirical antibiotic, antifungal, blood product transfusion, renal impairment correction, hepatic abnormality correction, dialysis, dose reduction or management of potential drug interactions, oral care, oxygen supply, and advising patients on MTX usage.It could be beneficial to better comprehend the therapeutic and toxic mechanisms of MTX action. Such an understanding would facilitate the identification of de novo targets for the treatment of inflammatory and autoimmune disorders, whilst additionally minimising adverse event profiles. This is highly significant for the design of new drugs and the development of more targeted therapies, as this will facilitate the reduction of drug toxicity whilst simultaneously preserving effectiveness [91-92].

5. CONCLUSION

It is concluded that a comprehensive understanding of the molecular mechanisms of methotrexate toxic effects was determined in this study. Study also discussed the management of this toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chan ES, Cronstein BN. Mechanisms of action of methotrexate. Bulletin of the Hospital for Joint Diseases. 2013;71.
2. Chan ES, Cronstein BN. Methotrexate—how does it really work?. Nature Reviews Rheumatology. 2010;6(3):175-178.
3. Genestier L, Paillot R, Quemeneur L, Izeradjene K, Revillard JP. Mechanisms of action of methotrexate. Immunopharmacology. 2000;47(2-3):247-257.
4. Sutaria RB, Amaral JK, Schoen RT. Emergence and treatment of chikungunya arthritis. Current opinion in rheumatology. 2018;30(3):256-263.
5. Marks M, Marks JL. Viral arthritis. Clinical medicine. 2016;16(2):129.
6. Perl A. Mechanisms of viral pathogenesis in rheumatic disease. Annals of the rheumatic diseases. 1999;58(8):454-461.
7. Suhrbier A, Jaffar-Bandjee MC, Gasque P. Arthritogenic alphaviruses— an overview. Nature Reviews Rheumatology. 2012;8(7):420-429.
8. Conway R, Carey JJ. Risk of liver disease in methotrexate treated patients. World Journal of Hepatology. 2017;9(26):1092.
9. Bijlsma JW, Jacobs JW. Methotrexate: still the anchor drug in RA treatment. Joint Bone Spine. 2009;76(5):452-454.
10. Singh JA, Furst DE, Bharat A, Curtis JR, Kavanaugh AF, Kremer JM, et al. update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis care & research. 2012;64(5):625-639.
11. Emery P, Sebba A, Huizinga TW. Biologic and oral disease-modifying antirheumatic drug monotherapy in rheumatoid arthritis. Annals of the Rheumatic Diseases. 2013;72(12):1897-1904.
12. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacological reviews. 2005;57(2):163-172.
13. Tian H, Cronstein BN. Understanding the mechanisms of action of methotrexate. Bull NYU Hosp Jt Dis. 2007;65(3):168-173.
14. Prey S, Paul C. Effect of folic or folinic acid Supplementation on methotrexate-associated safety and efficacy in

inflammatory disease: A Systematic Review. British Journal of Dermatology. 2009;160(3):622-628.

15. Schnabel A, Gross WL. April. Low-dose methotrexate in rheumatic diseases—efficacy, side effects, and risk factors for side effects. In Seminars in arthritis and rheumatism. WB Saunders. 1994;23(5):310-327.
16. Bedoui Y, Guillot X, Sélambarom J, Guiraud P, Giry C, Jaffar-Bandjee MC, Ralandison S, Gasque P. Methotrexate an old drug with new tricks. International journal of molecular sciences. 2019;20(20):5023.
17. Bannwarth B, Labat L, Moride Y, Schaeverbeke T. Methotrexate in rheumatoid arthritis. Drugs. 1994;47(1):25-50.
18. Grim J, Chládek J, Martínková J. Pharmacokinetics and pharmacodynamics of methotrexate in non-neoplastic diseases. Clinical pharmacokinetics. 2003;42(2):139-151.
19. Olsen EA. The pharmacology of methotrexate. Journal of the American Academy of Dermatology. 1991;25(2):306-318.
20. Hou Z, Matherly LH. Biology of the major facilitative folate transporters SLC19A1 and SLC46A1. Current topics in membranes. 2014;73:175-204.
21. Yamamoto T, Shikano K, Nanki T, Kawai S. Folylpolyglutamate synthase is a major determinant of intracellular methotrexate polyglutamates in patients with rheumatoid arthritis. Scientific reports. 2016;6(1):1-8.
22. Hawwa AF, AlBawab A, Rooney M, Wedderburn LR, Beresford MW, McElnay JC. Methotrexate polyglutamates as a potential marker of adherence to long-term therapy in children with juvenile idiopathic arthritis and juvenile dermatomyositis: An observational, cross-sectional study. Arthritis research & therapy. 2015;17(1):1-10.
23. Mori S, Arima N, Ito M, Fujiyama S, Kamo Y, Ueki Y. Non-alcoholic steatohepatitis-like pattern in liver biopsy of rheumatoid arthritis patients with persistent transaminitis during low-dose methotrexate treatment. PLoS One. 2018;13:e0203084.
24. Alturkistani HA, Abuzinadah O, Kelany AM, Abd El-Aziz GS, Alrafiah AR. The combined effect of honey and olive oil against methotrexate mediated hepatotoxicity in rats: a biochemical, histological and immunohistological study. Histol. Histopathol. 2019;34:1313–1327.
25. Karlsson Sundbaum J, Eriksson N, Hallberg P, Lehto N, Wadelius M, Baecklund E. Methotrexate treatment in rheumatoid arthritis and elevated liver enzymes: a long-term follow-up of predictors, surveillance, and outcome in clinical practice. Int. J. Rheum. Dis. 2019;22:1226–1232.
26. Moodi H, Hosseini M, Abedini MR, Hassanzadeh-Taheri M, Hassanzadeh-Taheri M. Ethanolic extract of Iris songarica rhizome attenuates methotrexate- induced liver and kidney damages in rats. Avicenna J. Phytomed. 2020;10:372–383.
27. Alam SS, Hafiz NA, Abd El-Rahim AH, Protective role of taurine against genotoxic damage in mice treated with methotrexate and tamoxfine. Environ. Toxicol. Pharmacol. 2011;31:143–152.
28. Raghunath A, Sundarraj K, Nagarajan R, Arfuso F, Bian J, Kumar AP, Sethi G, Perumal E. Antioxidant response elements: Discovery, classes, regulation and potential applications. Redox biology. 2018;17:297-314.
29. Ezhilarasan D, Evraerts J, Brice S, Buc-Calderon P, Karthikeyan S, Sokal E, Najimi M. Silibinin inhibits proliferation and migration of human hepatic stellate LX-2 cells. J. Clin. Exp. Hepatol. 2016;6:167–174.
30. Saha S, Buttari B, Panieri E, Profumo E, Saso L. An overview of Nrf2 signaling pathway and its role in inflammation. Molecules. 2020;25:5474.
31. Kozieł MJ, Kowalska K, Piastowska-Ciesielska AW. Nrf2: A main responsive element in cells to mycotoxin-induced toxicity. Arch. Toxicol; 2021.

    Available: https://doi.org/ 10.1007/s00204-021-02995-4.
32. Ebrahimi R, Sepand MR, Seyednejad SA, Omidi A, Akbariani M, Gholami M, Sabzevari O. Ellagic acid reduces methotrexate-induced apoptosis and mitochondrial dysfunction via up-regulating Nrf2 expression and inhibiting the IκBα/NFκB in rats. Daru. 2019;27:721–733.
33. Chauhan P, Sharma H, Kumar U, Mayachari A, Sangli G, Singh S.

Protective effects of Glycyrrhiza glabra supplementation against methotrexateinduced hepato-renal damage in rats: an experimental approach. J. Ethnopharmacol. 2020;263:113209.

34. Hussein OE, Hozayen WG, Bin-Jumah MN, Germoush MO, Abd El-Twab SM, Mahmoud AM. Chicoric acid prevents methotrexate hepatotoxicity via attenuation of oxidative stress and inflammation and up-regulation of PPARγ and Nrf2/HO-1 signaling. Environ. Sci. Pollut. Res. Int. 2020;27:20725–20735.
35. Mahmoud AM, Hussein OE, Hozayen WG. Ferulic acid prevents oxidative stress, inflammation, and liver injury via upregulation of Nrf2/HO-1 signaling in methotrexate-induced rats. Environ. Sci. Pollut. Res. Int. 2020;27:7910–7921.
36. Ahmed SMU, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: Pivotal roles in inflammation. Biochimica et Biophysica Acta (BBA)- Molecular Basis of Disease. 2017;1863(2):585-597.
37. Cure E, Kirbas A, Tumkaya L, Cure MC, Kalkan Y, Yilmaz A, Yuce S. Protective effect of infliximab on methotrexate-induced liver injury in rats: unexpected drug interaction. J. Cancer Res. Ther. 2015;11:164–169.
38. Goudarzi M, Kalantar M, Sadeghi E, Karamallah MH, Kalantar H. Protective effects of apigenin on altered lipid peroxidation, inflammation, and antioxidant factors in methotrexate-induced hepatotoxicity. Naunyn Schmiedebergs Arch. Pharmacol. 2021;394:523–531.
39. Devaraj E, Roy A, Royapuram Veeraragavan G, Magesh A, Varikalam Sleeba A, et al. β-Sitosterol attenuates carbon tetrachloride-induced oxidative stress and chronic liver injury in rats. Naunyn Schmiedebergs Arch. Pharmacol. 2020;393:1067–1075.
40. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: clinical and experimental perspective. Arab J. Gastroenterol. 2018;19:56–64.
41. Ezhilarasan D. Endothelin-1 in portal hypertension: the intricate role of hepatic stellate cells. Exp. Biol. Med. (Maywood). 2020b;245:1504–1512.
42. Van Ede AE, Laan RF, Blom HJ, Boers GH, Haagsma CJ, et al. Homocysteine and folate status in methotrexate- treated patients with rheumatoid arthritis. Rheumatology (Oxford). 2002v;41:658–665.
43. Friedman B, Cronstein B. Methotrexate mechanism in treatment of rheumatoid arthritis. Joint Bone Spine. 2019;86:301–307.
44. Chan ES, Montesinos MC, Fernandez P, Desai A, Delano DL, et al. Adenosine A(2A) receptors play a role in the pathogenesis of hepatic cirrhosis. Br. J. Pharmacol. 2006;148:1144–1155.
45. Labadie JG, Jain M. Noninvasive tests to monitor methotrexate-induced liver injury. Clin. Liver Dis. (Hoboken). 2019;13:67–71.
46. Cao L, Quan XB, Zeng WJ, Yang XO, Wang MJ. Mechanism of hepatocyte apoptosis. J. Cell Death. 2016;9:19–29.
47. Ali N, Rashid S, Nafees S, Hasan SK, Shahid A, Majed F, Sultana S. Protective effect of Chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: an experimental approach. Chem. Biol. Interact. 2017;272;80–91.
48. Abo-Haded HM, Elkablawy MA, Al-Johani Z, Al-Ahmadi O, El-Agamy DS, Hepatoprotective effect of sitagliptin against methotrexate induced liver toxicity. PLoS One. 2017;12:e0174295.
49. Allard J, Le Guillou D, Begriche K, Fromenty B. Drug-induced liver injury in obesity and nonalcoholic fatty liver disease. Adv. Pharmacol. 2019;85:75–107.
50. Yoshida S, Onuma K, Akahori K, Sakamoto H, Yamawaki Y, Shoji T, Nakagawa H, Hasegawa H, Amayasu H. Elevated levels of IL-8 in interstitial pneumonia induced by low-dose methotrexate. Journal of allergy and clinical immunology. 1999;103(5):952-954.
51. Yoshimura T, Matsushima K, Oppenheim JJ, Leonard EJ. Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL 1). The Journal of Immunology. 1987;139(3):788-793.
52. Standiford TJ, Kunkel SL, Basha MA, Chensue SW, Lynch J.3, Toews GB, Westwick J, Strieter RM. Interleukin-8 gene expression by a pulmonary epithelial cell line. A model for cytokine networks in

the lung. The Journal of clinical investigation. 1990;86(6):1945-1953.

53. Yamauchi Y, Okazaki H, Desaki M, Kohyama T, Kawasaki S, Yamamoto K, Takizawa H. Methotrexate induces interleukin-8 production by human bronchial and alveolar epithelial cells. Clinical Science. 2004;106(6):619-625.
54. Fujimori Y, Kataoka M, Tada S, Takehara H, Matsuo K, Miyake T, Okahara M, Yamadori I, Tanimoto M. The role of interleukin-8 in interstitial pneumonia. Respirology. 2003;8(1):33-40.
55. Nakagami H, Pitzschke A, Hirt H. Emerging MAP kinase pathways in plant stress signalling. Trends in plant science. 2005;10(7):339-346.
56. Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science. 2002;298(5600):1911-1912.
57. Ge B, Xiong X, Jing Q, Mosley JL, Filose A, Bian D, Huang S, Han J. TAB1β (transforming growth factor-β-activated protein kinase 1-binding protein 1β), a novel splicing variant of TAB1 that interacts with p38α but not TAK1. Journal of Biological Chemistry. 2003;278(4):2286-2293.
58. Kim YJ, Song M, Ryu JC. Inflammation in methotrexate-induced pulmonary toxicity occurs via the p38 MAPK pathway. Toxicology. 2009;256(3):183- 190.
59. Holtmann H, Enninga J, Kä lble S, Thiefes A. Dö rrie A, et al. The MAPK kinase kinase TAK1 plays a central role in coupling the interleukin-1 receptor to both transcriptional and RNA-targeted mechanisms of gene regulation. Journal of Biological Chemistry. 2001;276(5):3508-3516.
60. Choo MK, Sakurai H, Koizumi K, Saiki I. TAK1-mediated stress signaling pathways are essential for TNF-α-promoted pulmonary metastasis of murine colon cancer cells. International journal of cancer. 2006;8(11):2758-2764.
61. Alford KA, Glennie S, Turrell BR, Rawlinson L, Saklatvala J, Dean JL eat shock protein 27 functions in inflammatory gene expression and transforming growth factor-β-activated kinase-1 (TAK1)-mediated signaling. Journal of Biological Chemistry. 2007;282(9): 6232-6241.
62. Kim AL, Labasi JM, Zhu Y, Tang X, McClure K, Gabel CA, Athar M, Bickers DR. Role of p38 MAPK in UVB-induced inflammatory responses in the skin of SKH-1 hairless mice. Journal of investigative dermatology. 2005;124(6):1318-1325.
63. Yang J, Hooper WC, Phillips DJ, Talkington DF. Regulation of proinflammatory cytokines in human lung epithelial cells infected with Mycoplasma pneumoniae. Infection and immunity. 2002;70(7):3649-3655.
64. Auron PE, Warner SJ, Webb AC, Cannon JG, Bernheim HA, et al. Studies on the molecular nature of human interleukin 1. The Journal of Immunology. 1987;138(5:1447-1456.
65. Nagaoka I, Trapnell BC, Crystal RG. Upregulation of platelet-derived growth factor-A and-B gene expression in alveolar macrophages of individuals with idiopathic pulmonary fibrosis. The Journal of clinical investigation. 1990;85(6):2023- 2027.
66. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proceedings of the National Academy of Sciences. 1991;88(15):6642-6646.
67. Nagai S, Aung H, Takeuchi M, Kusume K, Izumi T. IL-1 and IL-1 inhibitory activity in the culture supernatants of alveolar macrophages from patients with interstitial lung diseases. Chest. 1991;99(3):674-680.
68. Lynch JP, Standiford TJ, Rolfe MW, Kunkel SL, Strieter RM. Neutrophilic alveolitis in idiopathic pulmonary fibrosis. Am Rev Respir Dis. 1992;145(6):1433-1439.
69. Zhang Y, Lee TC, Guillemin B, Yu MC, Rom WN. Enhanced IL-1 beta and tumor necrosis factor-alpha release and messenger RNA expression in macrophages from idiopathic pulmonary fibrosis or after asbestos exposure. The Journal of Immunology. 1993;150(9);4188-4196.
70. Iyonaga K, Takeya M, Saita N, Sakamoto O, Yoshimura T, Ando M, Takahashi K. Monocyte chemoattractant protein-1 in idiopathic pulmonary fibrosis and other interstitial lung diseases. Human pathology. 199425(5):455-463.
71. Gerards AH, de Lathouder S, De Groot ER, Dijkmans BAC, Aarden LA. Inhibition

of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis. Rheumatology. 2003;42(10):1189-1196.

72. Thakkar MM, Engemann SC, Walsh KM, Sahota PK. Adenosine and the homeostatic control of sleep: effects of A1 receptor blockade in the perifornical lateral hypothalamus on sleep–wakefulness. Neuroscience. 2008;153(4):875-880.
73. Kane BJ, Kuhn JG, Roush MK. Pentostatin: an adenosine deaminase inhibitor for the treatment of hairy cell leukemia. Annals of Pharmacotherapy. 1992;26(7-8):939-947.
74. Bernini JC, Fort DW, Griener JC, Kane BJ, Chappell WB, Kamen BA. Aminophylline for methotrexate-induced neurotoxicity. The Lancet. 1995;345(8949):544-547.
75. Quinn CT, Griener JC, Bottiglieri T, Hyland K, Farrow A, Kamen BA. Elevation of homocysteine and excitatory amino acid neurotransmitters in the CSF of children who receive methotrexate for the treatment of cancer. Journal of Clinical Oncology. 1997;15(8);2800-2806.
76. Millot F, Dhondt JL, Mazingue F, Mechinaud F, Ingrand P, Guilhot F. Changes of cerebral biopterin and biogenic amine metabolism in leukemic children receiving 5 g/m 2 intravenous methotrexate. Pediatric research. 1995;37(2):151-154.
77. Albrecht K, M ler-Ladner U. Side effects and management of side effects of methotrexate in rheumatoid arthritis. Clinical and Experimental Rheumatology-InclSupplements. 2010;28(5):S95.
78. Merrill JT, Shen C, Schreibman D, Coffey D, Zakharenko O, Fisher R, et al. Adenosine A1 receptor promotion of multinucleated giant cell formation by human monocytes. A mechanism for methotrexate-induced nodulosis in rheumatoid arthritis. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 1997;40(7):1308-1315.
79. Verstappen SMM, Bakker MF, Heurkens AHM, Van der Veen MJ, et al. Adverse events and factors associated with toxicity in patients with early rheumatoid arthritis treated with methotrexate tight control therapy: the CAMERA study. Annals of the rheumatic diseases. 2010;69(6):1044-1048.
80. Seideman P, Müller-Suur R, Ekman E. Renal effects of low dose methotrexate in rheumatoid arthritis. The Journal of rheumatology. 1993;20(7):1126- 1128.
81. Izzedine H, Launay-Vacher V, Karie S, Caramella C, De Person F, Deray G. Is low-dose methotrexate nephrotoxic? Case report and review of the literature. Clinical nephrology. 2005;64(4).
82. Abelson HT, Fosburg MT, Beardsley GP, Goorin AM, Gorka C, Link M, Link D. Methotrexate-induced renal impairment: clinical studies and rescue from systemic toxicity with high-dose leucovorin and thymidine. Journal of Clinical Oncology. 1983;1(3):208-216.
83. Cronstein BN. The mechanism of action of methotrexate. Rheumatic disease clinics of North America. 1997;23(4):739-755.
84. Li X, Abe E, Yamakawa Y, Yoneda G, Fujino R, et al. Effect of administration duration of low dose methotrexate on development of acute kidney injury in rats. J Kidney. 2016;2(3):3.
85. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. The oncologist. 2006;11(6):694-703.
86. Saag KG, Teng GG, Patkar NM, Anuntiyo J, Finney C, et al. American College of Rheumatology recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. Arthritis Care & Research: Official Journal of the American College of Rheumatology. 2008;59(6):762-784.
87. Lateef O, Shakoor N, Balk RA. Methotrexate pulmonary toxicity. Expert opinion on drug safety. 2005;4(4):723-730.
88. Madke B, Singh AL. Acute methotrexate toxicity. Indian Journal of Drugs in Dermatology. 2015;1(1):46.
89. Bateman DN, Page CB. Antidotes to coumarins, isoniazid, methotrexate and thyroxine, toxins that work via metabolic processes. British journal of clinical pharmacology. 2016;81(3):437-445.
90. Howard SC, McCormick J, Pui CH, Buddington RK, Harvey RD. Preventing and managing toxicities of high-dose methotrexate. The oncologist. 2016; 21(12):1471.
91. Dhir V, Sandhu A, Kaur J, Pinto B, Kumar P, Kaur P, Gupta N, et al. Comparison of two different folic acid doses with

methotrexate–a randomized controlled trial (FOLVARI Study). Arthritis research & therapy. 2015;17(1):1-9.

92. Sah SK, Subramanian R, Ramesh M. Methotrexate-induced organ toxicity in patients with rheumatoid arthritis: A review article. Drug Invention Today. 2020;14(1).

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