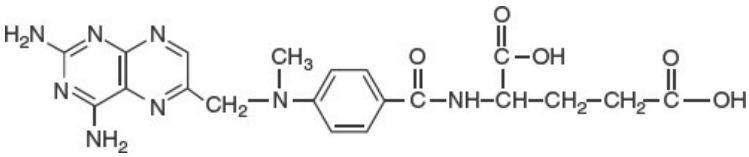
Chapter 51: Methotrexate, 5-Fluorouracil, and Capecitabine

Richard Y. Wang

INTRODUCTION

Methotrexate and the fluoropyrimidines (5-fluorouracil and capecitabine) belong to the chemotherapeutic class of antimetabolites. Patients affected by these chemotherapeutics present with significant clinical toxicities that are potentially fatal. Clinicians must recognize these patients early and be prepared to provide immediate interventions that include specific antidotal therapies. The oral formulations of methotrexate and capecitabine present a risk for intentional or unintentional chemotherapeutics ingestion in the home.

METHOTREXATE



Methotrexate (MTX) is commonly used for a many malignant conditions. Its immunosuppressive activity also allows it to be used for rheumatoid arthritis, organ transplantation, psoriasis, trophoblastic diseases, and termination of pregnancy.[20,47]

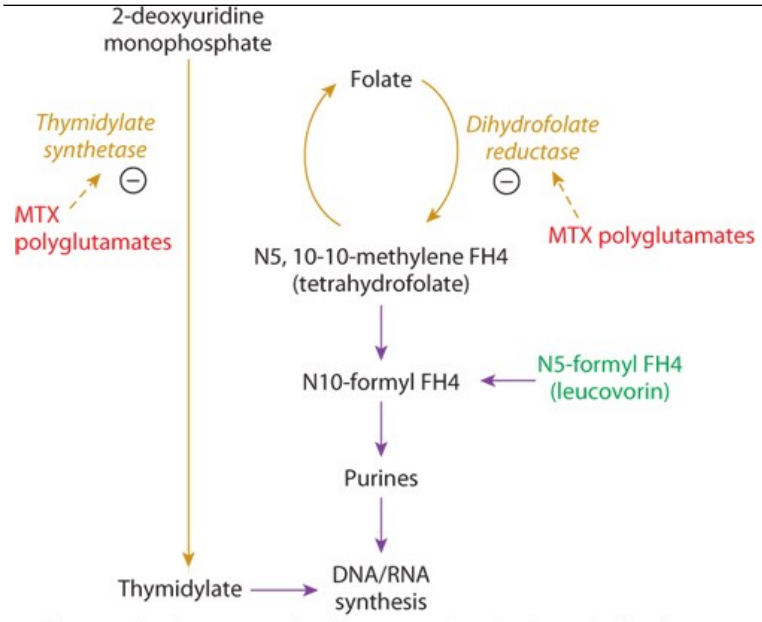
Risk factors for MTX toxicity include impaired kidney function (primary route of drug elimination); third compartment spacing: ascites and pleural effusions; concurrent use of nephrotoxins, such as nonsteroidal antiinflammatory drugs (NSAIDs) and aminoglycosides[55] and certain intravenous radiologic contrast agents;[30,40] age; folate deficiency; and concurrent infection.[95] Methotrexate toxicity depends on the dose, but even more on the duration of exposure.

Pharmacology

The therapeutic and toxic effects of methotrexate are based on its ability to limit DNA and RNA syntheses by inhibiting dihydrofolate reductase (DHFR) and thymidylate synthetase (Fig. 51–1). Dihydrofolate reductase reduces folic acid first to dihydrofolate and then to tetrahydrofolate (FH_4), which serves as an essential cofactor in the synthesis of purine nucleotides that are used in the synthesis of DNA and RNA. Methotrexate, a structural analog of folate, competitively inhibits DHFR by binding to the enzymatic site of action. Methotrexate polyglutamates are formed intracellularly by folylpolyglutamate synthetase and they also inhibit DHFR. The inhibition of DHFR diminishes reduced folate production, which is necessary for the formation of purine nucleotides. Reduced folates are also required by thymidyl synthetase to serve as methyl donors in the formation of thymidyl. Thymidyl is then used for DNA synthesis. In addition, thymidyl production is impaired by the direct inhibition of thymidylate synthetase by polyglutamated derivatives of methotrexate.

FIGURE 51–1.

Mechanism of MTX toxicity. Methotrexate inhibits dihydrofolate reductase activity, which is necessary for DNA and RNA synthesis. Leucovorin bypasses blockade to allow for continued synthesis. MTX = methotrexate.



Source: L.S. Nelson, M.A. Howland, N.A. Lewin, S.W. Smith, L.R. Goldfrank, R.S. Hoffman: Goldfrank's Toxicologic Emergencies, Eleventh Edition

The antiinflammatory effect of MTX likely involves the following mechanisms: (1) inhibition of trans-methylation (by depletion of intracellular folate stores) which causes the death of T cells and inhibits the formation of polyamines (spermine and spermidine) that are involved in the inflammatory cascade, (2) reduction in intracellular concentration of glutathione, and (3) inhibition of intracellular 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase, which leads to increased extracellular concentration of adenosine through a series of steps (see below). Adenosine binds to the A_{2a} receptor located on the surface of leukocytes, and it affects selected functions, where it inhibits the syntheses of cytokines (TNF α) and complements produced by macrophages, and inhibits lymphocyte proliferation and migration to targeted sites.[23,69]

The de novo synthesis of purine nucleotides involves the formylation of the intermediate AICAR to formyl-AICAR by AICAR transformylase in the latter steps of the pathway. Formyl-AICAR proceeds to form inosine and then adenosine. The latter step is facilitated by adenosine deaminase. Adenosine is phosphorylated to AMP, ADP, and ATP. These phosphorylated species of adenosine can translocate to the extracellular compartment, where they are converted to adenosine. Polyglutamates of MTX inhibit AICAR transformylase, which causes an increase in the concentration of AICAR and then the inhibition of AMP and adenosine (via AICA ribonucleoside) deaminases.[74] The inhibition of these deaminases increases the intracellular concentrations of adenosine and its phosphorylated derivatives, and contributes to the movement of these products to the extracellular compartment.

The bioavailability of methotrexate is limited by a saturable intestinal absorption mechanism. At oral doses less than 30 mg/m^2, the absorption is 90% and the peak plasma MTX concentration is achieved at one to 2 hours. In children administered oral MTX, peak blood concentrations mean (SD) of MTX were 0.71 (0.17) µmol/L and 1.48 (0.46) µmol/L for doses at less than or equal to 10 and 30 mg/m^2, respectively.[92,98] In adults, MTX administered as an oral dose of 7.5 mg, 15 mg, or 50 mg achieved peak blood concentrations (mean, SD) of 0.48 (0.09) µmol/L , 0.84 (0.3) µmol/L, and 1.84 (0.70) µmol/L, respectively.[4,99] At oral doses greater than 30 mg/m^2, gut bioavailability significantly decreases. For example, at doses greater than 80 mg/m^2, the absorption is less than 10% to 20%.[14]

Methotrexate dosing regimens for chemotherapy are variable, but high-dose therapy is equal to or is greater than 1,000 mg/m^2. Conventional intravenous doses of up to 100 mg/m^2 are often administered without leucovorin rescue. Doses of 1,000 mg/m^2 are considered potentially lethal. Much higher doses (2,000–3,000 mg g/m^2) can be given when MTX is followed by leucovorin "rescue" in order to prevent life-threatening toxicity. Mortality from high-dose MTX is approximately 6%, and occurs primarily when MTX concentrations are not monitored.[95,101]

Methotrexate has a triphasic plasma clearance. The initial plasma distribution half-life is short at 0.75 hours. The second half-life is 2 to 3.4 hours and represents renal clearance of the drug. The third phase has a half-life of about 8 to 10.4 hours and represents tissue redistribution into the plasma. This third phase is prolonged in the setting of kidney failure and is associated with bone marrow and gastrointestinal (GI) toxicity. The volume of distribution is 0.6 to 0.9 L/kg and protein binding is 50%. Healthy kidneys eliminate 50% to 80% of MTX unchanged within 48 hours of administration.

When the creatinine clearance is less than 60 mL/min, MTX clearance is delayed.[83,105] Ten percent to 30% of MTX is eliminated unchanged in the bile, which contributes to enterohepatic circulation of the chemotherapeutic. In the setting of kidney failure, the half-life of MTX is prolonged by the recirculation of MTX in the gut.

Hepatic aldehyde oxidase metabolizes a minor portion (<10%) of MTX to 7-hydroxy methotrexate (7-OH-MTX), which inhibits DHFR but to a lesser extent than MTX. Aldehyde oxidase is also found in renal tubules, but not in the glomerulus, and its contribution to the local production of 7-OH-MTX and kidney injury remains to be determined.[70] Another metabolite is 2,4-diamino-*N*(10)-methylpteroic acid (DAMPA), and it accounts for less than 5% of MTX. In the gut, bacterial carboxypeptidase acts on MTX to form DAMPA, which is reabsorbed into the blood compartment. Approximately 50% of -*N* (10) DAMPA is eliminated unchanged by the kidneys, and this metabolite is inactive at DHFR based on poor cellular uptake and inhibition of the enzyme when compared to MTX.[112]

Pathophysiology

At high doses, methotrexate and the insoluble drug metabolites 7-OH-MTX and DAMPA accumulate and precipitate in the renal tubules, causing reversible acute tubular necrosis. Methotrexate is one-tenth as soluble at a pH of 5.5 as it is at a pH of 7.5.[14,85] Expressed another way, the plasma concentration threshold for nephrotoxicity is 2.2 mmol/L when the urine pH is 5.5, and 22 mmol/L when the urine pH is 6.9. Thus, patients who are either inadequately hydrated or not alkalinized are at risk for acute kidney failure from high-dose MTX treatment.[3,48] Methotrexate is excreted unchanged in the urine by both glomerular filtration and active tubular secretion. A small amount of MTX is metabolized intracellularly to polyglutamate derivatives, which inhibit DHFR and thymidyl synthetase and are believed to be responsible for the persistent cytotoxic effect of MTX because they do not easily diffuse outside of the cell. The threshold concentration for MTX in plasma that inhibits DNA synthesis is lower for intestinal epithelial cells (0.005 µM/L) than for hematopoietic cells (0.01 µmol/L) by one order of magnitude.[20] Thus, patients with a significant exposure to MTX will develop GI toxicity before bone marrow toxicity.

The decreased production of reduced folates and diminished folate content in the hepatocyte likely contribute to injury, which leads to hepatic fibrosis from the stimulation of hepatic stellate cells by adenosine.[74,75] Patients on long-term therapy with MTX and elevated aminotransferases can develop hepatic fibrosis from persistent injury to the liver.

Clinical Manifestations

In the course of MTX therapy, a variety of disorders can occur, resulting from either increased patient susceptibility to toxicity or excessive administration. The clinical manifestations of MTX toxicity include stomatitis, esophagitis, kidney failure, myelosuppression, hepatitis, and central neurologic system dysfunction. In a group of 23 patients who received 45 courses of high-dose MTX therapy with leucovorin rescue, the most commonly observed signs included increased aspartate aminotransferase (AST) or alanine aminotransferase (ALT) (81%), nausea and vomiting (66%), mucositis (33%), dermatitis (18%), leukopenia (11%), thrombocytopenia (9%), and creatinine elevation (7%).[77]

Nausea and vomiting, considered rare from cancer therapy with MTX at 40 mg/m^2, typically begin 2 to 4 hours after high-dose therapy (1,000 mg/m^2) and last for about 6 to 12 hours. Mucositis, characterized by mouth soreness, stomatitis, or diarrhea, usually occurs in the first week of therapy, and can last for 4 to 7 days. Other gastrointestinal effects resulting from MTX therapy include pharyngitis, anorexia, gastrointestinal hemorrhage, and toxic megacolon.[9] Hepatocellular toxicity, as defined by increased hepatic enzymes (AST, ALT), and hyperbilirubinemia occur with both acute and chronic therapies.[62,71,77] It is usually associated with high dose regimens, and elevations in AST/ALT will begin within one to 3 days following an exposure to a significant dose of MTX. Laboratory abnormalities improve within 1 to 2 weeks of discontinuation of MTX. The mechanism is incompletely understood, but toxicity is attributed to reduced liver folate stores resulting from intracellular competition with polyglutamates of MTX for glutamyl conjugation in chronic exposures,[11] and cellular damage from oxidative stress[5,41] or the precipitation of 7-OH-MTX in the bile duct[17] in acute exposures. Factors associated with hepatotoxicity are sustained high plasma concentrations, increased cumulative dosages, chronic therapy, and host factors such as increase in age, obesity, alcoholism, and prior liver disease.[107]

Pancytopenia usually occurs within the first 2 weeks after an acute exposure. There are several reports demonstrating the occurrence of pancytopenia in individuals receiving chronic MTX therapy for rheumatoid arthritis and psoriasis.[27,54,62,80]

When used at intravenous (IV) doses of 40 to 60 mg/m^2, MTX is not associated with appreciable nephrotoxicity. However, at doses greater than 5,000 g/m^2 (approximately 130 mg/kg for an adult), several investigators report severe kidney injury, with oliguria, azotemia, and kidney failure.[13] The incidence of kidney injury (serum creatinine ≥1.5 to 3.0 × upper limit of normal) in patients with osteosarcoma and treated with high-dose MTX in conjunction with hydration, urinary alkalinization, and leucovorin is 1.8%.[109] Kidney function typically recovers over time. Patients at risk for nephrotoxicity include the elderly, those with underlying kidney disease defined as a glomerular filtration rate of less than 60 mL/min, and those who receive concurrent drug therapy that can delay MTX excretion, which includes agents that reduce renal blood flow such as NSAIDs, the nephrotoxins such as cisplatin, and the aminoglycosides, or weak organic acids such as salicylates and piperacillin which inhibit renal secretion.[46,95]

The neurologic complications associated with either high-dose systemic MTX therapy or intrathecal administration are the most consequential manifestations. The incidence of neurologic toxicity from high dose MTX therapy is approximately 5% to 15%.[49] The manifestations usually occur from hours to days after the initiation of therapy and include hemiparesis, paraparesis, quadriparesis, seizures, and dysreflexia.[49,61,96,103] These events are reversible to varying degrees.[2] Clinical findings occurring within several hours (usually within 12 hours) of therapy are attributed to chemical arachnoiditis, and they include acute onset of fever, meningismus, pleocytosis, and increased cerebrospinal fluid (CSF) protein concentration.[42] Leukoencephalopathy is associated with the onset of behavioral disorders and progressive dementia from months to years after treatment and is irreversible, although manifestations presenting soon after treatment can be reversible depending on the extent of involvement.[6,113] Patients with increased age and prior cranial radiation are at risk for this disorder.[37] Patients with leukoencephalopathy have findings consistent with edema, and demyelination or necrosis of the white matter on computed tomography (CT) and magnetic resonance imaging (MRI) of the brain.[6]

Diagnostic Testing

Plasma MTX concentrations are monitored during therapy to limit clinical toxicity. For example, patients with a plasma concentration greater than 1.0 µmol/L at 48 hours posttreatment are considered at risk for bone marrow and gastrointestinal mucosal toxicities.[95] In the former example, the MTX concentration since the time of administration is used in cancer therapy to minimize bone marrow toxicity (suppression) and maximize the efficacy of the therapeutic.[32] The drug concentration and the duration of exposure are indirect indicators of the efficacy of the drug during therapy. In patients with an unintentional exposure to MTX and not receiving MTX therapy, their exposure to MTX (concentration and duration) should be minimized because they are vulnerable to GI toxicity (mucositis). The threshold concentration of MTX for the inhibition of DNA synthesis is one order of magnitude lower for intestinal epithelial cells than hematopoietic cells.

There are several analytical methods available to measure the concentration of MTX, which is routinely conducted in blood (serum or plasma) (Table 51–1). It is important to select the appropriate analytical method to measure MTX because the performance characteristics are variable among these methods.

TABLE 51–1

Analytical Techniques Used to Measure Methotrexate and Selected Metabolites

Technique	Analyte	LOD (μM/L)	LOQ (μmol/L)	CV (MTX, μmol/L)*	AMR (μmol/L)	Matrix	Comments
FPIA[1,16]	MTX	0.02	0.03	14% (0.07)	0.03–1.0	Serum, plasma	Assay cross-reacts with DAMPA
				5.9% (0.8)			
EMIT[7,36]	MTX	0.02	0.04	11.7% (0.07)	0.04–1.2	Serum, plasma	Assay cross-reacts with DAMPA
				6.9% (0.82)			
CMIA[10,16]	MTX		0.04	4.1% (0.07)+	0.04–1.5	Plasma	Assay cross-reacts with DAMPA
				4.6% (0.45)+			
HPLC-UVD[12]	MTX	0.003	0.01	12.6% (0.05)+	0.025–5.0	Serum	
				5.5% (1.0)+			
LC-MS[15]	MTX		0.0025	12% (0.005)	0.006–1.0	Urine	
				1.7% (0.01)			
	7-OH-MTX		0.01				
LC-MS/MS[79]	MTX	0.0004		16.8% (0.0022)+, &	0.002–5.5	Plasma, CSF	
				4.1% (0.82)+			
	7-OH-MTX	0.0004					
	DAMPA	0.0023					

*CVs for inter- and intraday at selected MTX concentrations: +CV for interday; & for measurements made in plasma.

AMR = analytical measurement range; CSF = cerebrospinal fluid; CMIA = chemiluminescent microparticle immunoassay (Abbott); CV = coefficient of variation; DAMPA = 2,4-diamino-*N*(10)-methylpteroic acid; EMIT = enzyme multiplied immunoassay (ARK); FPIA = fluorescence polarization immunoassay (monoclonal antibody, Abbott); HPLC = high-performance liquid chromatography; LC = liquid chromatography; LOD = limit of detection; LOQ = limit of quantification; MS = mass spectrometry; MTX = methotrexate; 7-OH-MTX = 7-hydroxymethotrexate; UVD = ultraviolet detection.

The measurement of methotrexate concentrations in the clinical setting is routinely conducted using an immunoassay technique such as enzyme

multiplied immunoassay (EMIT), chemiluminescent microparticle immunoassay (CMIA), or fluorescence polarization immunoassay (FPIA) (Table 51–1).[1,7,10,16,36] These measurements are performed on serum or plasma. The presence of the MTX metabolite DAMPA but not 7-OH-MTX diminishes the specificity of these analytical methods for MTX. The amount by which DAMPA affects the MTX concentration depends on the assay.

At low concentrations of MTX, analytical methods based on high-performance liquid chromatography with ultraviolet detection (HPLC-UVD) or mass spectrometry (LC-MS or LC-MS/MS) provide increased sensitivity and specificity compared to the immunoassays (Table 51–1). The primary advantage of the HPLC-UVD technique over the immunoassay is the ability to measure MTX independent of DAMPA.[12] The advantages of mass spectrometry are increased sensitivity and specificity compared to HPLC-UVD or immunoassays. For example, analytical methods based on mass spectrometry detect or quantify MTX at a concentration one to 2 orders of magnitude lower than methods using either one of the former 2 techniques, and they can quantify 7-OH-MTX and DAMPA in a single analytical run.[15,79] The limitations of methods using HPLC (with UVD or mass spectrometry) are their availability and turnaround time for the test result.

When patients are treated with glucarpidase (carboxypeptidase G_2) (Antidotes in Depth: A13), it is preferable to use an HPLC method to measure MTX because of the presence of DAMPA during therapy. The FPIA method with monoclonal antibodies is recommended not to be used in patients who have developed antibodies to mouse monoclonal antibodies or elevated concentrations of DAMPA.

An elevated CSF methotrexate concentration (>100 µmol/L) is indicative of an excessive intrathecal dose or delayed cerebrospinal fluid outflow obstruction.[72] Radiologic imaging of the brain, such as computed tomography and magnetic resonance imaging, are useful to evaluate for meningeal inflammation, demyelination and necrosis of the white matter, or other pathologies such as a cerebrovascular accident.

Management

The initial approach to the patient exposed to MTX is to determine whether the exposure is acute or chronic because the priorities are different for these patients. For example, a patient with chronic MTX toxicity is more likely to die from overwhelming sepsis than the patient with an acute exposure because the former patient typically presents with bone marrow suppression and severe gastroenteritis. Fluid resuscitation, stabilization, and assessment for neutropenia and kidney and liver injuries are essential steps during the evaluation of the patient with chronic MTX toxicity.

The patient with an acute exposure to MTX typically presents early after an oral exposure. The essential steps during the evaluation of this patient are limiting further gut absorption of MTX, and administering leucovorin early. Leucovorin is most effective as a competitive antidote to MTX when the intracellular concentration of MTX is still low. The time to peak blood concentration of MTX from an oral therapeutic dose of MTX is about 2 hours (1–3 hours). Using the above perspective as a framework for the management of these patients, the following discussion will review the roles of activated charcoal, urinary alkalinization, colony-stimulating factor, and antidotal therapies.

Activated charcoal adsorbs methotrexate and administration is recommended as soon as possible to limit absorption in the setting of oral exposure.[38] The administration of multiple-dose activated charcoal and cholestyramine[29,89] can significantly decrease the elimination half-life of methotrexate by interrupting the enterohepatic circulation.[34,38] This approach can increase MTX clearance but is of most benefit to patients with diminished creatinine clearance. Multiple-dose activated charcoal is recommended for patients with evidence of delayed MTX clearance, such as kidney injury or prolonged half-life (based on blood MTX concentration). Activated charcoal should be withheld in patients with gastrointestinal hemorrhage.

Adequate hydration with 0.9% sodium chloride solution as well as urinary alkalinization with IV sodium bicarbonate (to urine pH 7 to 8) (Antidotes in Depth: A5) is recommended because these modalities prevent or limit kidney injury from the precipitation of MTX and its metabolites in patients who receive inadvertent high doses of glucarpidase.[21] Serial measurements of serum creatinine and blood MTX concentration will determine the duration of urinary alkalinization.

The complete blood count (CBC) should be monitored at least as frequently as days 7, 10, and 14 to assess the impact on the cells in the bone marrow.[56] Granulocyte-macrophage colony–stimulating factor (GM-CSF) was used in patient with a chronic MTX overdose and pancytopenia.[94] The patient had a serum MTX concentration of 1.25 µmol/L on admission and was in kidney failure. Bone marrow biopsy showed promyelocytes, but no mature white cells, and a marked reduction of megakaryocytes. Because of deteriorating conditions, GM-CSF (125 mcg/m^2/d) was administered when the MTX concentration fell below the reference value for toxicity. Seven days after the initiation of GM-CSF, the white blood cell (WBC) count rose and reached expected values within 10 days. Colony-stimulating factor is recommended for patients with febrile neutropenia and who are at high risk for

complications from an infection or are likely to have a poor outcome (eg, absolute neutrophil count <100 cells/µL, prolonged neutropenia {>10 days}, age 65 years or older, and hypotension with multiorgan dysfunction).[91]

Patients presenting with meningismus or altered mental status following MTX therapy should receive an initial MRI of the brain and then CSF analysis for infection.[52] Although not considered standard, it would be reasonable to measure the CSF concentration of MTX if excessive exposure to this compartment is suspected. The peak therapeutic concentration of methotrexate in CSF after the intrathecal administration of 12 mg MTX by lumbar puncture is 100 µmol/L, and the terminal half-life of MTX in the cerebrospinal compartment is 7 to 16 hours.[72] Magnetic resonance imaging of the brain can demonstrate a high signal throughout the pachymeningeal (dura mater) region, which is consistent with a chemical meningitis,[33] or a high signal of the white matter with a decreased diffusion coefficient in a diffusion-weighted image to indicate the presence of edema, which is an early finding of leukoencephalopathy.

Antidotes

The available antidotes or rescue agents (term used in cancer therapy for therapeutics that extend the use of chemotherapeutics) for methotrexate -toxicity are leucovorin (folinic acid) (Antidotes in Depth: A12) and glucarpidase (carboxypeptidase G_2) (Antidotes in Depth: A13). The effectiveness of these therapies depends on both the timing of administration and the dose, which warrants the monitoring of plasma MTX concentrations during the use of these antidotes. Leucovorin rescue therapy limits the bone marrow and gastrointestinal toxicity of MTX by allowing for the continuation of essential biochemical processes that are dependent on reduced folates. The purpose of the initial dose of leucovorin is to achieve a plasma concentration equal to the MTX and subsequent doses should be adjusted according to plasma MTX concentrations at 24 and 48 hours postexposure (Fig. 51–1).[31,108] Leucovorin treatment is continued until the MTX concentration is less than 0.01 µmol/L.[20] In patients with marrow toxicity and no cancer, leucovorin therapy should be continued until marrow recovery occurs, even if plasma MTX is no longer detectable,[60] because MTX polyglutamates can still be present in the cytosol and adversely affect cellular activity. Among 36 patients undergoing MTX therapy (amount varied from 2 to 13 mg MTX per week) for rheumatoid arthritis, all of these patients had indirectly detected MTX polyglutamates in red blood cells and nondetected MTX in the plasma by FPIA and MAB.[43,44]

Glucarpidase (carboxypeptidase G_2) is a recombinant bacterial enzyme that is used as a rescue therapy to inactivate MTX by hydrolyzing it to DAMPA and glutamate. Glucarpidase is used in patients with an elevated plasma MTX concentration (>1.0 µmol/L) and delayed renal clearance of MTX.[102] Glucarpidase is not indicated for patients with a plasma MTX concentration that is consistent with the expected clearance for MTX at the dose administered (ie, a concentration that is within 2 SD of the mean MTX elimination curve for the dose administered), or for patients with no more than mild kidney injury. Following glucarpidase therapy, plasma MTX concentrations need to be monitored because residual concentrations of MTX in the blood after initial enzymatic therapy can result from the fact that the action of glucarpidase occurs solely within the vascular compartment; inadequate dose of glucarpidase occurs in patients with large MTX exposures or following redistribution of MTX from tissue stores to the blood compartment.[18,86,110] Glucarpidase can also be successfully administered intrathecally to reduce elevated MTX concentrations in the cerebrospinal space (Special Considerations: SC7).[111]

Extracorporeal Elimination

The patient with delayed renal clearance of a toxic concentration of MTX and who is not a candidate for glucarpidase therapy can benefit the most from extracorporeal elimination of MTX when the procedure is instituted early during the patient's course. Once MTX distributes into the cell and peripheral tissue compartments, the procedure cannot remove intracellular polyglutamate derivatives of MTX, and multiple sessions can be required to clear additional MTX stored at peripheral sites.[108] In addition, the procedure can be performed safely in the patient before he or she develops pancytopenia and gastrointestinal hemorrhage from MTX toxicity.

There are several reports of the use of hemodialysis or hemoperfusion (or both) for patients with MTX toxicity.[51,68,78,97,105] Although the volume of distribution (0.6–0.9 L/kg) and protein binding (50% that is not concentration dependent) suggest that MTX is cleared by hemodialysis, older clinical evidence suggests otherwise.[94] In one report, less than 10% of an initial 700 mg dose of methotrexate was cleared in 12 sessions of hemodialysis.[97] The measured clearance was only 38 mL/min, which can be compared to 5 mL/min for peritoneal dialysis,[39] 0.28 to 24 mL/min for continuous venovenous hemodiafiltration,[50,53] and 180 mL/min for normal renal clearance.[57] The ability of hemofiltration to extract MTX from the blood is also limited by the

magnitude of the concentration gradient for MTX, which decreases as the concentrations of MTX decreases in the blood.[22] Plasma exchange transfusion is not recommended to remove MTX because the drug has a low degree of protein binding, which limits the efficacy of this procedure.[13,53,73,97]

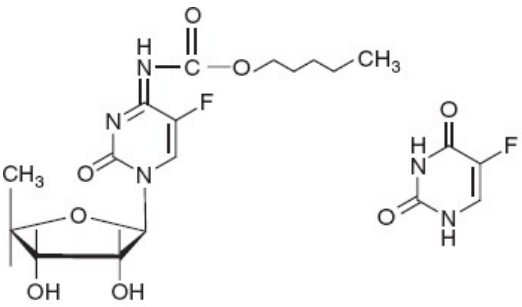
Acute intermittent hemodialysis with a (F-80B Fresenius Dialyzer) high-flux dialyzer membrane yielded an effective mean plasma MTX clearance of 92 ± 10.3 mL/min in 6 patients with kidney failure that was a result of either chronic disease or high-dose MTX therapy.[105] These patients received high-dose MTX therapy and had predialysis serum MTX concentrations ranging from 1.45 to 1,813 µmol/L. The time of dialysis initiation after MTX treatment was from 1 hour to 6 days in this patient population. A serum MTX concentration of 0.3 µmol/L was used as an end point for dialysis. The reported plasma MTX clearance by this technique closely approximates normal renal MTX clearance and is indicated to enhance the clearance of MTX in patients with diminished renal clearance and a toxic plasma MTX concentration when it can be conducted safely.[83]

Charcoal hemoperfusion removed more than 50% of MTX in 4 patients with impaired renal clearance during high dose MTX therapy.[26,78] This was thought to have prevented severe skin and mucosal toxicity. Sequential hemodialysis and hemoperfusion were used for a patient with substantial MTX toxicity.[38] These procedures decreased the half-life of elimination from 45 hours to 7.6 hours. In experimental animals, hemoperfusion significantly reduced the terminal half-life of methotrexate. In surgically anephric dogs, hemoperfusion decreased the half-life from more than 20 hours to 1.3 hours.[45] Unfortunately, hemoperfusion is currently typically unavailable but modern standard hemodialysis is quite effective in MTX removal (Chap. 6).[78]

In vitro studies indicate that the toxic effects of 100 µmol/L of MTX cannot be reversed by 1,000 µmol/L of leucovorin.[76] This suggests the need for extracorporeal elimination, such as high-flux hemodialysis, or glucarpidase (or both) to lower persistent plasma MTX concentrations of greater than 100 µmol/L.[78] It is important to perform high-flux hemodialysis early, prior to distribution into tissues. Rebound of MTX concentrations from tissues can be expected with intermittent hemodialysis, which typically begins at 2 hours postdialysis and plateau at 16 hours.[35,39,105]

Patients who are at the greatest risk for developing MTX toxicity despite leucovorin treatment should receive glucarpidase (Antidotes in Depth: A13). If the patient with diminished renal clearance and a toxic MTX concentration is not a candidate for glucarpidase therapy, it is reasonable to use hemodialysis early in the patient's course. Current standard hemodialysis can offer the additional benefit of correcting fluid and electrolyte disorders resulting from kidney failure. Other treatment options to limit additional organ toxicity, including leucovorin and urinary alkalinization, should be continued during extracorporeal MTX removal. Leucovorin needs to be replaced postdialysis because it is water-soluble and will be removed by hemodialysis.[24,78,88,90]

5-FLUOROURACIL AND CAPECITABINE



Capecitabine

5-Fluorouracil (5-FU)

The fluoropyrimidines used in chemotherapy are 5-fluorouracil (5-FU), capecitabine, tegafur, and floxuridine. The fluoropyrimidines are competitive analogues to uridine and they disrupt the syntheses of DNA (during the S-phase of the cell cycle) and RNA (Chap. 50). Although floxuridine is metabolized to 5-FU, it has limited systemic toxicity because it is administered to the target organ by the arterial route. These chemotherapeutics are used to treat breast and selected GI cancers, such as colorectal. Common risk factors for toxicity from these chemotherapeutics are selected genetic polymorphisms (ie, dihydropyrimidine dehydrogenase {DPD} deficiency), and underlying liver, kidney, and coronary artery diseases.

Pharmacology

Capecitabine is an oral pro-drug of 5-FU; it is well absorbed by the gut, and then metabolized to 5-FU. In comparison, 5-FU has an erratic oral absorption in the gut and needs to be administered by the intravenous route as a bolus or a continuous infusion. Technical problems with the infusion pump or a pump programming error are reported causes of clinical toxicity from an overdose of 5-FU.[59,84]

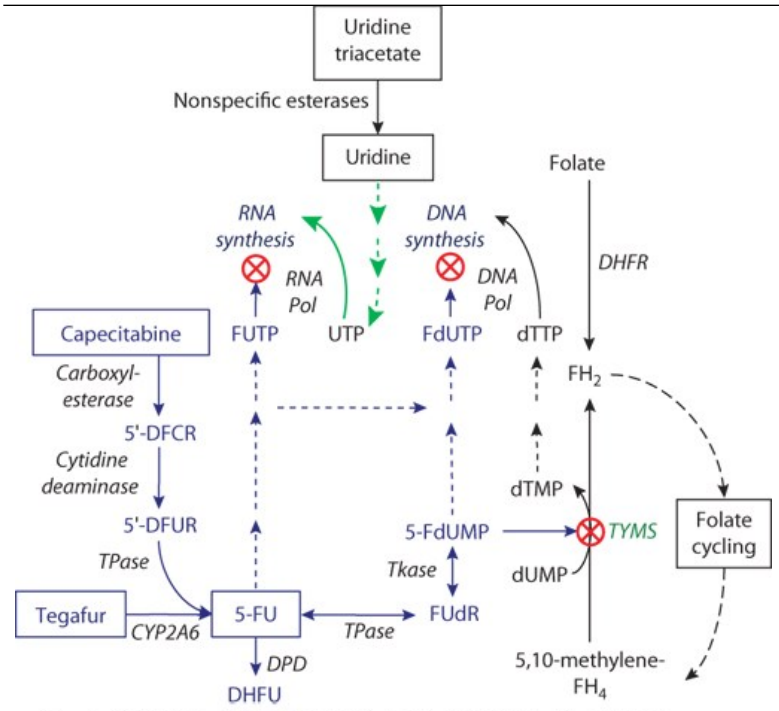
The time to peak blood concentration for capecitabine is 1.5 to 2 hours (3.9 mg/L).[104] A median peak blood 5-FU concentration was 55.4 mg/L for a group of 18 patients receiving bolus therapy (400 mg/m^2 5-FU) for colon cancer.[19] 5-Fluorouracil has an apparent volume of distribution of 8–11 L/m^2, distributes to third spaces or compartments, such as peritoneal and pleural fluids, and is approximately 10% protein bound. The plasma protein binding for capecitabine is 60%. The terminal half-lives for 5-FU and capecitabine in the blood compartment are about 15 and 45 minutes, respectively.

Whether a patient experiences therapeutic effects or toxic effects from the use of these chemotherapeutics depends on the balance between the enzymatic reactions involved with the formation of active metabolites and the enzymatic reaction that degrades these metabolites and the parent compound. This is important because these chemotherapeutics require enzymes that can have variable levels of potential to activate and inactivate them.

Capecitabine undergoes 3 enzymatic reactions in the liver to form 5-FU. Capecitabine is initially metabolized by carboxylesterase to 5′-deoxy-5-fluorocytidine (5-DFCB) and then to 5′-deoxy-5-fluorouridine (5′-DFUR) by cytidine deaminase (Fig. 51–2). In the liver or at the tumor site, thymidine phosphorylase converts 5′-DFUR to 5-FU. Inside the cell, 5-FU is converted to active metabolites: FdUMP, FTP, and FdUTP. Floxuridine (5-FUDR) is metabolized to 5-FU and then to FdUTP and FdUMP. Tegafur is an additional prodrug that is bioactivated by CYP2A6 to 5-FU. CYP2C9 is also capable of metabolizing tegafur, but it did not appear to significantly contribute to overall activation of tegafur in a human liver microsome system.

FIGURE 51–2.

5-Fluorouracil, capecitabine, and tegafur chemotherapeutic mechanisms of action and uridine triacetate antidotal rescue. 5′-DFCR = 5′-deoxy-5-fluorocytidine; 5′-DFUR = 5′-deoxy-5-fluorouridine; 5-FdUMP = fluorodeoxyuridine monophosphate; 5-FU = 5-fluorouracil; 5,10-methylene-FH4 = 5,10-methylenetetrahydrofolate; CYP2A6 = cytochrome P450, family 2, subfamily A, polypeptide 6; DHFR = dihydrofolate reductase; DHFU = 5,6-dihydrofluorouracil; DNA Pol = DNA polymerase; DPD = dihydropyrimidine dehydrogenase; dTMP = deoxythymidine monophosphate; dTTP = deoxythymidine triphosphate; dUMP = deoxyuridine monophosphate; FdUTP = fluorodeoxyuridine triphosphate; FH2 = dihydrofolate; FUdR = floxuridine; FUTP = fluorouridine triphosphate; RNA Pol = RNA polymerase; TKase = thymidine kinase; TPase = thymidine phosphorylase; TYMS = thymidylate synthase; UTP = uridine triphosphate. (Used with the permission from Silas W. Smith, MD.)



Source: L.S. Nelson, M.A. Howland, N.A. Lewin, S.W. Smith, L.R. Goldfrank, R.S. Hoffman: Goldfrank's Toxicologic Emergencies, Eleventh Edition

Hepatic metabolism by DPD inactivates approximately 80% of 5-FU to 5,6-dihydro-5-fluorouracil and alpha-fluoro-beta-alanine (FBAL), and these products are excreted in the urine. The enzyme DPD has nonlinear kinetics because of saturation, and its level of activity depends on the genetic variant. The frequencies of genetic variants with complete and partial DPD deficiencies in adults are 0.1% to 0.5% and 3% to 5%, respectively.[63] Patients with a significant DPD deficiency based on certain genetic variants will present with early-onset and severe manifestations of toxicity from 5-FU or capecitabine.[58,64]

Cytidine deaminase is another enzyme with a variable level of expression in the general population. Patients with a markedly elevated level of activity for this enzyme can develop capecitabine toxicity. Patients with certain genetic variants of cytidine deaminase are "ultra-metabolizers" of capecitabine, and they form higher than expected concentrations of 5-FU and present with early-onset toxicities, including severe gastroenteritis and bone marrow suppression.[25,58,65,87] Lastly, certain thymidylate synthase polymorphisms are associated with more severe toxicity.

The proportions of unchanged 5-FU that are eliminated in the urine and bile are less than 10% and approximately 2% to 3%, respectively. Capecitabine and its metabolites are eliminated primarily by the kidneys (84% in the first 24 hours).[104] Capecitabine is contraindicated when the creatinine clearance is less than 30 mL/min.

Pathophysiology

The therapeutic and toxic effects of 5-FU (and capecitabine) are based on its ability to limit DNA and RNA activities by serving as an antagonist to the pyrimidine uracil in these macromolecules (Fig. 51–2). The specific sites of action and consequence are as follows: (1) 5-FU (as FdUMP) replaces dUMP and it irreversibly inhibits thymidylate synthetase with the cofactor 5,10-methylenetetrahydrofolate (affects DNA synthesis and repair), (2) FdUTP replaces dUTP and it inhibits DNA replication (because of depleted thymidine triphosphate), and (3) fluorouridine triphosphate (FUTP) replaces UTP and inhibits RNA and protein syntheses. Although the primary mechanism of action in chemotherapy for 5-FU is inhibition of thymidylate synthetase, the impairment of RNA synthesis causes significant toxicity to the host.

Clinical Manifestations

The clinical manifestations of toxicity from 5-FU or capecitabine are primarily gastroenteritis and bone marrow suppression.[28,66] In the setting of an overdose (5-FU), nausea and diarrhea present early (<3 days) after the completion of the administration of the chemotherapeutic.[84]

Patients with a significant genetic variant of a critical enzyme (eg, DPD, cytidine deaminase or thymidylate synthase) present with severe manifestations of toxicity that are similar to an overdose of 5-FU or capecitabine.[81] However, these clinical findings typically appear soon after the administration of the first course of treatment. For capecitabine, patients susceptible to toxicity will develop manifestations later than patients on 5-FU—usually on days 3 to 9 during a 14 day course of treatment.[25,59,65]

Hand-and-foot syndrome (palmar plantar erythrodysesthesia) presents with erythema, edema, and pain on the palms of the hand and soles of the feet. The skin desquamates and blisters, and the lesions can appear at other locations on the body. The dermatitis is more common with capecitabine than 5-FU therapy.[28] It has a variable time of onset (from 11 to 360 days), and is reversible upon discontinuation of the chemotherapeutic.[104]

Cardiac and central nervous system (CNS) manifestations of toxicity are uncommon in patients treated with these chemotherapeutics. The incidence of cardiotoxicity from 5-FU varies from 1.6% to 18%, and patents with an underlying coronary artery disease are at risk for toxicity.[82,93] Cardiac manifestations of toxicity include dysrhythmias, myocardial infarction, myocardial ischemia (from coronary artery vasospasm), heart failure, and cardiogenic shock.[59,82] In patients with documented cardiotoxicity from 5-FU, it is recommended not to resume therapy because the recurrence rate is high, varying from 82% to 100%.[93] The manifestations of CNS toxicity include confusion, encephalopathy, and coma. Although the causes of these cardiac and neurologic manifestations are unknown, toxicity from the metabolite fluoroacetate and genetic polymorphisms, such as significant DPD deficiency, are under consideration.[59,67,81]

Diagnostic Testing

Clinical laboratory tests for 5-FU or capecitabine and their metabolites (FBAL), and the type of genetic variant or level of activity for selected enzymes, such as DPD[8,63] or cytidine deaminase[25,87] are not routinely available.

Management

It is reasonable to administer activated charcoal as soon as possible to a patient presenting early after an oral overdose of capecitabine because it can limit gut absorption of the chemotherapeutic. Initiate supportive management with IV fluid hydration for dehydration and an antiemetic for vomiting (as needed) and assess for electrolyte disorders, cardiac and kidney injuries, neutropenia, and sepsis. If the patient presents with an acute coronary syndrome, conservative antianginal therapy (eg, nitrates and calcium channel blockers) should be initiated. The patient will require a cardiac monitored setting, and serial ECGs and cardiac enzymes.[82,93] Patients with a new-onset altered mental status should receive a computed tomography scan of the brain and possibly a lumbar puncture to assess for CNS infection.

Antidotes

Uridine triacetate is administered in a timely manner in an attempt to improve the survival of patients adversely affected by 5-FU or capecitabine. In an open-label, multicenter study of patients with 5-FU or capecitabine toxicity and treated with uridine triacetate, there were 117 overdoses and 18 early-onset severe symptoms. An overdose was defined as the administration of 5-FU at a dose or rate (via infusion pump) greater than the maximum tolerated dose for the patient's intended regimen of 5-FU or capecitabine. Patients with early onset of signs or symptoms were presumed susceptible to toxicity from a genetic variant of a critical enzyme. In the study, there were 130 survivors at 30 days among the 135 patients treated with uridine triacetate.[59] By comparison, when uridine triacetate was not available in a historical reference group of patients with 5-FU or capecitabine toxicity, there were 38 deaths among 42 patients.[59] When uridine triacetate was administered within 96 hours of the last dose of the chemotherapeutic to patients with early onset of severe toxicity, all 18 patients survived. However, only 3 of 8 patients survived when uridine acetate was administered after 96 hours.[59]

Uridine triacetate is available for the emergency treatment of patients with (1) an overdose of 5-FU or capecitabine regardless of the presence of clinical toxicity, (2) an early onset (<96 hours) of severe or life-threatening manifestations of toxicity (cardiovascular, central nervous system), or (3) an early onset of an unusually severe adverse reaction, such as gastroenteritis or neutropenia, within 96 hours of the administration of 5-FU or capecitabine during the course of chemotherapy.[100] Uridine triacetate is not recommended for the treatment of patients with common or anticipated manifestations of these chemotherapeutics during therapy.[100]

Uridine triacetate is administered orally and it is deacetylated by nonspecific esterases in the gut and blood to form uridine. Upon entry into the cell, uridine is activated by phosphorylation to uridine triphosphate (UTP) (Fig. 51–2). Uridine competes with FUTP as UTP during the synthesis of RNA and serves as a source of UTP for the synthesis of new RNA. A plasma uridine concentration of at least 70 µmol/L is desired for treatment of 5-FU toxicity.[59] A single oral dose of 6 to 10 g of uridine triacetate yields a peak plasma uridine concentration of approximately 150 µmol/L at about 2 hours.[84,106] The half-life for uridine is about 3 hours.[106]

The amount of uridine triacetate indicated for 5-FU or capecitabine toxicity in adults for each single dose is 10 g administered every 6 hours for 20 doses for a total of 5 days. For children, a single dose of uridine triacetate is 6.2 g/m^2 of body surface area up to a maximum of 10 g per dose. The dose and schedule for uridine triacetate are not adjusted by sex, amount of 5-FU or capecitabine, or hepatic or renal clearance.[102] A common side effect from this therapy is nausea, vomiting, and diarrhea.

SUMMARY

- The number of patients with potential MTX toxicity is anticipated to increase because of the expanding therapeutic indications and available multiple formulations of this chemotherapeutic.
- Specific interventions for MTX include supportive care, monitoring plasma MTX concentration, urinary alkalinization to limit kidney toxicity, enhanced elimination, and antidotal therapy with leucovorin and enzymatic cleavage with glucarpidase.
- Patients with significant genetic variants of critical enzymes involved with 5-FU or capecitabine typically present with severe clinical toxicity soon after the administration of the first course of treatment.
- Specific interventions for 5-FU or capecitabine toxicity include supportive care and antidotal therapy with uridine triacetate.
- The early recognition of these patients and institution of these therapies can offer the patient the best outcome.

Disclaimer

The findings and conclusions in this chapter are those of the author and do not necessarily represent the official position of the Centers for Disease - Control and Prevention.

REFERENCES

1. AbbottLaboratories. Methotrexate II. 2005; http://www.ilexmedical.com/files/PDF/TDX_Methotrexate.pdf. Accessed August 18, 2017.

2. Abelson HT. Methotrexate and central nervous system toxicity. *Cancer Treat Rep.* 1978;62:1999–2001. [PubMed: 376128]

3. Abelson HT, et al. Methotrexate-induced renal impairment: clinical studies and rescue from systemic toxicity with high-dose leucovorin and thymidine. *J Clin Oncol.* 1983;1:208–216. [PubMed: 6607976]

4. Ahern M, et al. Methotrexate kinetics in rheumatoid arthritis: is there an interaction with nonsteroidal antiinflammatory drugs? *J Rheumatol.* 1988;15:1356–1360. [PubMed: 3199396]

5. Akbulut S, et al. Cytoprotective effects of amifostine, ascorbic acid and *N*-acetylcysteine against methotrexate-induced hepatotoxicity in rats. *World J Gastroenterol.* 2014;20:10158–10165. [PubMed: 25110444]

6. Allen JC, et al. Leukoencephalopathy following high-dose IV methotrexate chemotherapy with leucovorin rescue. *Cancer Treat Rep.* 1980;64:1261–1273. [PubMed: 6970616]

7. ARK Diagnostics Inc. Methotrexate assay. http://www.ark-tdm.com/pdfs/2017/Methotrexate/ARK_Methotrexate_Calibrator_Rev05_February_2017.pdf. Published 2017. Accessed September 8, 2017.

8. ARUP Laboratories. 5-Fluorouracil toxicity and chemotherapeutic response panel. http://ltd.aruplab.com/Tests/Pub/2007228. Published 2014. Accessed August 18, 2017.

9. Atherton LD, et al. Toxic megacolon associated with methotrexate therapy. *Gastroenterology.* 1984;86:1583–1588. [PubMed: 6714582]

10. Aumente MD, et al. Evaluation of the novel methotrexate architect chemiluminescent inmunoassay: clinical impact on pharmacokinetic monitoring. *Ther Drug Monit.* 2017;39:492–498. [PubMed: 28682926]

11. Barak AJ, et al. Methotrexate hepatotoxicity. *J Am Coll Nutr.* 1984;3:93–96. [PubMed: 6201520]

12. Begas E, et al. Simple and reliable HPLC method for the monitoring of methotrexate in osteosarcoma patients. *J Chromatogr Sci.* 2014;52:590–595. [PubMed: 23800772]

13. Benezet S, et al. Inefficacy of exchange-transfusion in case of a methotrexate poisoning [in French]. *Bull Cancer.* 1997;84:788–790. [PubMed: 9339182]

14. Bleyer WA. The clinical pharmacology of methotrexate: new applications of an old drug. *Cancer.* 1978;41:36–51. [PubMed: 342086]

15. Bluett J, et al. A HPLC-SRM-MS based method for the detection and quantification of methotrexate in urine at doses used in clinical practice for patients with rheumatological disease: a potential measure of adherence. *Analyst.* 2015;140:1981–1987. [PubMed: 25671614]

16. Bouquie R, et al. Evaluation of a methotrexate chemiluminescent microparticle immunoassay: comparison to fluorescence polarization immunoassay and liquid chromatography-tandem mass spectrometry. *Am J Clin Pathol.* 2016;146:119–124. [PubMed: 27357291]

17. Bremnes RM, et al. Acute hepatotoxicity after high-dose methotrexate administration to rats. *Pharmacol Toxicol.* 1991;69:132–139. [PubMed: 1775433]

18. Buchen S, et al. Carboxypeptidase G2 rescue in patients with methotrexate intoxication and renal failure. *Br J Cancer.* 2005;92:480–487. [PubMed: 15668713]

19. Casale F, et al. Plasma concentrations of 5-fluorouracil and its metabolites in colon cancer patients. *Pharmacol Res.* 2004;50:173–179. [PubMed: 15177306]

20. Chabner BA, Young RC. Threshold methotrexate concentration for in vivo inhibition of DNA synthesis in normal and tumorous target tissues. *J Clin Invest.* 1973;52:1804–1811. [PubMed: 4719662]

21. Christensen ML, et al. Effect of hydration on methotrexate plasma concentrations in children with acute lymphocytic leukemia. *J Clin Oncol.* 1988;6:797–801. [PubMed: 3163362]

22. Connors NJ, et al. Methotrexate toxicity treated with continuous venovenous hemofiltration, leucovorin and glucarpidase. *Clin Kidney J.* 2014;7:590–592. [PubMed: 25859377]

23. Cronstein BN, et al. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. *J Clin Invest.* 1993;92:2675–2682. [PubMed: 8254024]

24. Cunningham J, et al. Do patients receiving haemodialysis need folic acid supplements? *Br Med J (Clin Res Ed).* 1981;282:1582. [PubMed: 6786568]

25. Dahan L, et al. Sudden death related to toxicity in a patient on capecitabine and irinotecan plus bevacizumab intake: pharmacogenetic implications. *J Clin Oncol.* 2012;30:e41–e44. [PubMed: 22184401]

26. Djerassi I, et al. Removal of methotrexate by filtration-adsorption using charcoal filters or by hemodialysis. *Cancer Treat Rep.* 1977;61:751–752.

[PubMed: 889610]

27. Doolittle GC, et al. Methotrexate-associated, early-onset pancytopenia in rheumatoid arthritis. *Arch Intern Med.* 1989;149:1430–1431. [PubMed: 2730263]

28. Ducreux M, et al. Capecitabine plus oxaliplatin (XELOX) versus 5-fluorouracil/leucovorin plus oxaliplatin (FOLFOX-6) as first-line treatment for metastatic colorectal cancer. *Int J Cancer.* 2011;128:682–690. [PubMed: 20473862]

29. Erttmann R, Landbeck G. Effect of oral cholestyramine on the elimination of high-dose methotrexate. *J Cancer Res Clin Oncol.* 1985;110:48–50. [PubMed: 4019569]

30. Fong CM, Lee AC. High-dose methotrexate-associated acute renal failure may be an avoidable complication. *Pediatr Hematol Oncol.* 2006;23:51–57. [PubMed: 16326413]

31. Frei E 3rd, et al. High dose methotrexate with leucovorin rescue. Rationale and spectrum of antitumor activity. *Am J Med.* 1980;68:370–376. [PubMed: 6965819]

32. Frei E 3rd, et al. New approaches to cancer chemotherapy with methotrexate. *N Engl J Med.* 1975;292:846–851. [PubMed: 1078714]

33. Fukushima T, et al. A magnetic resonance abnormality correlating with permeability of the blood-brain barrier in a child with chemical meningitis during central nervous system prophylaxis for acute leukemia. *Ann Hematol.* 1999;78:564–567. [PubMed: 10647882]

34. Gadgil SD, et al. Effect of activated charcoal on the pharmacokinetics of high-dose methotrexate. *Cancer Treat Rep.* 1982;66:1169–1171. [PubMed: 7083219]

35. Gibson TP, et al. Hemoperfusion for methotrexate removal. *Clin Pharmacol Ther.* 1978;23:351–355. [PubMed: 627142]

36. Godefroid MJ, et al. Multicenter method evaluation of the ARK Methotrexate Immunoassay. *Clin Chem Lab Med.* 2014;52:e13–e16. [PubMed: 24114913]

37. Gowan GM, et al. Methotrexate-induced toxic leukoencephalopathy. *Pharmacotherapy.* 2002;22:1183–1187. [PubMed: 12222556]

38. Grimes DJ, et al. Survival after unexpected high serum methotrexate concentrations in a patient with osteogenic sarcoma. *Drug Saf.* 1990;5:447–454. [PubMed: 2285498]

39. Hande KR, et al. Methotrexate and hemodialysis. *Ann Intern Med.* 1977;87:495–496. [PubMed: 907253]

40. Harned TM, Mascarenhas L. Severe methotrexate toxicity precipitated by intravenous radiographic contrast. *J Pediatr Hematol Oncol.* 2007;29:496–499. [PubMed: 17609630]

41. Hess JA, Khasawneh MK. Cancer metabolism and oxidative stress: insights into carcinogenesis and chemotherapy via the non-dihydrofolate reductase effects of methotrexate. *BBA Clin.* 2015;3:152–161. [PubMed: 26674389]

42. Hughes PJ, Lane RJ. Acute cerebral oedema induced by methotrexate. *BMJ.* 1989;298:1315. [PubMed: 2500219]

43. Inoue S, et al. Erythrocyte methotrexate-polyglutamate assay using fluorescence polarization immunoassay technique: application to the monitoring of patients with rheumatoid arthritis. *Yakugaku Zasshi.* 2009;129:1001–1005. [PubMed: 19652507]

44. Inoue S, et al. Preliminary study to identify the predictive factors for the response to methotrexate therapy in patients with rheumatoid arthritis. *Yakugaku Zasshi.* 2009;129:843–849. [PubMed: 19571519]

45. Isacoff W. Effects of extracorporeal charcoal hemoperfusion on plasma methotrexate [abstract]. *Proc Am Assoc Cancer Res.* 1977;18:1.

46. Iven H, Brasch H. The effects of antibiotics and uricosuric drugs on the renal elimination of methotrexate and 7-hydroxymethotrexate in rabbits. *Cancer Chemother Pharmacol.* 1988;21:337–342. [PubMed: 3370743]

47. Jackson RC, Grindley GB. The biochemical basis for methotrexate cytotoxicity. In: Sirotnak FM, et al, eds. *Folate Antagonists as Therapeutic Agents*. New York, NY: Academic Press; 1984:289–315.

48. Jacobs SA, et al. 7-Hydroxymethotrexate as a urinary metabolite in human subjects and rhesus monkeys receiving high dose methotrexate. *J Clin Invest.* 1976;57:534–538. [PubMed: 1062383]

49. Jaffe N, et al. Transient neurologic disturbances induced by high-dose methotrexate treatment. *Cancer.* 1985;56:1356–1360. [PubMed: 3875390]

50. Jambou P, et al. Removal of methotrexate by continuous venovenous hemodiafiltration. *Contrib Nephrol.* 1995;116:48–52. [PubMed: 8529381]

51. Kawabata K, et al. A case of methotrexate-induced acute renal failure successfully treated with plasma perfusion and sequential hemodialysis. *Nephron.* 1995;71:233–234. [PubMed: 8569964]

52. Kelkar R, et al. Epidemic iatrogenic *Acinetobacter* spp. meningitis following administration of intrathecal methotrexate. *J Hosp Infect.* 1989;14:233–243. [PubMed: 2575105]

53. Kepka L, et al. Successful rescue in a patient with high dose methotrexate-induced nephrotoxicity and acute renal failure. *Leuk Lymphoma.* 1998;29:205–209. [PubMed: 9638991]

54. Kevat SG, et al. Pancytopenia induced by low-dose methotrexate for rheumatoid arthritis. *Aust N Z J Med.* 1988;18:697–700. [PubMed: 3245825]

55. Kremer JM, Hamilton RA. The effects of nonsteroidal antiinflammatory drugs on methotrexate (MTX) pharmacokinetics: impairment of renal clearance of MTX at weekly maintenance doses but not at 7.5 mg. *J Rheumatol.* 1995;22:2072–2077. [PubMed: 8596147]

56. Langslow A. Nursing and the law. Deadly doses of methotrexate. *Aust Nurs J.* 1995;2:32–34. [PubMed: 7627329]

57. Liegler DG, et al. The effect of organic acids on renal clearance of methotrexate in man. *Clin Pharmacol Ther.* 1969;10:849–857. [PubMed: 5349626]

58. Loganayagam A, et al. Pharmacogenetic variants in the DPYD, TYMS, CDA and MTHFR genes are clinically significant predictors of fluoropyrimidine toxicity. *Br J Cancer.* 2013;108:2505–2515. [PubMed: 23736036]

59. Ma WW, et al. Emergency use of uridine triacetate for the prevention and treatment of life-threatening 5-fluorouracil and capecitabine toxicity. *Cancer.* 2017;123:345–356. [PubMed: 27622829]

60. MacKinnon SK, et al. Pancytopenia associated with low dose pulse methotrexate in the treatment of rheumatoid arthritis. *Semin Arthritis Rheum.* 1985;15:119–126. [PubMed: 3877983]

61. Massenkeil G, et al. Transient tetraparesis after intrathecal and high-dose systemic methotrexate. *Ann Hematol.* 1998;77:239–242. [PubMed: 9858151]

62. McIntosh S, et al. Methotrexate hepatotoxicity in children with leukemia. *J Pediatr.* 1977;90:1019–1021. [PubMed: 870655]

63. Mercier C, Ciccolini J. Profiling dihydropyrimidine dehydrogenase deficiency in patients with cancer undergoing 5-fluorouracil/capecitabine therapy. *Clin Colorectal Cancer.* 2006;6:288–296. [PubMed: 17241513]

64. Mercier C, Ciccolini J. Severe or lethal toxicities upon capecitabine intake: is DPYD genetic polymorphism the ideal culprit? *Trends Pharmacol Sci.*

2007;28:597–598. [PubMed: 18001850]

65. Mercier C, et al. Early severe toxicities after capecitabine intake: possible implication of a cytidine deaminase extensive metabolizer profile. *Cancer Chemother Pharmacol.* 2009;63:1177–1180. [PubMed: 19107485]

66. Meulendijks D, et al. Renal function, body surface area, and age are associated with risk of early-onset fluoropyrimidine-associated toxicity in patients treated with capecitabine-based anticancer regimens in daily clinical care. *Eur J Cancer.* 2016;54:120–130. [PubMed: 26761784]

67. Millart H, et al. The effects of 5-fluorouracil on contractility and oxygen uptake of the isolated perfused rat heart. *Anticancer Res.* 1992;12:571–576. [PubMed: 1580574]

68. Molinari A, et al. New antineoplastic prenylhydroquinones. Synthesis and evaluation. *Bioorg Med Chem.* 2000;8:1027–1032. [PubMed: 10882014]

69. Montesinos MC, et al. The antiinflammatory mechanism of methotrexate depends on extracellular conversion of adenine nucleotides to adenosine by ecto-5'-nucleotidase: findings in a study of ecto-5'-nucleotidase gene-deficient mice. *Arthritis Rheum.* 2007;56:1440–1445. [PubMed: 17469101]

70. Moriwaki Y, et al. Widespread cellular distribution of aldehyde oxidase in human tissues found by immunohistochemistry staining. *Histol Histopathol.* 2001;16:745–753. [PubMed: 11510964]

71. Nesbit M, et al. Acute and chronic effects of methotrexate on hepatic, pulmonary, and skeletal systems. *Cancer.* 1976;37(suppl):1048–1057. [PubMed: 1062231]

72. O'Marcaigh AS, et al. Successful treatment of intrathecal methotrexate overdose by using ventriculolumbar perfusion and intrathecal instillation of carboxypeptidase G2. *Mayo Clin Proc.* 1996;71:161–165. [PubMed: 8577190]

73. Park ES, et al. Carboxypeptidase-G2 resuce in a patient with a high dose methotrexate-induced nephrotoxicty. *Cancer Res Treat.* 2005;37:2.

74. Peng Z, et al. Ecto-5'-nucleotidase (CD73)-mediated extracellular adenosine production plays a critical role in hepatic fibrosis. *Nucleosides Nucleotides Nucleic Acids.* 2008;27:821–824. [PubMed: 18600546]

75. Perez-Aso M, et al. Adenosine 2A receptor promotes collagen production by human fibroblasts via pathways involving cyclic AMP and AKT but independent of Smad2/3. *FASEB J.* 2014;28:802–812. [PubMed: 24200882]

76. Pinedo HM, et al. The reversal of methotrexate cytotoxicity to mouse bone marrow cells by leucovorin and nucleosides. *Cancer Res.* 1976;36:4418–4424. [PubMed: 1087180]

77. Reggev A, Djerassi I. The safety of administration of massive doses of methotrexate (50 g) with equimolar citrovorum factor rescue in adult patients. *Cancer.* 1988;61:2423–2428. [PubMed: 3259154]

78. Relling MV, et al. Removal of methotrexate, leucovorin, and their metabolites by combined hemodialysis and hemoperfusion. *Cancer.* 1988;62:884–888. [PubMed: 3261621]

79. Roberts MS, et al. Determination of methotrexate, 7-hydroxymethotrexate, and 2,4-diamino-n10-methylpteroic acid by LC-MS/MS in plasma and cerebrospinal fluid and application in a pharmacokinetic analysis of high-dose methotrexate. *J Liq Chromatogr Relat Technol.* 2016;39:745–751. [PubMed: 28824272]

80. Roenigk HH Jr, et al. Methotrexate therapy for psoriasis. Guideline revisions. *Arch Dermatol.* 1973;108:35. [PubMed: 4716739]

81. Saif MW, Diasio RB. Benefit of uridine triacetate (Vistogard) in rescuing severe 5-fluorouracil toxicity in patients with dihydropyrimidine dehydrogenase (DPYD) deficiency. *Cancer Chemother Pharmacol.* 2016;78:151–156. [PubMed: 27278667]

82. Saif MW, et al. Fluoropyrimidine-associated cardiotoxicity: revisited. *Expert Opin Drug Saf.* 2009;8:191–202. [PubMed: 19309247]

83. Saland JM, et al. Effective removal of methotrexate by high-flux hemodialysis. *Pediatr Nephrol.* 2002;17:825–829. [PubMed: 12376811]

84. Santos C, et al. The successful treatment of 5-fluorouracil (5-FU) overdose in a patient with malignancy and HIV/AIDS with uridine triacetate. *Am J Emerg Med.* 2017;35:802.e807–802.e808.

85. Sasaki K, et al. Theoretically required urinary flow during high-dose methotrexate infusion. *Cancer Chemother Pharmacol.* 1984;13:9–13. [PubMed: 6733846]

86. Schwartz S, et al. Glucarpidase (carboxypeptidase G2) intervention in adult and elderly cancer patients with renal dysfunction and delayed methotrexate elimination after high-dose methotrexate therapy. *Oncologist.* 2007;12:1299–1308. [PubMed: 18055849]

87. Serdjebi C, et al. Role of cytidine deaminase in toxicity and efficacy of nucleosidic analogs. *Expert Opin Drug Metab Toxicol.* 2015;11:665–672. [PubMed: 25495470]

88. Sheikh-Hamad D, et al. Cisplatin-induced renal toxicity: possible reversal by *N*-acetylcysteine treatment. *J Am Soc Nephrol.* 1997;8:1640–1644. [PubMed: 9335396]

89. Shinozaki T, et al. Successful rescue by oral cholestyramine of a patient with methotrexate nephrotoxicity: nonrenal excretion of serum methotrexate. *Med Pediatr Oncol.* 2000;34:226–228. [PubMed: 10696135]

90. Skoutakis VA, et al. Folic acid dosage for chronic hemodialysis patients. *Clin Pharmacol Ther.* 1975;18:200–204. [PubMed: 1098832]

91. Smith TJ, et al. Recommendations for the use of WBC growth factors: American Society of Clinical Oncology Clinical Practice Guideline Update. *J Clin Oncol.* 2015;33:3199–3212. [PubMed: 26169616]

92. Sonneveld P, et al. Pharmacokinetics of methotrexate and 7-hydroxy-methotrexate in plasma and bone marrow of children receiving low-dose oral methotrexate. *Cancer Chemother Pharmacol.* 1986;18:111–116. [PubMed: 3791556]

93. Sorrentino MF, et al. 5-fluorouracil induced cardiotoxicity: review of the literature. *Cardiol J.* 2012;19:453–458. [PubMed: 23042307]

94. Steger GG, et al. GM-CSF in the treatment of a patient with severe methotrexate intoxication. *J Intern Med.* 1993;233:499–502. [PubMed: 8501421]

95. Stoller RG, et al. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. *N Engl J Med.* 1977;297:630–634. [PubMed: 302412]

96. Teh HS, et al. Transverse myelopathy following intrathecal administration of chemotherapy. *Singapore Med J.* 2007;48:e46–e49. [PubMed: 17304378]

97. Thierry FX, et al. Acute renal failure after high-dose methotrexate therapy. Role of hemodialysis and plasma exchange in methotrexate removal. *Nephron.* 1989;51:416–417. [PubMed: 2918955]

98. Tukova J, et al. Methotrexate bioavailability after oral and subcutaneous dministration in children with juvenile idiopathic arthritis. *Clin Exp Rheumatol.* 2009;27:1047–1053. [PubMed: 20149329]

99. Vakily M, et al. Coadministration of lansoprazole and naproxen does not affect the pharmacokinetic profile of methotrexate in adult patients with rheumatoid arthritis. *J Clin Pharmacol.* 2005;45:1179–1186. [PubMed: 16172183]

100. Vistogard(TM). (uridine triacetate) [package insert]. Gaithersburg, MD: Wellstat Therapeutics Corp; 2015. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208159s000lbl.pdf. Accessed August 18, 2017.

101. Von Hoff DD, et al. Incidence of drug-related deaths secondary to high-dose methotrexate and citrovorum factor administration. *Cancer Treat Rep.* 1977;61:745–748. [PubMed: 301783]

102. Voraxaze(TM). (glucaripdase) [package insert]. West Conshohocken, PA: BTG International Inc; 2012. https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125327lbl.pdf. Accessed September 8, 2017.

103. Walker RW, et al. Transient cerebral dysfunction secondary to high-dose methotrexate. *J Clin Oncol.* 1986;4:1845–1850. [PubMed: 3465876]

104. Walko CM, Lindley C. Capecitabine: a review. *Clin Ther.* 2005;27:23–44. [PubMed: 15763604]

105. Wall SM, et al. Effective clearance of methotrexate using high-flux hemodialysis membranes. *Am J Kidney Dis.* 1996;28:846–854. [PubMed: 8957036]

106. Weinberg ME, et al. Enhanced uridine bioavailability following administration of a triacetyluridine-rich nutritional supplement. *PLoS One.* 2011;6:e14709. [PubMed: 21379380]

107. Weinstein GD. Methotrexate. *Ann Intern Med.* 1977;86:199–204. [PubMed: 319725]

108. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. *Oncologist.* 2006;11:694–703. [PubMed: 16794248]

109. Widemann BC, et al. High-dose methotrexate-induced nephrotoxicity in patients with osteosarcoma. *Cancer.* 2004;100:2222–2232. [PubMed: 15139068]

110. Widemann BC, et al. Carboxypeptidase-G2, thymidine, and leucovorin rescue in cancer patients with methotrexate-induced renal dysfunction. *J Clin Oncol.* 1997;15:2125–2134. [PubMed: 9164227]

111. Widemann BC, et al. Treatment of accidental intrathecal methotrexate overdose with intrathecal carboxypeptidase G2. *J Natl Cancer Inst.* 2004;96:1557–1559. [PubMed: 15494606]

112. Widemann BC, et al. Pharmacokinetics and metabolism of the methotrexate metabolite 2, 4-diamino-N(10)-methylpteroic acid. *J Pharmacol Exp Ther.* 2000;294:894–901. [PubMed: 10945838]

113. Ziereisen F, et al. Reversible acute methotrexate leukoencephalopathy: atypical brain MR imaging features. *Pediatr Radiol.* 2006;36:205–212. [PubMed: 16369780]