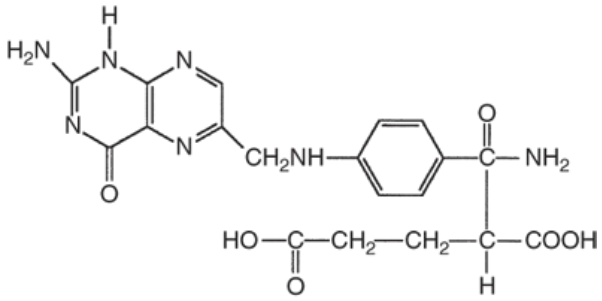
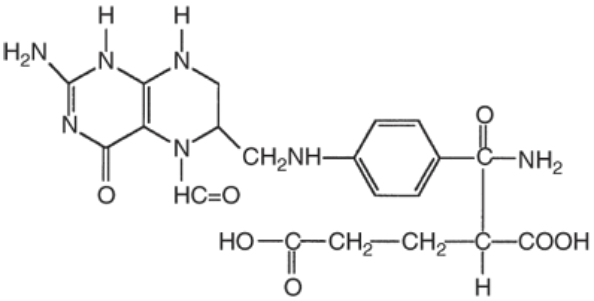
Chapter A12: Folates: Leucovorin (Folinic Acid) and Folic Acid

Silas W. Smith; Mary Ann Howland

INTRODUCTION



Folic Acid



Folinic Acid

Folates refer to the metabolically active reduced forms of folic acid, including dihydrofolate and tetrahydrofolate. These folates are vital to cellular biochemistry, including the synthesis of purines and DNA. Folic acid must be reduced in vivo by dihydrofolate reductase to tetrahydrofolate. Dihydrofolate reductase inhibitors such as methotrexate (MTX), pyrimethamine and pemetrexed prevent this reduction. Leucovorin (folinic acid) and levoleucovorin, do not require dihydrofolate reductase for activation. Therefore, either leucovorin or levoleucovorin is the primary antidote for a patient who receives an overdose of MTX or another dihydrofolate reductase inhibitor.

Methanol is metabolized to the active and toxic formic acid. Folates, including folic acid and leucovorin, speed up the conversion of formic acid to nontoxic metabolites. Because methanol does not interfere with the synthesis of tetrahydrofolate, either folic acid or leucovorin is acceptable for a patient poisoned by methanol. Preliminary evidence also suggests a role for folic acid to enhance arsenic elimination.

HISTORY

In 1930–1931, Lucy Wills, while studying pregnant textile workers with macrocytic anemia in Mumbai, India, discovered that a yeast extract provided to

these nutritionally deficient individuals cured and prevented their anemia.[105] Mitchell isolated the active ingredient from spinach in 1941 and named it folic acid from the Latin *folium*, meaning leaf.[35] Subsequently, the synthesis and chemical structure of folate was described in 1945–1946.[3] In 1948, the first reported clinical success in inducing temporary remission of acute leukemia by the antifolate aminopterin was reported, soon followed by success with the less toxic amethopterin (ie, MTX).[24] That same year, in studies exploring links to anemia, a factor in the gram-positive bacteria *Leuconostoc citrovorum* was identified as required in growth media for deficient species.[79] Two years later in 1950, this "citrovorum factor"—later named leucovorin (folinic acid)—successfully reversed aminopterin and MTX toxicity, which had resisted folate therapy.[82] In the 1960s, the concept emerged of providing higher doses of MTX for improved chemotherapeutic efficacy, which was then coupled with subsequent leucovorin "rescue" to mitigate toxicity.[53] Since then, the many roles of folate and natural or induced folate deficiency continue to be studied.

PHARMACOLOGY

Folic acid (pteroylglutamic acid), an essential water-soluble vitamin, consists of a pteridine ring joined to PABA (*para*-aminobenzoic acid) and glutamic acid.[3] Folic acid is the most common of the many folate congeners that exist in nature and are essential for normal cellular metabolic functions. Folic acid is rarely called vitamin B_9. After absorption, folic acid is reduced by dihydrofolic acid reductase (DHFR) to dihydrofolic acid and then tetrahydrofolic acid (THF), which accepts one-carbon groups. Tetrahydrofolic acid serves as the precursor for several biologically active forms of folic acid, including 5-formyltetrahydrofolic acid (5-formyl THF), which is best known as folinic acid, leucovorin, and citrovorum factor. The biologically active forms of folate are enzymatically interconvertible and function as cofactors, providing the one-carbon groups necessary for many intracellular metabolic reactions, including the synthesis of thymidylate and purine nucleotides, which are essential precursors of DNA.[69,76,86,89,99] The minimum daily requirement of folic acid is normally approximately 50 mcg, but nutritionally deprived, acutely ill patients may require 100 to 200 mcg, and women who are preconception or pregnant are advised to take 400 mcg to 1,000 mcg of folic acid daily.[18,37]

Leucovorin is a mixture of the active and inactive diastereoisomers of 5-formyl-THF, of which the levo-(6*S*)-form is active and available as levoleucovorin.[88] Both are available as the calcium salt, with the same chemical formula. The molecular weight (MW) is 511.5 Da; levoleucovorin for injection is supplied as the calcium pentahydrate form for a total MW of 601.6.[88] Absent metabolic inhibition, folate and leucovorin are rapidly metabolized to several active folates, including 5-methyltetrahydrofolate (5-methyl-THF). The dose of levoleucovorin is half of leucovorin. Leucovorin *increases* the toxicity of 5-fluorouracil (5-FU). Leucovorin is converted to (6*R*)-5,10-methylene-THF, which stabilizes the ternary complex with the 5-FU metabolite, fluoro-deoxyuridine monophosphate (5-FdUMP) and thymidylate synthase, leading to thymidylate depletion.[20,80]

After a DHFR inhibitor such as MTX inhibits the formation of tetrahydrofolic acid, the intracellular machinery for the synthesis of indispensable thymidylate and purine nucleotides comes to a halt, and DNA production ceases. Leucovorin and levoleucovorin are biologically active forms of folic acid and bypass this inhibition of DHFR caused by MTX.

Folate catalyzes the formation of carbon dioxide and water from formic acid, the final metabolic step in methanol elimination. Because there is no inhibition of the formation or recycling of active folate, either folic acid or leucovorin is beneficial (Chap. 106).

Investigations suggest that folic acid aids in the methylation and subsequent elimination of arsenic. Folate supplementation in folate-deficient subjects enhanced the elimination of arsenic and potentially decreased chronic arsenic toxicity (Chap. 86).[27,70]

Related Xenobiotics

The predominant form of dietary folate, *levo*-(6*S*)-5-methyl-THF, which is required for methionine biosynthesis from homocysteine, is available commercially both as the natural form and as mefolinate, the diastereoisomeric, 1:1 mixture (6*R*,*S*)-5-methyl-THF.[20] It is also found in combination with pharmaceuticals (eg, oral contraceptive pills).[26] (6*R*)-5,10-methylene-THF also bypasses activation to produce higher (6*R*)-5,10-methylene-THF concentrations compared to leucovorin in order to enhance 5-FU efficacy.[100] It is also undergoing evaluations as an MTX rescue agent (ClinicalTrials.gov Identifier: NCT01987102).

Leucovorin Pharmacokinetics

Whereas leucovorin is naturally formed in the body as the active *levo* (*l*)-(6*S*)-isomer, the initial commercial preparation was a racemic mixture

consisting of equal amounts of the inactive *dextro* (*d*)-(6*R*) and active (*l*) isomers. The pharmacokinetics of the racemic mixture of leucovorin and its active metabolite were studied after a single intravenous (IV) infusion and as a constant infusion in healthy human volunteers.[91,92] During a constant infusion of 500 mg/m^2/day, the steady-state concentration for the active (*l*) isomer was 2.33 µmol/L, the half-life was 35 minutes, and the volume of distribution (V_d) was 13.6 L. The active isomer is metabolized to an active metabolite, *l*-(6*S*)-5-methyl-THF, which achieved a steady-state concentration of 4.85 µmol/L and a half-life of 227 minutes. Similar values were achieved for half-life and V_d after single IV doses ranging from 25 to 100 mg. The inactive *d*-isomer achieved higher concentrations and had a much longer half-life with oral administration, which is saturable and stereoselective, resulting in absorption of the active isomer that is 4 to 5 times greater than that of the inactive isomer. Studies of stereospecific oral absorption demonstrate that 100% of the *l*-leucovorin is absorbed, but only 20% of the *d*-leucovorin is absorbed at this dose.[95] One study detected no adverse effects of the inactive isomer on the intracellular uptake of the active isomer and concluded that giving the active isomer provided no pharmacokinetic advantage over the racemic mixture.[81] In 5 normal subjects given 1,000 mg of leucovorin as a 2-hour intravenous infusion, peak plasma concentrations of *l*-(6*S*) leucovorin, *d*-(6*R*) leucovorin, and 5-methyl-THF were 5 9.1 ± 22 µmol/L, 148 ± 32 µmol/L, and 17.8 ± 17 µmol/L, respectively.[80] Intravenous leucovorin results in an area under the curve (AUC) for inactive (6*R*) LV that was 4.16 ± 1.4 times greater than the combined AUCs for *l*-(6*S*) leucovorin and 5-methyl-THF.[80] Two hundred milligrams of levoleucovorin was compared to 400 mg of leucovorin, each administered as a 2-hour IV infusion as a crossover study in 40 healthy volunteers; the area under the curve and the maximum serum concentrations of *l*-5-CH_3-THF were similar for both.[88]

The pharmacokinetics of IV leucovorin was compared with intramuscular (IM) and oral administration in male volunteers given 25 mg. At this dose, oral leucovorin was 92% bioavailable and the mean peak active *l*-5-methyl-THF concentration of 258 ng/mL (5.5×10^{-1} µmol/L) at 1.3 hours after IV administration, 226 ng/mL at 2.8 hours for IM, and 367 ng/mL at 2.4 hours for oral administration, respectively.[61] The pharmacokinetics of orally administered leucovorin was studied in healthy, fasted male volunteers in single doses ranging from 20 to 100 mg and 200 mg IV over 5 minutes compared with 200 mg orally.[61,74] Bioavailability decreased from 100% for the 20-mg dose to 78% for the 40-mg dose and ultimately to 31% for the 200-mg dose. A microbiologic assay was used to measure total tetrahydrofolates (reduced and active folates). Normal serum folate concentrations are approximately 0.05 µmol/L.[44] The 200-mg oral dose produced a peak serum concentration of 1.82 µmol/L compared with 0.66 µmol/L for the 20-mg oral dose and 27.1 µmol/L for the 200-mg IV dose.[61,74]

After IV administration of both leucovorin and levoleucovorin, *l*-5-methyl-THF enters the CSF. The concentration of CSF *l*-5-methyl-THF achieved was 100 to 1,000-fold less than that obtained after direct MTX intra-Ommaya reservoir administration during a period of 2 days of observation.[64] Because *l*-5-methyl-THF is cleared slowly from CSF (half-life = 85 hours), progressive accumulation occurs from chemotherapy cycle to cycle.[96] Thus, the significant reductions in *l*-5-methyl-THF that occurs with IV and intrathecal MTX can recover with ongoing leucovorin treatment.[9,93] The limitations of oral leucovorin therapy were demonstrated in one study in which oral leucovorin (10 mg every 6 hours for 11 doses) failed to provide adequate *l*-5-methyl-THF to exceed CSF MTX concentrations in 6 of 9 patients.[2] Intrathecal administration of leucovorin and levoleucovorin is contraindicated, as it can lower seizure threshold and is associated with fatal neurotoxicity.[25,45,88,97]

ROLE IN METHOTREXATE TOXICITY

Methotrexate, an antimetabolite, is a structural analog of folic acid, differing only in the substitution of an amino group for a hydroxyl group at the number 4 position of the pteridine ring (Chap. 51). Methotrexate binds to the active site of DHFR, rendering it incapable of reducing folic acid to its biologically active forms and incapable of regenerating the necessary active forms required for the synthesis of purine nucleotides and thymidylate.[87] Under various conditions, the binding between MTX and DHFR is competitive and very tight, with an inhibition constant ranging from 0.0034 nmol/L to 0.093 ± 0.021 nmol/L.[5,19] 7-HydroxyMTX, a major MTX metabolite, more weakly inhibits DHFR (K_i = 8.9 nM).[5] This compares to a K_m of 2.7 ± 0.5 µmol/L for dihydrofolic acid.[19] Leucovorin is a reduced, active form of folate. As such, it does not require DHFR for enzymatic interconversion to the form required for purine nucleotide and thymidylate formation. Folic acid is unable to counteract MTX toxicity because after MTX therapy, DHFR is unavailable to convert folic acid to an active reduced form. High-dose MTX therapy provides chemotherapeutic benefit, although it is associated with significant toxicity. *Leucovorin rescue* describe the standard practice of limiting the toxic effects associated with high-dose MTX, by providing leucovorin after the initial MTX infusion and in cases of diminished MTX elimination, which can be due to MTX toxicity itself or other factors.[102]

ROLE IN METHANOL AND FORMATE TOXICITY

The formate produced from methanol is metabolized to 10-formyl tetrahydrofolate in the presence of tetrahydrofolate, which can be acted upon by 10-formyltetrahydrofolate dehydrogenase to produce carbon dioxide and to regenerate tetrahydrofolate.[7] Higher folate activity correlates with a faster formate elimination half-life across multiple species.[90] Monkeys experimentally rendered folate deficient develop methanol toxicity at lower methanol concentrations.[62] Folic acid supplementation (2.5 mg/kg IV) provided to dogs poisoned with 2 g/kg methanol lowered maximum formate accumulation in plasma from 3 to 1 mmol/L.[90] Similarly, administering folic acid to healthy monkeys accelerates formate metabolism.[62] Pretreatment with folic acid or leucovorin decreased both formate concentrations and the accompanying metabolic acidosis without affecting the rate of methanol elimination.[62] In monkeys given repeated injections of leucovorin, either before or concurrent with the administration of methanol, decreased formate concentrations and an absence of metabolic acidosis were observed.[4] Leucovorin remained effective in monkeys in hastening the metabolism of formate when given as late as 10 hours after methanol administration.[67]

In methanol-poisoned humans, the hepatic concentrations of total folate, leucovorin, and folate dehydrogenase (which increases leucovorin concentrations) are all diminished.[46] In a methanol-poisoned patient treated without folic acid, the formate half-life was 2.8 hours.[42] In 6 methanol-poisoned patients treated without folic acid, the mean serum formate half-life was 2.6 hours.[38] By comparison, in a single methanol-poisoned patient who was given folic acid, ethanol, and hemodialyzed, the half-life of formate was 1.1 hours during hemodialysis.[68] In another methanol-poisoned patient, the formate half-life was 3.9 hours, which decreased to 1.2 hours after leucovorin treatment.[38] In an intentional ingestion of 110 g of formic acid treated with folinic acid (1 mg/kg every 4 hours for the first 24 hours), low plasma formic acid concentrations were present during leucovorin administration, which rose significantly after leucovorin cessation.[66] In human placental ex vivo experiments, folic acid reversed the toxic effects of formic acid on the placenta of decreased maternal hCG secretion.[41] These comparative data are inadequate to draw definitive conclusions. However, the evidence supports the continued recommendation for a therapeutic role of folate or leucovorin in addition to definitive therapy with fomepizole (Antidotes in Depth: A33) and/or hemodialysis.[7]

ROLE IN ARSENIC TOXICITY

Arsenic contamination of drinking water has plagued millions, causing increased manifestations of chronic arsenic toxicity, including cancer and cardiovascular, dermatologic, and neurologic disorders.[17,60] In a folate-dependent mechanism, arsenic undergoes methylation to monomethylarsonic acid (MMA) and then to dimethylarsinic acid (DMA) via one-carbon metabolism, and this methylation facilitates urinary arsenic excretion.[28,33] Patients with higher intakes of folate-related nutrients had lower percentages of inorganic arsenic and higher MMA to inorganic arsenic ratios.[33] Folate deficiency, hyperhomocysteinemia, and low urinary creatinine, each of which is associated with decreased arsenic methylation capacity, are risk factors for arsenic-induced skin lesions.[71] In a study of arsenic's epigenetic effects on histone modification, arsenic concentration in women were associated with lower total plasma histone concentrations only among women with folate deficiency.[94] The absolute methylation amount and product probably depends on the degree of upregulation, age, and available methyl groups (Chap. 86).[31,40] In animal models, folic acid supplementation decreased arsenic embryotoxicity, malformations, and abnormal cardiac and neural development.[58] This effect appeared to be mediated by decreasing subcellular reactive oxygen species when tested in human embryonic kidney cells.[58]

These findings encouraged the study of folate supplementation, along with other nutrients, with the aim to diminish the chronic health effects of arsenic toxicity. A randomized, double-blind, placebo-controlled trial of folic acid supplementation (400 mcg daily for 12 weeks) to participants with low plasma folate concentrations demonstrated enhanced arsenic methylation.[27] In a randomized, controlled trial of 622 patients, daily folic acid of 800 mcg for 12 and 24 weeks' treatment lowered blood arsenic to a greater extent than placebo, an effect that was sustained without rebound for 12 weeks after treatment cessation.[70]

ROLE IN TOXICITY FROM DIHYDROFOLATE REDUCTASE INHIBITOR ANTIBIOTICS

Pyrimethamine is a dihydrofolate reductase competitive inhibitor used to treat infections from *Toxoplasma*, *Isospora*, and *Pneumocystis*. In a dose-dependent fashion, leucovorin significantly reduces cytogenetic aberrations associated with pyrimethamine in vitro.[21] Leucovorin is routinely added

to pyrimethamine therapy for toxoplasmosis.[73,98] One large outbreak of pyrimethamine toxicity reported a 23% fatality rate in 664 cardiac patients who received isosorbide mononitrate contaminated with 50 mg of pyrimethamine.[48] Once the contaminant was determined, leucovorin was administered at a dose of 30 mg every 6 hours for 2 days and then every 12 hours until complete recovery.[48] Trimetrexate glucuronate, another dihydrofolate reductase inhibitor used in to treat *Pneumocystis*, has been discontinued in the United States. It required administration with concurrent IV leucovorin in order to preclude serious or life-threatening bone marrow suppression, oral and gastrointestinal mucosal ulceration, and renal and hepatic dysfunction (20 mg/m^2 over 5–10 minutes every 6 hours for a total daily dose of 80 mg/m^2, or orally as 4 doses of 20 mg/m^2 spaced equally throughout the day for 24 days).[63]

ADVERSE EFFECTS AND SAFETY ISSUES

Reports of adverse reactions to parenteral injections of folic acid, leucovorin, or levoleucovorin are uncommon. However, reported adverse reactions include allergic or anaphylactoid reactions.[95] Seizures are also rarely associated with leucovorin or levoleucovorin administration.[65] The calcium content of leucovorin and levoleucovorin warrants a slow IV infusion at a rate not faster than 160 mg/min in adults. There are 0.004 mEq of calcium per milligram of leucovorin calcium injection. Extremely large doses of leucovorin on the order of 1,000 mg every 3 hours might lead to hypercalcemia.[106] Neither leucovorin nor levoleucovorin should be administered intrathecally due to neurotoxic adverse effects.[25,45,52,77,97] As described in the pharmacology section, leucovorin and levoleucovorin are not antidotes for 5-FU, and both enhance the chemotherapeutic and toxic effects of fluoropyrimidines including 5-FU and 5-FU prodrugs such as capecitabine and tegafur.[20,57,88,95,100]

Protocols recommend separating leucovorin and levoleucovorin from glucarpidase by 2 hours (Antidotes in Depth: A13). Otherwise, leucovorin acts as a substrate for glucarpidase, which cleaves the active *l*-(6*S*)-leucovorin approximately 50% faster than the inactive *d*-(6*R*)-form.[1,23,34,84]

There is a potential for dosing errors when interchanging leucovorin and levoleucovorin. The dose of levoleucovorin is one-half the dose of leucovorin.[88]

PREGNANCY AND LACTATION

Folic acid is a Food and Drug Administration (FDA) category A drug and is safe and essential during pregnancy and compatible with breastfeeding. Leucovorin and levoleucovorin are FDA pregnancy category C drugs.[88,95] Although definitive reproductive studies do not exist, folinic acid has successfully treated megaloblastic anemia during gestation[83] and is considered compatible with pregnancy.[13] In addition, there are case reports of maternal administration of leucovorin, in combination with 5-FU and oxaliplatin chemotherapy during pregnancy, with subsequent healthy fetal delivery.[49,59] Breast milk excretion is unstudied, but leucovorin is considered compatible with breastfeeding.[13]

DOSING AND ADMINISTRATION

After MTX overdose, a dose of leucovorin estimated to produce the same plasma concentration as the MTX dose should be given as soon as possible, preferably within one hour. One mole of MTX weighs 454.4 Da, and 1 mole of leucovorin calcium weighs 511.5 Da, with the MW of the leucovorin portion equal to 471.4 Da. Because of the safety of leucovorin and because of the toxicity of MTX, underdosing leucovorin should be avoided. Although serum MTX concentrations are often closely followed in patients on diverse oncologic regimens,[11,12] in the overdose setting, or in MTX toxicity related to treatment for tubal pregnancies, it is inappropriate to wait for a serum concentration before initiating treatment with leucovorin.[6] The toxic threshold for MTX is reported as 1×10^{-8} mol/L (0.01 µmol/L or 10 nmol/L), based on mouse studies evaluating DNA synthesis.[15] Normal serum folic acid concentrations are in the range of 13 to 43 nmol/L. Oral MTX doses as low as 2 mg in adults resulted in mean maximal MTX serum drug concentration of 0.215 µmol/L at one to 2 hours after ingestion.[85] The MTX half-life for the 2 mg dose rose from 2.4 hours to 3.2 hours when three 2-mg tablets were ingested 12 hours apart.[85] If the patient's MTX exposure is not for chemotherapeutic intent, there is no rationale to permit MTX to remain unantagonized by leucovorin. Cases and case series demonstrate that organ-level toxicity is rare, but still possible from single-adult and pediatric oral intentional and unintentional ingestions of MTX.[8,30,32,75] Even in light of the known MTX oral saturation limits, in one retrospective series of 19 cases of children and adults with MTX ingestions with subsequent MTX concentrations, all were above 0.01 µmol/L, the concentrations known to impair DNA

and RNA synthesis in GI epithelium and bone marrow when present for as little as 10 hours, and several MTX concentrations were above 1 µmol/L.[16] A second poison control center review determined MTX concentrations in 15 of 103 oral ingestions, which ranged from 0.02 to 3.23 µmol/L.[32] Even when not associated with acute organ toxicity or symptoms, these MTX concentrations present a gametogenetic and stochastic carcinogenic risk.[15]

Methotrexate bioavailability decreases from 100% with oral doses less than 30 mg/m^2 to approximately 10% to 20% with doses greater than 80 mg/m^2.[10,78] A compilation of pharmacokinetic studies reported that the bioavailable dose appears to saturate at approximately 14.4 ± 1.64 mg/m^2, with several notable outliers.[16] As an example, if a child unintentionally ingests 100 (2.5 mg) MTX tablets for a total dose of 250 mg, only part of this dose is absorbed because MTX absorption is saturable.[30] In this case, it is reasonable to assume that a bioavailability of 50% or less would result in an absorbed dose of MTX of less than 125 mg. For this exposure, an IV dose of 125 mg of leucovorin would be reasonable to provide over 15 to 30 minutes. This dose of IV leucovorin would be expected to produce serum concentrations in excess of that of the MTX, given that the V_d of leucovorin is about 25% less than MTX and the MWs are similar. It is reasonable to repeat this dose of IV leucovorin every 3 to 6 hours until the serum MTX concentration is less than 0.01 µmol/L, preferably zero.[55,95] This differs from recommendations in patients receiving MTX therapeutically (see later discussion).

The MTX half-life varies from 5 to 45 hours, depending on the dose and the patient's kidney function. For this reason, it is reasonable to continue leucovorin therapy for 12 to 24 doses (3 days) or longer if MTX concentrations are unavailable. Patients can develop third-space storage in ascites or pleural effusions, and thus can require leucovorin dosing for an extended period of time. Patients with bone marrow toxicity require more prolonged dosing because plasma half-lives of MTX do not reflect persistent intracellular concentrations.

Therapeutic Methotrexate Therapy

The dose of leucovorin for "leucovorin rescue" after "high-dose" MTX therapy (doses of 500 mg/m^2 or greater) ranges from 10 to 25 mg/m^2 IM or IV every 6 hours for 72 hours up to 1,000 mg/m^2 every 6 hours in patients with renal compromise and delayed elimination.[14,39,95,101,104] If a neonate must be treated, a benzyl alcohol–free preparation must be used because of the toxicity of benzyl alcohol in neonates (Chap. 46).[29,51] For MTX overdoses, equimolar serum leucovorin concentrations theoretically provide adequate protection, but because precise determinations are invariably delayed, leucovorin administration should be initiated without delay.

As a rough guide, a single dose of 25 mg of IV leucovorin in an adult produces a peak concentration of the active *l*-5-CH_3-THF metabolite of approximately 258 ng/mL, which is 0.55 µmol/L.[95] A dosage of about 150 mg every 4 hours in an adult achieves a steady-state concentration of about 4.85 µmol/L.[91] Although the dose of leucovorin can be as high as 1,000 mg/m^2 every 6 hours, this is rarely warranted and cannot adequately compete with serum concentrations of MTX above 100 µmol/L.[72] Under these circumstances, it is recommended to administer glucarpidase (Antidotes in Depth: A13). An IV leucovorin dose of 150 mg/m^2 every 3 to 6 hours is anticipated to be effective in all but the most severe overdoses and should be administered IV as soon as possible over 15 to 30 minutes, but not faster than 160 mg/min in adults because of the calcium content. We recommend that this dose be continued a minimum of several days or until the serum MTX concentration falls below 0.01 µmol/L.[55,95] One case series of 11 patients receiving MTX over 4 hours at 10 to 12 g/m^2 for osteosarcoma or 3.5 g/m^2 for central nervous system lymphoma required high-dose leucovorin rescue for MTX concentrations at high risk for toxicity at 24, 48, or 72 hours, usually because of acute kidney injury. The dosage of leucovorin ranged from 0.24 to 10 g/day and was titrated downward as the MTX concentration fell. It took an average of 11 ± 3 days for the MTX concentration to drop below 0.1 µmol/L (Table A12–1). Table A12–2 is offered in recognition of the importance and complexity associated with switching complex methotrexate concentrations precisely.

TABLE A12–1

Leucovorin Dosage and Administration with Chemotherapeutic Methotrexate Use[a,b]

Clinical Situation	Laboratory Findings	Leucovorin Dosage
Normal methotrexate elimination	Serum [methotrexate] ~10 µmol/L at 24 hours after administration, 1 µmol/L at 48 hours, and <0.2 µmol/L at 72 hours	15 mg PO, IM, or IV every 6 hours for 60 hours (10 doses starting at 24 hours after the start of methotrexate infusion)
Delayed late methotrexate elimination	Serum [methotrexate] remaining >0.2 µmol/L at 72 hours and >0.05 µmol/L at 96 hours after administration	Continue 15 mg PO, IM, or IV every 6 hours until [methotrexate] is <0.05 µmol/L
Delayed early methotrexate - elimination or evidence of acute kidney injury	Serum [methotrexate] of ≥50 µmol/L at 24 hours or ≥5 µmol/L at 48 hours after administration *or* a ≥100% increase in serum [creatinine] at 24 hours after methotrexate administration (eg, an increase from 0.5 to ≥1 mg/dL)	150 mg IV every 3 hours until [methotrexate] is <1 µmol/L; then 15 mg IV every 3 hours until [methotrexate] is <0.05 µmol/L

[a]Leucovorin should not be administered intrathecally. [b]Data from references 36, 88, and 95. IM = intramuscular; IV = intravenous; PO = oral.

TABLE A12–2

Rapid Calculations

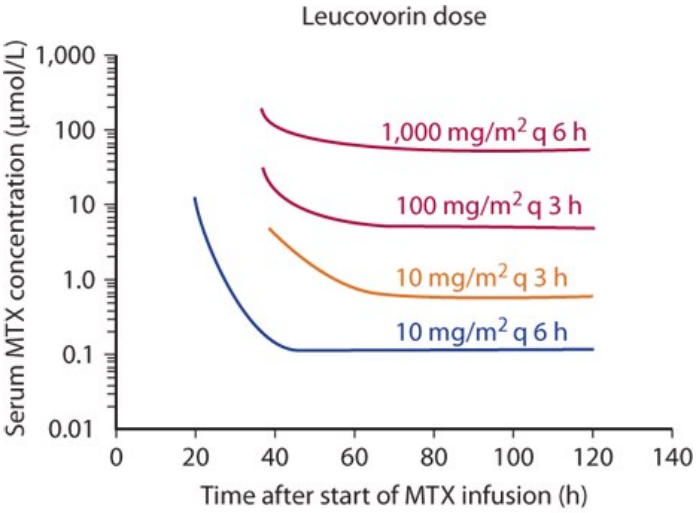
1 mole = 1 g molecular weight
1 molar = 1 mole/L
1×10^{-3} moles = 1 millimole = 1 mmol
1×10^{-6} moles = 1 micromole = 1 µmol
1×10^{-9} moles = 1 nanomole = 1 nmol
1 mole of methotrexate weighs 455 Da; 1 mole methotrexate = 455 g
1 molar methotrexate = 455 g/L = 455 mg/mL
0.01×10^{-6} molar methotrexate = 455×10^{-8} g/L = 455×10^{-8} mg/mL = 455×10^{-5} mcg/mL = 455×10^{-2} ng/mL = 4.55 ng/mL
To convert methotrexate concentrations in mg/L (mcg/mL) to µmol/L, multiply by 2.2
To convert methotrexate concentrations in mg/L (mcg/mL) to nmol/L, multiply by 2,200

Leucovorin rescue strategies after high-dose MTX are aligned in the leucovorin, levoleucovorin, and methotrexate package inserts, with 15 mg leucovorin (7.5 mg of levoleucovorin) provided at baseline every 6 hours for 10 doses beginning 24 hours after MTX administration, so as not to compromise chemotherapeutic efficacy.[36,88,95] In the setting of early or delayed methotrexate elimination or acute kidney injury, leucovorin dosing must be increased to counteract the persistent adverse effects of MTX (Table A12–1).[36,88,95] Other protocols call for leucovorin rescue using a 4-level tiered pharmacokinetically guided rescue strategy depending upon the MTX concentration at different time points (Fig. A12–1).[11,39,101] Healthcare facilities have also implemented leucovorin rescue stratified by multiple checkpoint MTX concentrations at 24, 36, 42, 48, and 72 hours, with multiple leucovorin dosing adjustments depending upon the MTX concentrations at these timepoints.[14,39] Administration of high-dose MTX by continuous infusion over 24 hours may also invoke different leucovorin rescue protocols. Regardless of the specific rescue dosing regimen chosen, systematic protocol implementation in treatment sites reduces variance in the time to MTX concentration measurements and time to first leucovorin rescue dose and increases appropriate leucovorin dose escalation and appropriate leucovorin at discharge.[14] A constant IV infusion of 21 mg/m^2/h has been safely administered for 5 days. A transition to oral administration of leucovorin depends on the serum concentration of MTX and whether adequate serum

concentrations of leucovorin can be achieved orally. In adults, a 200-mg oral dose of leucovorin produces a peak serum concentration of 1.82 µmol/L compared with 27.1 µmol/L with a 200-mg IV dose.

FIGURE A12–1.

Example of a nomogram developed by Bleyer[10] for pharmacokinetically guided leucovorin rescue after high-dose methotrexate (MTX) administration.



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

Levoleucovorin, the active *l*-isomer of folinic acid, is available and should be dosed at half the dose of racemic leucovorin.[88] Because of the calcium content of the levoleucovorin solution, the rate of intravenous infusion should not exceed more than 16 mL (160 mg of levoleucovorin) per minute.[88,95]

Administration of activated charcoal (AC) limits the benefit of subsequent administration of oral leucovorin. In addition to leucovorin, other modalities to treat patients with MTX overdoses include AC (Antidotes in Depth: A1), urinary alkalinization (Antidotes in Depth: A5), glucarpidase (Antidotes in Depth: A13), and extracorporeal removal (Chap. 51).

Intrathecal Methotrexate Overdose

Unintentional overdose with intrathecal MTX is potentially quite serious and is dose dependent.[45,54] In these cases, IV leucovorin and *not* intrathecal leucovorin should be administered. Intrathecal leucovorin was considered a major factor in the death of a child given a slightly higher dose of intrathecal MTX than was prescribed.[52] Although some cases have been managed with IV leucovorin with or without additional drainage procedures, it is reasonable to administer intrathecal glucarpidase in cases of significant intrathecal MTX overdose or signs of neurotoxicity.[22,43,77,103] In intrathecal MTX overdoses, consultation with experienced hematologists/oncologists and medical toxicologists is warranted (Special Considerations: SC7).[50]

Pemetrexed Toxicity

Pemetrexed toxicity is similar to that of methotrexate. Toxicity is attenuated initially with a low dose of vitamin B_{12} and leucovorin as described below. In clinical trials, leucovorin was permitted for NCI Common Terminology Criteria (CTC) Grade 4 leukopenia lasting ≥3 days, CTC Grade 4 neutropenia lasting ≥3 days, and immediately for CTC Grade 4 thrombocytopenia, bleeding associated with Grade 3 thrombocytopenia, or Grade 3 or 4 mucositis.[56] Leucovorin was administered as 100 mg/m^2 IV once, followed by 50 mg/m^2 IV every 6 hours for 8 days.[56]

Methanol Toxicity

Either folic acid or leucovorin (folinic acid) parenterally is recommended at the first suspicion of methanol poisoning. Folic acid is most commonly used. No complications were reported with the use of 50 to 70 mg of IV folic acid every 4 hours for the first 24 hours in the treatment of methanol-poisoned patients.[68] The precise dosage necessary is unknown, but 1 to 2 mg/kg every 4 to 6 hours is probably sufficient. Given that case reports

demonstrate that plasma formic acid concentrations can rise after leucovorin cessation,[66] it is recommended to continue folic/folinic acid until the methanol and formate are eliminated. Because the first dose is usually administered before hemodialysis, a second dose is recommended at the completion of hemodialysis because this highly water-soluble vitamin will have been eliminated.

FORMULATION AND ACQUISITION

Leucovorin (folinic acid) powder for injection is available in 50-, 100-, 200-, and 350-mg vials. Each milligram of leucovorin contains 0.004 mEq of calcium. Reconstitution with sterile water for injection—5 mL to the 50-mg vial, 10 mL to the 100-mg vial, or 20 mL to the 200-mg vial—results in a final concentration of 10 mg/mL. Adding 17.5 mL of sterile water for injection to the 350-mg vial results in a final concentration of 20 mg/mL, where each milliliter contains 0.002 mmol of leucovorin.[95] These lyophilized products contain no preservatives. The only inactive ingredient is sodium chloride added to adjust tonicity. Reconstitute with Sterile Water for Injection, USP; when doses greater than 10 mg/m^2 are used (to avoid excess benzyl alcohol contained in Bacteriostatic Water for Injection, USP) and use immediately.[88] Further dilute in 100 to 1,000 mL of 0.9% sodium chloride or D_5W for infusion.[55] When protected from light, leucovorin both undiluted in glass containers and when diluted with 0.9% sodium chloride (250 mL) in polyethylene bags, has remained stable, with less than 10% degradation for at least 30 days at room and refrigerator temperatures.[47] Leucovorin is also available in a single-use vial as a solution for injection at a concentration of 10 mg/mL in a 50-mL vial. Because of the calcium content, the rate of IV administration should not be faster than 160 mg/min in adults.[95] Leucovorin is also available orally in a variety of strengths, including 5-, 10-, 15-, and 25-mg tablets.

Levoleucovorin lyophilized powder for injection is available in a single-use 50-mg vial containing the equivalent of 50 mg of levoleucovorin as the calcium pentahydrate salt and 50 mg of mannitol.[88] Reconstitution with 5.3 mL of 0.9% sodium chloride injection yields a concentration of 10 mg/mL.[88] Levoleucovorin is also available as a sterile solution in a single-use 175-mg vial that contains 17.5 mL of sterile solution in which each milliliter contains levoleucovorin calcium pentahydrate equivalent to 10 mg of levoleucovorin and 8.3 mg of sodium chloride. Because of the calcium content, the rate of IV administration should not be faster than 160 mg/min (16 mL of reconstituted solution/min).[88] Further dilution to concentrations of 0.5 mg/mL in 0.9% sodium chloride injection or 5% dextrose injection is acceptable, but should be used within 4 hours when stored at room temperature.[88]

Folic acid is available parenterally in 10-mL multidose vials with 1.5% benzyl alcohol in concentrations of 5 or 10 mg/mL from a variety of manufacturers. Once opened, the vial must be kept refrigerated.

If administration to neonates is necessary, a benzyl alcohol–free preparation must be used because of the toxicity of benzyl alcohol in neonates (Chap. 46).

SUMMARY

- Leucovorin (folinic acid) is the primary antidote for a patient who receives an overdose of methotrexate.
- Leucovorin is the biologically active, reduced form of folic acid, the synthesis of which is prevented by methotrexate.
- Only leucovorin (folinic acid) is an acceptable antidote for a patient with methotrexate toxicity, but either folic acid or leucovorin is acceptable for a patient poisoned by methanol.
- After a methanol overdose, folic acid enhances the elimination of formate.
- Leucovorin increases the toxicity of 5-FU. Uridine triacetate (Antidotes in Depth: A14) is the appropriate antidote in the case of 5-FU overdose.

REFERENCES

1. Albrecht AM, et al. Carboxypeptidase displaying differential velocity in hydrolysis of methotrexate, 5-methyltetrahydrofolic acid, and leucovorin. *J Bacteriol.* 1978;134:506–513. [PubMed: 26657]

2. Allen J, et al. The inability of oral leucovorin to elevate CSF 5-methyl-tetrahydrofolate following high dose intravenous methotrexate therapy. *J Neurooncol.* 1983;1:39–44. [PubMed: 6611387]

3. Angier RB, et al. The structure and synthesis of the liver *L. casei* factor. *Science.* 1946;103:667–669.

4. Anonymous. From the NIH: use of folate analogue in treatment of methyl alcohol toxic reactions is studied. *JAMA.* 1979;242:1961–1962. [PubMed: 113572]

5. Appleman JR, et al. Kinetics of the formation and isomerization of methotrexate complexes of recombinant human dihydrofolate reductase. *J Biol Chem.* 1988;263:10304–10313. [PubMed: 3292526]

6. Bachman EA, Barnhart K. Medical management of ectopic pregnancy: a comparison of regimens. *Clin Obstet Gynecol.* 2012;55:440–447. [PubMed: 22510626]

7. Barceloux DG, et al. American Academy of Clinical Toxicology practice guidelines on the treatment of methanol poisoning. *J Toxicol Clin Toxicol.* 2002;40:415–446. [PubMed: 12216995]

8. Bebarta VS, et al. Acute methotrexate ingestions in adults: a report of serious clinical effects and treatments. *J Toxicol.* 2014;2014:214574. [PubMed: 24829573]

9. Belz S, et al. High-performance liquid chromatographic determination of methotrexate, 7-hydroxymethotrexate, 5-methyltetrahydrofolic acid and folinic acid in serum and cerebrospinal fluid. *J Chromatogr B Biomed Appl.* 1994;661:109–118. [PubMed: 7866539]

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

11. Bleyer WA. New vistas for leucovorin in cancer chemotherapy. *Cancer.* 1989;63:995–1007. [PubMed: 2645044]

12. Booser DJ, et al. Continuous-infusion high-dose leucovorin with 5-fluorouracil and cisplatin for relapsed metastatic breast cancer: a phase II study. *Am J Clin Oncol.* 2000;23:40–41. [PubMed: 10683074]

13. Briggs GG, et al. *Drugs in Pregnancy and Lactation*. Philadelphia, PA: Lippincott Williams & Wilkins; 2017.

14. Cerminara Z, et al. A single center retrospective analysis of a protocol for high-dose methotrexate and leucovorin rescue administration. *J Oncol Pharm Pract.* 2017 Jan 1:1078155217729744.

15. 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]

16. Chan BS, et al. What can clinicians learn from therapeutic studies about the treatment of acute oral methotrexate poisoning? *Clin Toxicol (Phila).* 2017;55:88–96. [PubMed: 28084171]

17. Chen Y, et al. Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: a population-based, cross-sectional study. *Am J Epidemiol.* 2007;165:541–552. [PubMed: 17164464]

18. Chitayat D, et al. Folic acid supplementation for pregnant women and those planning pregnancy: 2015 update. *J Clin Pharmacol.* 2016;56:170–175. [PubMed: 26272218]

19. Cody V, et al. Correlations of inhibitor kinetics for *Pneumocystis jirovecii* and human dihydrofolate reductase with structural data for human active site mutant enzyme complexes. *Biochemistry.* 2009;48:1702–1711. [PubMed: 19196009]

20. Danenberg PV, et al. Folates as adjuvants to anticancer agents: chemical rationale and mechanism of action. *Crit Rev Oncol Hematol.* 2016;106:118–

131. [PubMed: 27637357]

21. Egel C, et al. Inhibitory effects of ascorbic acid and folinic acid on chromosome aberrations induced by pyrimethamine in vitro. *Teratog Carcinog Mutagen.* 2002;22:353–362. [PubMed: 12210498]

22. Ettinger LJ, et al. Intrathecal methotrexate overdose without neurotoxicity: case report and literature review. *Cancer.* 1978;41:1270–1273. [PubMed: 346194]

23. European Medicines Agency. *Pre-authorisation Evaluation of Medicines for Human Use. Withdrawal Assessment Report for Voraxaze*. London, UK: European Medicines Agency (EMEA); 2008 June 26, 2008. Report No.: EMEA/CHMP/171907/2008\.

24. Farber S, et al. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (aminopterin). *N Engl J Med.* 1948;238:787–793. [PubMed: 18860765]

25. Finkelstein Y, et al. Intrathecal methotrexate neurotoxicity: clinical correlates and antidotal treatment. *Environ Toxicol Pharmacol.* 2005;19:721–725. [PubMed: 21783548]

26. Food and Drug Administration Center for Drug Evaluation and Research. Application number: 022532Orig1s000. Medical Review(s). Clinical Review. Daniel Davis. NDA 22-532. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/022532orig1s000medr.pdf2011. Accessed September 2, 2017.

27. Gamble MV, et al. Folate and arsenic metabolism: a double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. *Am J Clin Nutr.* 2006;84:1093–1101. [PubMed: 17093162]

28. Gamble MV, et al. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. *Environ Health Perspect.* 2005;113:1683–1688. [PubMed: 16330347]

29. Gershanik J, et al. The gasping syndrome and benzyl alcohol poisoning. *N Engl J Med.* 1982;307:1384–1388. [PubMed: 7133084]

30. Gibbon BN, Manthey DE. Pediatric case of accidental oral overdose of methotrexate. *Ann Emerg Med.* 1999;34:98–100. [PubMed: 10382002]

31. Hall MN, et al. Folate, cobalamin, cysteine, homocysteine, and arsenic metabolism among children in Bangladesh. *Environ Health Perspect.* 2009;117:825–831. [PubMed: 19479028]

32. Hays H, et al. Evaluation of toxicity after acute accidental methotrexate ingestions in children under 6 years old: a 16-year multi-center review. *Clin Toxicol (Phila).* 2018;56:120–125. [PubMed: 28730845]

33. Heck JE, et al. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. *Am J Clin Nutr.* 2007;85:1367–1374. [PubMed: 17490975]

34. Hempel G, et al. Interactions of carboxypeptidase G2 with 6S-leucovorin and 6R-leucovorin in vitro: implications for the application in case of methotrexate intoxications. *Cancer Chemother Pharmacol.* 2005;55:347–353. [PubMed: 15723260]

35. Hoffbrand AV, Weir DG. The history of folic acid. *Br J Haematol.* 2001;113:579–589. [PubMed: 11380441]

36. Hospira I. Methotrexate Injection, USP (Contains Preservative) [presecribing information]. Lake Forest, IL: Hospira, Inc; 2014.

37. Houben PF, et al. Anticonvulsant drugs and folic acid in young mentally retarded epileptic patients. A study of serum folate, fit frequency and I.Q. *Epilepsia.* 1971;12:235–247. [PubMed: 5292449]

38. Hovda KE, et al. Methanol and formate kinetics during treatment with fomepizole. *Clin Toxicol (Phila).* 2005;43:221–722. [PubMed: 16035197]

39. Howard SC, et al. Preventing and managing toxicities of high-dose methotrexate. *Oncologist.* 2016;21:1471–1482. [PubMed: 27496039]

40. Howe CG, et al. Folate and cobalamin modify associations between *S*-adenosylmethionine and methylated arsenic metabolites in arsenic-exposed Bangladeshi adults. *J Nutr.* 2014;144:690–697. [PubMed: 24598884]

41. Hutson JR, et al. Adverse placental effect of formic acid on hCG secretion is mitigated by folic acid. *Alcohol Alcohol.* 2013;48:283–287. [PubMed: 23408242]

42. Jacobsen D, McMartin KE. Methanol and ethylene glycol poisonings. Mechanism of toxicity, clinical course, diagnosis and treatment. *Med Toxicol.* 1986;1:309–334. [PubMed: 3537623]

43. Jakobson AM, et al. Cerebrospinal fluid exchange after intrathecal methotrexate overdose. A report of two cases. *Acta Paediatr.* 1992;81:359–361. [PubMed: 1606401]

44. Janinis J, et al. Second-line chemotherapy with weekly oxaliplatin and high-dose 5-fluorouracil with folinic acid in metastatic colorectal carcinoma: a Hellenic Cooperative Oncology Group (HeCOG) phase II feasibility study. *Ann Oncol.* 2000;11:163–167. [PubMed: 10761750]

45. Jardine LF, et al. Intrathecal leucovorin after intrathecal methotrexate overdose. *J Pediatr Hematol Oncol.* 1996;18:302–304. [PubMed: 8689347]

46. Johlin FC, et al. Studies on the role of folic acid and folate-dependent enzymes in human methanol poisoning. *Mol Pharmacol.* 1987;31:557–561. [PubMed: 3574297]

47. Karbownik A, et al. Stability of calcium folinate (Teva) in concentrate after re-use and in dilute infusions in 0.9% NaCl in polyethylene bags. *Acta Pol Pharm.* 2013;70:301–307. [PubMed: 23614286]

48. Khan Assir MZ, et al. An outbreak of pyrimethamine toxicity in patients with ischaemic heart disease in Pakistan. *Basic Clin Pharmacol Toxicol.* 2014;115:291–296. [PubMed: 24490639]

49. Kim EY, et al. Laparoscopic gastrectomy followed by chemotherapy for advanced gastric cancer diagnosed during pregnancy: a case report. *Anticancer Res.* 2016;36:4813–4816. [PubMed: 27630333]

50. Lampkin BC, Wells R. Intrathecal leucovorin after intrathecal methotrexate. *J Pediatr Hematol Oncol.* 1996;18:249. [PubMed: 8689335]

51. LeBel M, et al. Benzyl alcohol metabolism and elimination in neonates. *Dev Pharmacol Ther.* 1988;11:347–356. [PubMed: 3229281]

52. Lee AC, et al. Intrathecal methotrexate overdose. *Acta Paediatr*. 1997;86:434–7. [PubMed: 9174236]

53. Lefkowitz E, et al. Head and neck cancer. 3. Toxicity of 24-hour infusions of methotrexate (NSC-740) and protection by leucovorin (NSC-3590) in patients with epidermoid carcinomas. *Cancer Chemother Rep.* 1967;51:305–311. [PubMed: 4863189]

54. Levitt M, et al. Transport characteristics of folates in cerebrospinal fluid; a study utilizing doubly labeled 5-methyltetrahydrofolate and 5-formyltetrahydrofolate. *J Clin Invest.* 1971;50:1301–1308. [PubMed: 5314166]

55. Lexicomp Online. *Leucovorin Calcium*. Alphen aan den Rijn, Netherlands: Wolters Kluwer Clinical Drug Information, Inc.; 2017. Accessed September 5, 2017.

56. Lilly USA LLC. ALIMTA- pemetrexed disodium heptahydrate injection, powder, lyophilized, for solution [prescribing information]. Indianapolis, IN: Lilly USA, LLC; 2013.

57. Lonardi F, et al. Toxicity of laevo-leucovorin and dose-lowering. *Eur J Cancer.* 1992;28:1007–1008.

58. Ma Y, et al. Folic acid protects against arsenic-mediated embryo toxicity by up-regulating the expression of Dvr1. *Sci Rep.* 2015;5:16093. [PubMed: 26537450]

59. Makoshi Z, et al. Chemotherapeutic treatment of colorectal cancer in pregnancy: case report. *J Med Case Rep.* 2015;9:140. [PubMed: 26070460]

60. Mazumdar M, et al. Arsenic is associated with reduced effect of folic acid in myelomeningocele prevention: a case control study in Bangladesh. *Environ Health.* 2015;14:34. [PubMed: 25885259]

61. McGuire BW, et al. Absorption kinetics of orally administered leucovorin calcium. *NCI Monogr.* 1987:47–56.

62. McMartin KE, et al. Methanol poisoning. V. Role of formate metabolism in the monkey. *J Pharmacol Exp Ther.* 1977;201:564–572. [PubMed: 405471]

63. MedImmune Oncology I. Neutrexin® (trimetrexate glucuronate for injection). Gaithersburg, MD: MedImmune Oncology, Inc; 2005.

64. Mehta BM, et al. Serum and cerebrospinal fluid distribution of 5-methyltetrahydrofolate after intravenous calcium leucovorin and intra-Ommaya methotrexate administration in patients with meningeal carcinomatosis. *Cancer Res.* 1983;43:435–438. [PubMed: 6600162]

65. Meropol NJ, et al. Seizures associated with leucovorin administration in cancer patients. *J Natl Cancer Inst.* 1995;87:56–58. [PubMed: 7666465]

66. Moore DF, et al. Folinic acid and enhanced renal elimination in formic acid intoxication. *J Toxicol Clin Toxicol.* 1994;32:199–204. [PubMed: 8145360]

67. Noker PE, et al. Methanol toxicity: treatment with folic acid and 5-formyl tetrahydrofolic acid. *Alcohol Clin Exp Res.* 1980;4:378–383. [PubMed: 7004236]

68. Osterloh JD, et al. Serum formate concentrations in methanol intoxication as a criterion for hemodialysis. *Ann Intern Med.* 1986;104:200–203. [PubMed: 3946945]

69. Patel R, et al. Pharmacology and phase I trial of high-dose oral leucovorin plus 5-fluorouracil in children with refractory cancer: a report from the Children's Cancer Study Group. *Cancer Res.* 1991;51:4871–4875. [PubMed: 1893377]

70. Peters BA, et al. Folic acid and creatine as therapeutic approaches to lower blood arsenic: a randomized controlled trial. *Environ Health Perspect.* 2015;123:1294–1301. [PubMed: 25978852]

71. Pilsner JR, et al. Folate deficiency, hyperhomocysteinemia, low urinary creatinine, and hypomethylation of leukocyte DNA are risk factors for arsenic-induced skin lesions. *Environ Health Perspect.* 2009;117:254–260. [PubMed: 19270796]

72. 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]

73. Podzamczer D, et al. Thrice-weekly sulfadiazine-pyrimethamine for maintenance therapy of toxoplasmic encephalitis in HIV-infected patients. Spanish Toxoplasmosis Study Group. *Eur J Clin Microbiol Infect Dis.* 2000;19:89–95. [PubMed: 10746493]

74. Priest DG, et al. Pharmacokinetics of leucovorin metabolites in human plasma as a function of dose administered orally and intravenously. *J Natl Cancer Inst.* 1991;83:1806–1812. [PubMed: 1744924]

75. Pruitt AW, et al. Accidental ingestion of methotrexate. *J Pediatr.* 1974;85:686–688. [PubMed: 4547412]

76. Reynolds EH. Effects of folic acid on the mental state and fit-frequency of drug-treated epileptic patients. *Lancet.* 1967;1:1086–1088. [PubMed: 4164775]

77. Riva L, et al. Successful treatment of intrathecal methotrexate overdose with folinic acid rescue: a case report. *Acta Paediatr.* 1999;88:780–782. [PubMed: 10447141]

78. Roenigk HH Jr, et al. Methotrexate in psoriasis: consensus conference. *J Am Acad Dermatol.* 1998;38:478–485. [PubMed: 9520032]

79. Sauberlich HE, Baumann CA. A factor required for the growth of leuconostoc citrovorum. *J Biol Chem.* 1948;176:165–173. [PubMed: 18886154]

80. Schilsky RL, Ratain MJ. Clinical pharmacokinetics of high-dose leucovorin calcium after intravenous and oral administration. *J Natl Cancer Inst.* 1990;82:1411–1415. [PubMed: 2388292]

81. Schleyer E, et al. Impact of the simultaneous administration of the (+)- and (–)-forms of formyl-tetrahydrofolic acid on plasma and intracellular pharmacokinetics of (–)-tetrahydrofolic acid. *Cancer Chemother Pharmacol.* 2000;45:165–171. [PubMed: 10663632]

82. Schoenbach EB, et al. Reversal of aminopterin and amethopterin toxicity by citrovorum factor. *J Am Med Assoc.* 1950;144:1558–1560. [PubMed: 14794378]

83. Scott JM. Folinic acid in megaloblastic anaemia of pregnancy. *Br Med J.* 1957;2:270–272. [PubMed: 13446456]

84. Sherwood RF, et al. Purification and properties of carboxypeptidase G2 from *Pseudomonas* sp. strain RS-16. Use of a novel triazine dye affinity method. *Eur J Biochem.* 1985;148:447–453. [PubMed: 3838935]

85. Shiozawa K, et al. Serum levels and pharmacodynamics of methotrexate and its metabolite 7-hydroxy methotrexate in Japanese patients with rheumatoid arthritis treated with 2-mg capsule of methotrexate three times per week. *Mod Rheumatol.* 2005;15:405–409. [PubMed: 17029103]

86. Smith DB, Racusen LC. Folate metabolism and the anticonvulsant efficacy of phenobarbital. *Arch Neurol.* 1973;28:18–22. [PubMed: 4682068]

87. Smith SW, Nelson LS. Case files of the New York City Poison Control Center: antidotal strategies for the management of methotrexate toxicity. *J Med Toxicol.* 2008;4:132–140. [PubMed: 18570175]

88. Spectrum Pharmaceuticals Inc. Fusilev® (levoleucovorin) [prescribing information]. Irvine, CA: Spectrum Pharmaceuticals, Inc; 2011.

89. Stover P, Schirch V. The metabolic role of leucovorin. *Trends Biochem Sci.* 1993;18:102–106. [PubMed: 8480361]

90. Stratemann K, et al. The folate content as limiting factor for formate detoxication and methanol metabolism. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol.* 1968;260:208–209. [PubMed: 4239236]

91. Straw JA, et al. Pharmacokinetics of leucovorin (D,L-5-formyltetrahydrofolate) after intravenous injection and constant intravenous infusion. *NCI Monogr.* 1987:41–45.

92. Straw JA, et al. Pharmacokinetics of the diastereoisomers of leucovorin after intravenous and oral administration to normal subjects. *Cancer Res.* 1984;44:3114–3119. [PubMed: 6609768]

93. Surtees R, et al. Demyelination and single-carbon transfer pathway metabolites during the treatment of acute lymphoblastic leukemia: CSF studies. *J Clin Oncol.* 1998;16:1505–1511. [PubMed: 9552059]

94. Tauheed J, et al. Associations between post translational histone modifications, myelomeningocele risk, environmental arsenic exposure, and folate deficiency among participants in a case control study in Bangladesh. *Epigenetics.* 2017;12:484–491. [PubMed: 28387569]

95. Teva Parenteral Medicines Inc. Leucovorin Calcium for Injection [package insert]. Teva Parenteral Medicines, Inc. Irvine, CA; 2014.

96. Thyss A, et al. Evidence for CSF accumulation of 5-methyltetrahydrofolate during repeated courses of methotrexate plus folinic acid rescue. *Br J*

Cancer. 1989;59:627–630. [PubMed: 2785400]

97. Trinkle R, Wu JK. Intrathecal leukovorin after intrathecal methotrexate overdose. *J Pediatr Hematol Oncol.* 1997;19:267–269. [PubMed: 9201155]

98. Van Delden C, Hirschel B. Folinic acid supplements to pyrimethamine-sulfadiazine for *Toxoplasma encephalitis* are associated with better outcome. *J Infect Dis.* 1996;173:1294–1295. [PubMed: 8627092]

99. Weh HJ, et al. Neurotoxicity following weekly therapy with folinic acid and high-dose 5-fluorouracil 24-h infusion in patients with gastrointestinal malignancies. *Eur J Cancer.* 1993;29A:1218–1219. [PubMed: 8518040]

100. Wettergren Y, et al. A pharmacokinetic and pharmacodynamic investigation of Modufolin(R) compared to Isovorin(R) after single dose intravenous administration to patients with colon cancer: a randomized study. *Cancer Chemother Pharmacol.* 2015;75:37–47. [PubMed: 25342290]

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

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

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

104. Widemann BC, et al. Efficacy of glucarpidase (carboxypeptidase G_2) in patients with acute kidney injury after high-dose methotrexate therapy. *Pharmacotherapy.* 2014;34:427–439. [PubMed: 24132809]

105. Wills L. Treatment of “pernicious anaemia of pregnancy” and “tropical anaemia” with special reference to yeast extract as a curative agent. *Nutrition.* 1931;7:323–327; discussion 328.

106. Zoubek A, et al. Successful carboxypeptidase G_2 rescue in delayed methotrexate elimination due to renal failure. *Pediatr Hematol Oncol.* 1995;12:471–477. [PubMed: 8519632]