Chapter A13: Glucarpidase (Carboxypeptidase G_2)

Silas W. Smith

INTRODUCTION

Glucarpidase (carboxypeptidase G_2, $CPDG_2$) is indicated for the management of methotrexate (MTX) toxicity. When given intravenously or intrathecally, it rapidly enzymatically inactivates MTX, folates, and folate analogues. It does not substitute for, and must be used in conjunction with, leucovorin (Antidotes in Depth: A12). Leucovorin should not be administered within 2 hours before or after a dose of glucarpidase.

HISTORY

Soon after the description of the structure and synthesis of folate,[6] a *Flavobacterium* species capable of removing the glutamate moiety of folate was discovered.[47] From 1955 to 1956, the inactivation of folate analogues (including chemotherapeutic aminopterin) was demonstrated in bacteria and yeasts.[61,92] Purification of "carboxypeptidase G," a pseudomonad-derived zinc-dependent enzyme responsible for MTX cleavage, was reported in 1967.[35,48] Other bacterial carboxypeptidases that differed in their substrate specificity and kinetics were isolated and purified in 1971 (*Pseudomonas stutzeri* carboxypeptidase G_1),[52] 1978 (*Flavobacterium* carboxypeptidase),[5] and 1992 (*Pseudomonas* sp. M-27 carboxypeptidase G_3).[104] By 1976, carboxypeptidase G_1 was scaled to pilot manufacturing production.[25] Carboxypeptidase G_1 ($CPDG_1$) was initially explored as a chemotherapeutic to deprive growing tumors of folate.[9,10,20,42] Human usage of $CPDG_1$ for this purpose was reported in 1974.[10] The antidotal potential of carboxypeptidase was first suggested in 1972 when it was noted that $CPDG_1$ rapidly decreased serum MTX concentrations and improved survival in mice injected with lethal MTX doses.[21] Carboxypeptidase G_1 was first used for rescue in a patient receiving MTX with kidney failure in 1978.[40] Carboxypeptidase G_1 was subsequently used to selectively eliminate systemic MTX in patients treated with high dosages targeting central nervous system (CNS) malignancies.[1,2] Unfortunately, the enzyme source of $CPDG_1$ was then lost.[4,106] The carboxypeptidase currently used in clinical practice ($CPDG_2$) was cloned from *Pseudomonas* strain R-16 and sequenced, characterized, and expressed in *Escherichia coli* in the early 1980s.[57-59,81] The preliminary crystal structure was provided in 1991, with a characterization (at 2.5 Å) and description of the active site, and biochemical mechanism of action in 1997.[49,74,89] Following the renewed availability of the recombinant $CPDG_2$ product, it underwent nonhuman primate testing for both intravenous (IV) and intrathecal (IT) rescue of MTX overdose.[3,4] Reports of successful use in human IV and IT MTX overdose rapidly emerged.[26,40,44,45,60,62,75,97,99,106] The US FDA designated glucarpidase an Orphan Product in 2003 and granted marketing approval as Voraxaze in January 2012.[16]

PHARMACOLOGY

Chemistry/Preparation

Glucarpidase is produced by recombinant DNA technology. The enzyme cloned from *Pseudomonas* strain R16 is expressed in *Escherichia coli* strain RV308.[31] The final commercial drug product is freeze-dried, and packaged in the United States.[31,39]

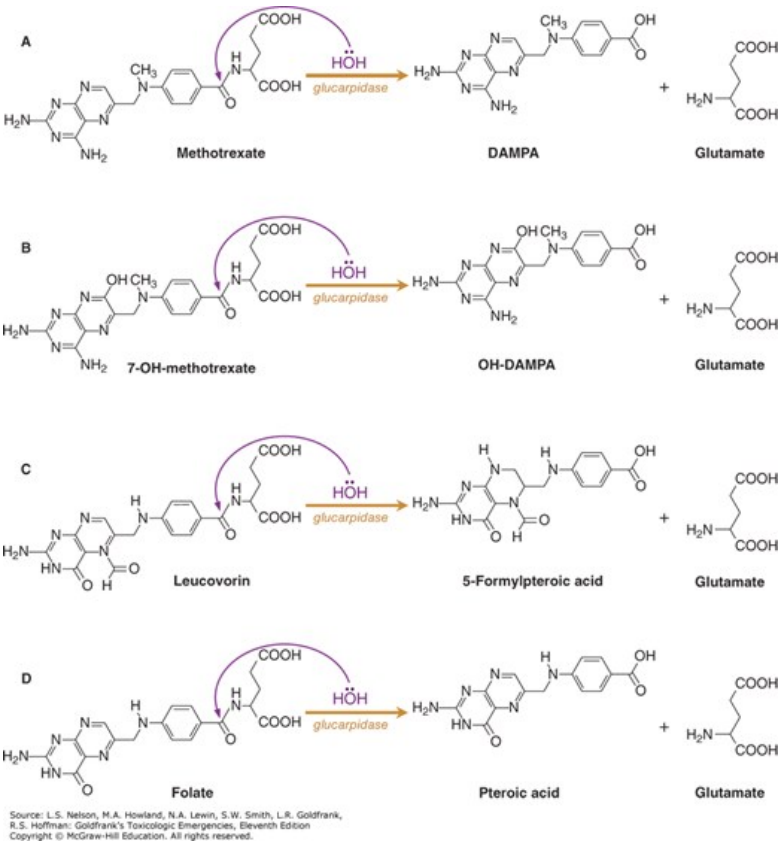
Mechanism of Action

Glucarpidase is a dimerized protein structure with 2 domains—a β-sheet interaction site and a zinc-dependent catalytic domain.[74] As a peptidase, the catalytic domain of glucarpidase hydrolyzes the C-terminal glutamate residues of folate and folate analogues such as MTX. Molecular modeling

suggests that the 2 zinc (2^+) ions bind a water molecule, promoting its polarization and nucleophilic attack on the carbonyl group of the substrate (Fig. A13–1).[90] Methotrexate and its metabolite 7-OH-MTX are thus split into inactive DAMPA (2,4-diamino-*N*(10)-methylpteroic acid) and OH-DAMPA plus glutamate.[102] DAMPA undergoes subsequent hepatic metabolism. Glucarpidase similarly inactivates leucovorin and folate by cleaving their terminal glutamate residues (Fig. A13–1).[5]

FIGURE A13–1.

The catalytic domain of glucarpidase permits hydrolysis of the C-terminal glutamate residue of folate and folate analogues such as MTX via hypothesized nucleophilic attack of a zinc-bound water molecule.[90] (A, B) MTX and its metabolite 7-hydroxy-MTX are split into inactive DAMPA (2,4-diamino-*N*(10)-methylpteroic acid) and hydroxy-DAMPA plus glutamate. Glucarpidase similarly inactivates (C) leucovorin (folinic acid) and (D) folate by cleavage of terminal glutamate residues.



Pharmacokinetics

In one study, 50 units/kg of glucarpidase was given IV to 8 volunteer subjects with normal kidney function and 4 volunteers with impaired function (calculated creatinine clearance <30 mL/min, range 9.8–27.4 mL/min).[71] Those with normal kidney function achieved a mean maximum serum concentration of glucarpidase of 3.1 mcg/mL, with a mean half-life of 9.0 hours. These values were essentially unaffected in the setting of an impaired glomerular filtration rate (GFR); therefore, renal dosing is not indicated.[16] Glucarpidase restriction to the plasma compartment was implied by a -volume of distribution of 3.6 L.[71] The large protein size (83-kDa dimer) of glucarpidase precludes traversing the blood–brain barrier, crossing the cell membranes to act intracellularly, acting on MTX within the gut lumen or urinary collecting system, or treating MTX extravasation.[1,21,26,56]

Pharmacodynamics

One unit of glucarpidase activity catalyzes the hydrolysis of 1 µmol of MTX per minute at 37°C.[3] The mean enzymatic activity half-life of glucarpidase was 5.6 hours in normal volunteers and 8.2 hours in volunteers with impaired GFR.[71] Its activity is optimal at a pH between 7.0 and 7.5, compatible with

human physiology. After glucarpidase administration, serum MTX concentrations rapidly declined by 71% to 99% with the original Center for Applied Microbiology and Research (CAMR) product, with the concurrent appearance of inactive DAMPA in both serum and urine.[17,45,77,82,94,95,97] DAMPA undergoes subsequent hepatic metabolism and renal elimination (~50%).[102] Commercial glucarpidase rapidly decreases serum MTX concentrations by greater than 97% within 15 minutes (Table A13–1).[16,23,32,66,82,85] In the primary efficacy study of 22 patients used as the basis for approval, glucarpidase induced MTX reductions of greater than 95% for up to 8 days, although 5 patients also received hemodialysis and 12 of 22 patients (54.5%) did not meet the primary endpoint of an MTX concentration ≤1 µmol/L in all samples, either because of a high baseline MTX concentration and/or significant MTX redistribution and rebound.[32] The ongoing glucarpidase enzymatic activity, which persists after the initial rapid MTX concentration declines, has led authors to explore glucarpidase efficacy at doses lower than the FDA-approved dose (50 units/kg). In one reported case of a patient who received late administration of approximately 8 units/kg glucarpidase, the serum MTX as measured by immunoassay declined from 1.2 to 0.5 µmol/L.[87] In 11 patients who received lower glucarpidase doses (10–31 units/kg) because of a supply shortage, there was no difference in MTX pharmacokinetics, -toxicity, or survival.[77] In a retrospective evaluation of 26 patients who received glucarpidase after high-dose MTX, a multivariable analysis could not find any statistically significant relationship between glucarpidase dose and the percentage decrease in plasma MTX concentration as measured by immunoassay or high-performance liquid chromatography (HPLC).[80] Higher dosages of glucarpidase did not lead to more rapid recovery of kidney function, and lower doses (13–50 units/kg) were equally efficacious.[80] These findings were confirmed in another clinical trial in which almost half of the patients were treated with glucarpidase doses below 50 units/kg (as low as 20 units/kg), and the dose per kilogram did not correlate with the time for serum MTX concentration to decrease below 0.2 µmol/L or with the overall reduction in MTX concentration.[85]

TABLE A13–1

Glucarpidase Efficacy Trials[a]

Trial	2000–2003 FDA Tr001[32] "Berlin"[77]	1993–2004 FDA Tr002[32] "NCI"[65,96]	1997–2002 FDA Tr003[32] "Bonn"[17]	2004–2007 FDA Tr006[32] "NCI II"	2007–(2010) FDA Tr016[32] BTG IND 11557	2008–2010 "St. Jude"[23]	2008–2014 NOPHO ALL 2008[85]
Lot	CAMR	CAMR	CAMR	Commercial	Commercial	Commercial	Commercial
Sites (n)	29	149	50	55	NR	1	7b
Safety data (n)	43	214	65	149	141	20	47c
Malignancies	ALL: 13; L: 12; +CNS: 16; others: 2	L/L: 111; OS/S: 75; others: 3	ALL: 26; NHL: 21; OS: 12; others: 6	L/L: 93; OS/S: 47; others: 9	L/L: 88; OS/S: 46; others: 7	ALL: 10; OS: 6; L:4	ALL: 47
Age (years)	18–78 (54)	0.4–82 (17)	0.9–71.8 (15.4)	0.08–85 (18)	0.5–85 (16)	4.1–20.4 (12.1)	1–17.9 (8)
TTT (hours)	27–176 (56)	NR	25–178 (52)	27–86 (48)	NR	26.3–95 (45.9)	32–82 (45)
Dose (units/kg)	10–58 (50)	NR	33–60 (50)	18–98 (49)	6–189 (50)	13–65.6 (51.6)	44.3–53.5 (50)
[MTX] (µmol/L)	1–1187	1–849 (35)	0.52–901 (11.93)	3.9–708 (38.9)	NR	1.3–590.6 (29.1)	102–200 (132)

[MTX] ↓ from -baseline (%) [MTX] RSCIR	N = 24 18–99 (>97%) 83%	N = 70 NR 57%	N = 25 73–99 (97%) NR	N = 22 ≥97% 45%	NR NR NR	N = 6 99.2–99.9 (99.6) 67%	N= 8 100% NR
Leucovorin dosing	mg = [MTX_S](μM) × (kg); [MTX_S] ≤ 5 μM: 15–75 mg/m2 Q 6	1 g/m2 IV Q 6 h; then 250 mg/m2 Q 6 × 48 h	None 4 h prior; post 1 h @ 100 mg/m2 Q 6 × 24 h	NR	NR	NR	mg = [MTX_S] (μM) × (kg) until MTX < 0.2 μM
Heme/myelo	60.4%	NR	4.6%	NR	NR	10%	76%
Infection	16.2%	NR	12.5%	NR	NR	20%	18%
Mucositis	34.9%	NR	15.3%	NR	NR	5%	19%
Nephrotoxicity	18.6%	NR	34.1%	NR	NR	35%	100%
Hepatotoxicity	16.2%	NR	32.5%	NR	NR	NR	35%
MTX-death[d]	23.2%	5.1%	6.1%	4.0%	2.1%	0%	0%

[a]Parenthetical values denote medians.

[b]The value refers to countries (Denmark, Estonia, Finland, Iceland, Lithuania, Norway, and Sweden).

[c]50 glucarpidase courses in 47 unique patients.

[d]Methotrexate-associated death or death not specifically reported as malignancy related.

ALL = acute lymphoblastic leukemia; CAMR = Center for Applied Microbiology and Research (UK) lot 004; FDA = Food and Drug Administration; heme/myelo = hematological toxicity/myelosuppression; L/L = leukemia/lymphoma; MTX = methotrexate; NCI = National Cancer Institute; NHL = non-Hodgkin lymphoma; NOPHO = Nordic Organization for Pediatric Hematology and Oncology; NR = not reported in FDA summary; OS/S = osteosarcoma/sarcoma; RSCIR = rapid and sustained clinically important reduction; Tr = Trial (FDA identifier); TTT = time to treatment.

ROLE IN METHOTREXATE TOXICITY

Patients receiving high-dose MTX therapy are routinely "rescued" with leucovorin (eg, 10–15 mg every 6 hours).[105] Treatment nomograms and institutional algorithms recommend higher leucovorin doses when MTX concentrations are excessive or the elevation of the concentration is prolonged (Antidotes in Depth: A12).[11,19,94,105] However, at MTX concentrations above 100 μmol/L (1×10^{-4} mol/L), data suggest that adequate leucovorin concentrations cannot be achieved for competitive and complete reversal of toxicity.[17,45,50,72] Also, leucovorin administration provides 0.004 mEq calcium per milligram of leucovorin and is rarely associated with hypercalcemia in extremely high-dose therapy.[106] Necessary urine alkalinization and diuresis can also be limited by serum pH, sodium, and fluid administration ceilings once patients develop toxicity.[24,79] Thus, despite adequate leucovorin rescue, alkalization, diuresis, and supportive care, additional antidotal strategies are required in the setting of persistently elevated MTX concentrations. High-dose MTX-induced kidney dysfunction developed in 1.8% of patients with osteosarcoma enrolled in clinical trials.[95] In a 10-year retrospective review of 1,982 patients who received high-dose MTX (HDMTX) for leukemia, lymphoma, and osteosarcoma, 21 patients (1.1%) required glucarpidase rescue for delayed MTX elimination.[22] In a recent clinical trial with prespecified $CPDG_2$ administration when the serum

MTX concentration was greater than 250 µmol/L at 24 hours, greater than 30 µmol/L at 36 hours, or greater than 10 µmol/L at 42 hours in combination with reduced kidney function (at least 50% creatinine increase), glucarpidase was required in 47 of 1,286 children (3.7%).[85] This yielded a relative risk for delayed MTX elimination of approximately 0.5% per each HDMTX cycle.[85] Chemotherapeutic regimens that combine MTX with renal toxic medications such as cisplatin may also increase the risk. Thus, it is reasonable to anticipate the need for glucarpidase in approximately 1% to 4% of patients receiving HDMTX, depending on the specific indication, dose, and regimen.

Glucarpidase is labeled for patients with serum MTX concentrations greater than 1 µmol/L in the setting of impaired kidney function (as evidenced by an MTX concentration not falling within 2 standard deviations of the mean MTX excretion curve for the specific administered MTX protocol).[16] In the absence of an MTX concentration, significant mucositis, gastrointestinal distress, myelosuppression, hepatitis, or neurotoxicity should prompt administration of glucarpidase in addition to aggressive leucovorin therapy, while awaiting confirmation. The primary glucarpidase efficacy and safety studies in adults and children are summarized in Table A13–1. These trials provide data for glucarpidase use in the setting of elevated MTX concentrations and the failure of leucovorin rescue and standard care approach. The CAMR product, which was used in initial clinical trials,[17,77,96] was not demonstrated to be bioequivalent to the current commercial product.[32] Although no fully reported trial has yet demonstrated the superiority of glucarpidase as adjuvant therapy to leucovorin and supportive care alone, the pharmacodynamic efficacy is clear: glucarpidase immediately decreases serum MTX concentrations by greater than 97%.[16,23,32,66,82,85,96]

The expansion of glucarpidase use to include routine provision at 24 hours in addition to leucovorin rescue after HDMTX was studied in a double-blind, randomized, crossover phase II clinical trial, in which HPLC plasma [MTX] were determined daily until less than 0.2 µmol/L.[68] In 16 patients, routine glucarpidase use reduced delays to subsequent chemotherapy cycles and the severity of mucositis, but it did not reduce MTX-associated nephrotoxicity or the overall total MTX area under the plasma drug concentration-time curve (AUC).[66-68] Upon further exploration, although glucarpidase decreased the MTX $AUC_{24\text{-}72\ hours}$, the overall MTX AUC was not statistically significantly changed, primarily because of the contribution of MTX exposure in the first 24 hours (prior to glucarpidase).[66-68] Clinical trials have also highlighted pharmacokinetic limitations beyond glucarpidase's impressive immediate decrease in [MTX]. In studies, a rapid and sustained clinically important reduction in MTX concentration (RSCIR, [MTX] < 1 µmol/L), occurred in only 45%,[32] 59%,[100] and 67%[23] of patients. This failure of RSCIR reflects MTX redistribution, and underscores the need for continued leucovorin therapy.[79] Patients with [MTX] of greater than or equal to 50 µmol/L were less likely to achieve RSCIR than those with of less than 50 µmol/L (although glucarpidase did lead to a higher percentage reduction in those with higher [MTX]).[100] In exploratory analysis, patients with osteosarcoma—who received higher MTX doses (eg, 8–12 g/m^2)—experienced less benefit with glucarpidase.[32] Another evaluation concluded that glucarpidase was unable to prevent fatal MTX toxicity in 3% of patients.[16] However, overall, the data support the use of glucarpidase to treat those at risk for toxicity from MTX because of either persistently elevated [MTX] or kidney dysfunction.[17,36,77,95] More recent trials have demonstrated more favorable outcomes. In one trial where glucarpidase intervention was required in 50 cycles of HDMTX in 47 of a total of 1286 HDMTX-treated children, mortality was 0%.[85] The use of glucarpidase to manage MTX toxicity has also permitted earlier resumption of MTX chemotherapy.[7,23,28,68,84]

Intracellular MTX is polyglutamated, which hinders transmembrane transport and increases intracellular half-life. This MTX pool is inaccessible to glucarpidase (and hemodialysis) and can persist, causing cytotoxicity and a rebound in serum [MTX] for up to 85 hours after glucarpidase administration.[34,77,96,98] In one trial, MTX rebound was seen in 6 of 28 patients (21%) at a median of 24 hours after glucarpidase administration.[66] Delaying glucarpidase more than 96 hours after MTX initiation, once intracellular MTX is established, is associated with failure to prevent significant MTX toxicity.[77,96] This emphasizes the need for close monitoring of [MTX] to ensure early administration of glucarpidase as soon as indicated. Persistent intracellular or otherwise inaccessible MTX requires ongoing leucovorin therapy for 48 hours at the same leucovorin dose as that prior to glucarpidase, which is continued until the [MTX] is below the leucovorin treatment threshold (50–100 nmol/L, 0.05–0.1 µmol/L; $0.05–0.1 \times 10^{-6}$ molar) for a minimum of 3 days.[16,17,23,32,38,43,66,77,80,85,86,94,96,100,103]

In the setting of oral MTX overdose, gastrointestinal decontamination should be performed (Chap. 51), because glucarpidase has no intraluminal activity. Leucovorin is contraindicated for IT administration, and it has rarely been associated with seizures.[30,41,55,88] Simian studies and human case reports demonstrate that IT glucarpidase provides an effective means to rapidly lower cerebrospinal fluid [MTX] in cases of overdose or prolonged CNS persistence.[3,13,62,98] Reductions in CSF [MTX] after glucarpidase administration range from 72% to 99.8%.[13,98] Intravenous leucovorin with or without steroid administration has also been employed to minimize toxicity.[46,73] Patent applications revealed experiments demonstrating

glucarpidase cleavage of pemetrexed and raltitrexed.[53,54] In vitro, glucarpidase hydrolyses pemetrexed (K_m = 25 μM, k_{cat} = 1,808 s^{-1}) with a similar reaction kinetic profile to MTX (K_m = 10 μM, k_{cat} = 1,039 s^{-1}).[8] These promising kinetic data await animal and human investigations before a definitive recommendation can be made regarding glucarpidase treatment of toxicity because of other chemotherapeutic antifolates.[14]

ADVERSE EFFECTS AND SAFETY ISSUES

Antidotal Compromise

The affinity of $CPDG_2$ for MTX is 10- to 15-fold higher than for leucovorin; however, its affinity for the active metabolite of leucovorin, 5-methyltetrahydrofolate (5-mTHF) and folate are similar.[5,29,81] Although racemic leucovorin is commonly administered, the active enantiomer is also commercially available. Because glucarpidase cleaves active *levo*-(6*S*)-leucovorin approximately 50% faster than inactive *dextro*-(6*R*)-leucovorin,[37] glucarpidase will compromise leucovorin rescue if both antidotes are administered contemporaneously. Further, once the MTX has been rapidly cleaved, glucarpidase persistence (recalling its enzymatic activity half-life of 5.6 hours in volunteers and 8.2 hours in kidney failure) would risk compromising the endogenous folate pool and exogenously administered leucovorin. Healthy volunteers provided leucovorin 2 hours after glucarpidase had their leucovorin concentrations decreased by 50%, and activated *levo*-5-MeTHF became undetectable. When leucovorin therapy was delayed 26 hours after glucarpidase administration, enzymatic cleavage by glucarpidase still decreased leucovorin and 5-MeTHF concentrations to 80% and 75%, respectively.[29] In MTX trials within 15 minutes after administration of glucarpidase, the median leucovorin concentrations fell by 8% to 18%, with the remaining leucovorin likely being the inactive *d*-isomer.[94,96] This is of concern, as the *d*-isomer may accumulate and inhibit passive transport of the active "l"-isomer.[12] Active 5-methyltetrahydrofolate concentrations also declined precipitously by greater than 97%.[94,96] Given these concerns of antidotal leucovorin destruction, a later trial of 20 patients evaluated leucovorin and [(6*S*)-5-MeTHF] for a period of 3 hours after leucovorin administration in patients who had received HDMTX, glucarpidase, and leucovorin or HDMTX and leucovorin alone.[78,91] Leucovorin was not provided within 2 hours of glucarpidase (median, 2.2 hours later). The study's interpretation is limited by unbalanced trial arms. In the glucarpidase group, pretreatment [MTX] were higher; body surface areas (BSAs) and leucovorin administration were greater; and serious and other adverse events were more common. When accounting for unequal BSAs, and despite the greater than 2+-hour delay in subsequent leucovorin administration, the patients given glucarpidase experienced a decrease in dose-normalized *levo*-(6*S*)-leucovorin $AUC_{0\text{-}3\ hours}$, *levo*-(6*S*)-leucovorin C_{max}, active (6*S*)-5MeTHF $AUC_{0\text{-}3\ hours}$, and 5MeTHF C_{max} by 33%, 52%, 92%, and 93%, respectively.[18] This trial and others confirmed the recommendation that leucovorin should not be administered for at least 2 hours before or after glucarpidase and that the ongoing leucovorin dose should be based on the pre-glucarpidase [MTX].[16,18,29,32,38]

Immunogenicity

Initial studies reported adverse effects in 4 of 9 patients treated with $CPDG_1$, including development of inactivating antibodies, "sensitization" to $CPDG_1$, and anaphylactoid reactions.[1,2,10,40] The current recombinant glucarpidase ($CPDG_2$) imparts a much lower incidence of adverse effects than the initial $CPDG_1$ enzyme, including paresthesias (2%), flushing (2%), nausea/vomiting (2%), hypotension (1%), headache (1%), and rash (0.3%).[16,32] - However, in intentional repeated dosing of glucarpidase with HDMTX, 2 of 4 patients experienced allergic reactions.[32] In patients administered $CPDG_2$ fused to a murine single-chain Fv antibody, 36% (11 of 30) developed anti-$CPDG_2$ antibodies, but no antimurine antibodies were detected.[51] Studies using the noncommercial (CAMR) lot reported antiglucarpidase antibody (AGA) development in none of 28 patients[96] and in 3 of 7 patients,[77] respectively. In clinical trials using the commercial product, 19% (19 of 99) developed AGAs following a single glucarpidase dose and 28.5% (6 of 21) developed AGAs after 2 doses, consistent with increased antibody development with multiple exposures.[16,32] Neutralizing antibodies were found in 11 of the 25 patients who tested positive for AGA-binding antibodies, of whom 8 had received only a single dose.[16] Antiglucarpidase antibodies were found in 43% (6 of 14) of assessed patients in another trial, all of whom became positive following the second glucarpidase dose.[66] In a broader evaluation of 205 patients treated with glucarpidase from 2007 to 2012, AGAs developed in 32 of 176 (18%) patients following a single glucarpidase dose an in 11 of 29 (37.9%) after 2 or more doses; 14 of 176 (8.0%) single-dose patients had neutralizing antibodies, which increased to 8 of 29 (27.6%) after 2 or more doses.[101] Although AGAs might decrease clinical efficacy or predispose to allergic reaction upon re-exposure,[2,4,29,77] many patients

have been successfully treated with more than one dose of glucarpidase for persistently elevated MTX concentrations.[17,26,45,64,70,77,83,85,99,106]

Other Considerations

Because "inactive" DAMPA has a pH-dependent urinary solubility 8 to 10 times less than MTX,[37,95] in the absence of a contraindication (eg, volume overload), alkalinization and saline diuresis should be continued to prevent DAMPA precipitation and further renal compromise.[17,94] Although the supplied product contains lactose and Tris–HCl with zinc buffer, lactose-intolerant patients can receive glucarpidase. Previous concerns of allergic reactions to lactose-containing xenobiotics and patients with rare hereditary problems of fructose intolerance, galactose intolerance, galactosemia, or glucose–galactose malabsorption, are unaddressed in prescribing guidelines.[16]

PREGNANCY AND LACTATION

Glucarpidase carries a pregnancy category C designation, although formal human and animal data are lacking. The excretion of glucarpidase in breast milk is unknown.

DOSING AND ADMINISTRATION

Glucarpidase is dosed in units per kilogram in both children and adults. After reconstituting each 1,000-unit vial with 1 mL of sterile sodium chloride (0.9%), a single dose of 50 units/kg is administered immediately by IV injection over 5 minutes. If not used immediately, reconstituted glucarpidase can be stored under refrigeration at 36° to 46°F (2°–8°C) for up to 4 hours.[16] Although clinical trials permitted additional glucarpidase doses 24 to 48 hours later in cases of persistent elevated MTX concentrations, repeat administration has not demonstrated significant efficacy and is not recommended.[16,17,65,76] The substantial cost of glucarpidase[93] and the apparent glucarpidase efficacy of lower doses[77,80,85,87] has led some authors to advocate rounding the glucarpidase doses down (eg, to the nearest vial size) or capping glucarpidase doses (eg, at 2,000 units), although this practice is not consistent with the FDA-approved dose (50 units/kg). Although this approach awaits further formal study, this approach would be reasonable in cases of glucarpidase shortage or the need to triage dosing. In cases of IT MTX overdose, a fixed dose of glucarpidase (2,000 units) reconstituted in sterile 0.9% sodium chloride has been administered intrathecally (off label) over 5 minutes and is recommended in this scenario.[13,62,98] A lack of compatibility studies precludes glucarpidase mixing with other xenobiotics.

Monitoring and Analytical Considerations

False elevations of [MTX] are reported with all of the various immunoassay techniques after glucarpidase administration.[28,37,45,70,99,106] The DAMPA metabolite significantly cross-reacts with both the MTX radioimmunoassay and competitive dihydrofolate reductase–binding assays.[27] Both MTX metabolites (7-OH-MTX and DAMPA) appreciably interfere with fluorescence polarization immunoassay (FPIA) and enzyme-multiplied immunoassay technique (EMIT) assays. For DAMPA, the cross-reactivity rates are 100% (EMIT) and 36% to 44% (FPIA).[69] The cross-reactivity of 7-OH-MTX using EMIT is 4% to 31%, and 0.6% to 3% with FPIA.[33,69] This interference persists despite newer immunoassays.[63] Clinically, the concentrations of DAMPA detected are comparable to those of MTX after administration of $CPDG_2$.[26] Thus, HPLC should be used to determine actual [MTX] when glucarpidase is given.[15,16]

FORMULATION AND ACQUISITION

Branded glucarpidase (Voraxaze) is available in single-use glass vials each containing lyophilized glucarpidase (1,000 units) with lactose monohydrate (10 mg), buffered to pH 6.5 to 8.0 with Tris–HCl (0.6 mg) and zinc acetate dihydrate (0.002 mg). It should be maintained at 36° to 46°F (2°–8°C), but not frozen. The manufacturer's website details acquisition information for inside and outside of the United States (http://www.btgplc.com/products/specialty-pharmaceuticals/voraxaze and http://www.btgplc.com/contact-us/contacts, respectively).

SUMMARY

- Glucarpidase is a bacterially derived enzyme used in the treatment of MTX toxicity. It cleaves MTX in the serum compartment to rapidly reduce serum [MTX].

- Glucarpidase does not substitute for leucovorin, which should be continued to counteract persistent intracellular and CNS MTX.
- Leucovorin should not be administered within 2 hours before or after a dose of glucarpidase to avoid the enzymatic destruction of leucovorin and its active metabolites.
- The measurement of [MTX] after glucarpidase administration will be unreliable unless HPLC is used.

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