Published in final edited form as:

Cancer Chemother Pharmacol. 2014 June ; 73(6): 1307–1313. doi:10.1007/s00280-014-2464-2.

Successful challenges using native *E. coli* asparaginase after hypersensitivity reactions to PEGylated *E. coli* asparaginase

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Abstract

Purpose—Asparaginase is an essential component of pediatric acute lymphoblastic leukemia (ALL) therapy. However, asparaginase-induced hypersensitivity reactions can compromise its efficacy either by directly influencing the pharmacokinetics of asparaginase or by leading to a discontinuation of asparaginase treatment. Here, we report successful challenges using native *E. coli* asparaginase after previous hypersensitivity reactions to both PEGylated *E. coli* asparaginase and *Erwinia* asparaginase.

Patients and methods—The two patients included in this case report were diagnosed with B-precursor ALL at St. Jude Children's Research Hospital and were treated with a common regimen. Both patients developed hypersensitivity reactions to PEGylated *E. coli* asparaginase and *Erwinia* asparaginase early in treatment, and they were challenged with native *E. coli* asparaginase. Serum samples were collected for estimating the pharmacokinetic parameters of each patient during native *E. coli* asparaginase therapy.

Results—Challenges with native *E. coli* asparaginase were successful and asparaginase serum concentrations above therapeutic levels were attained in both patients.

Conclusions—These two cases suggest that some patients can be given native *E. coli* asparaginase after hypersensitivity reactions to PEGylated asparaginase and achieve therapeutic concentrations of the drug in serum.

Keywords

acute lymphoblastic leukemia; pediatric; asparaginase; hypersensitivity; anti-asparaginase antibody; asparaginase activity

Introduction

Asparaginase is one of the key therapeutic agents for childhood acute lymphoblastic leukemia (ALL) [1]. Asparaginase is an active bacterial enzyme drug that systemically depletes the non-essential amino acid asparagine and leads to leukemic cell death possibly due to the low levels of asparagine synthetase in these cells [2]. Asparaginase regimens achieving a serum concentration at or above 0.1 IU/mL are believed to be sufficient to deplete asparagine [3].

The pharmacokinetic properties of asparaginase are dependent on several different factors, including the bacterial source used to generate the protein and on the particular manufacturer of the preparation. Oncaspar is a PEGylated form of the native *E. coli* asparaginase that has a half-life of about a week, whereas the native *E. coli* asparaginase (Elspar) and *Erwinia* asparaginase (Erwinase) have a half-life of 1.3 days and 0.65 days, respectively [1,4,5]. Asparaginase-induced hypersensitivity reactions can decrease exposure to asparaginase. Hypersensitivity reactions to asparaginase can be as frequent as 63%, and patients with reactions to asparaginase have been reported to have faster drug clearances compared to patients that did not have reactions [6,7]. Furthermore, patients that develop neutralizing antibodies to asparaginase, even in the absence of a clinical reaction, may have sub-therapeutic serum concentrations due to an accelerated asparaginase clearance [1,2,8]. Neutralizing antibodies generated against the native *E. coli* preparation have been shown to cross-react with Oncaspar asparaginase but not with Erwinase asparaginase [9].

Patients with low asparaginase exposure due to toxicities have been reported to have worse outcomes [10]. Therefore, substituting a different preparation of asparaginase after a hypersensitivity reaction or after the detection of neutralizing antibodies can extend asparaginase therapy and possibly improve patient outcome. In clinical trials, PEGylated asparaginase has been associated with a decreased frequency of hypersensitivity [10], and Oncaspar is approved for use in patients who develop allergies to native *E. coli* asparaginase. *Erwinia* asparaginase is generally the first choice to use in patients who react to primary treatment with Oncaspar, but some of these patients subsequently develop allergies to *Erwinia* asparaginase. It is unknown whether patients with past hypersensitivity reactions to Oncaspar asparaginase will cross-react to Elspar asparaginase. Herein, we report successful challenges using Elspar in two pediatric ALL patients with previous hypersensitivity reactions to both Oncaspar and Erwinase preparations of asparaginase. Asparaginase serum concentrations above therapeutic levels were attained in both patients, a finding suggesting that selected patients with Oncaspar hypersensitivities may continue asparaginase therapy with Elspar asparaginase without cross-reactivity.

Case Reports

The two patients included in this case report were diagnosed with B-precursor ALL at St. Jude Children's Research Hospital. Patient A was enrolled on the Total XVI acute leukemia protocol [11], and Patient B was not enrolled on a clinical trial but did provide consent for similar treatment regimen and asparaginase serum measures. Table 1 summarizes the

pertinent characteristics of the two patients. Oncaspar (3,000 IU/m², IV) was given at day 3 of remission induction in both patients.

Patient A tolerated the first dose of Oncaspar during induction but reacted to the second dose of Oncaspar given during week 1 of continuation (Figure 1A and Supplemental Table 1). The patient had a grade 3 reaction with symptoms including urticaria and difficulty breathing. She was then given Erwinase (42,000 IU/m² per dose) but developed a grade 3 hypersensitivity reaction to the second dose of Erwinase. The symptoms of the second reaction included itchy eyes, bronchospasm with wheezing, and difficulty breathing. She was challenged with intravenous Elspar during reinduction I treatment (39,000 IU/m² per dose) with hydrocortisone (200 mg, PO) and diphenhydramine (50 mg, PO) premedication approximately two hours before each dose of Elspar. She received Elspar twice weekly for 5 doses during reinduction I (weeks 7-9 of continuation treatment) before the drug was held due to mild pancreatitis (Figure 1A, Supplemental Table 1). Elspar was resumed twice weekly during weeks 10-16 of continuation treatment without hydrocortisone and diphenhydramine and an additional 9 doses of Elspar were given before the drug was completely discontinued due to a second and more severe episode of pancreatitis (Figure 1A, Supplemental Table 1). The Elspar substitution resulted in an additional 14 doses of asparaginase administered for this patient.

Patient B had a severe reaction to the first dose of Oncaspar (Figure 1B and Supplemental Table 1). The symptoms of the grade 4 reaction included anaphylaxis, rash, and shortness of breath. He was then treated with Erwinase (20,000 IU/m² per dose) thrice weekly for 6 doses during remission induction. He developed a hypersensitivity reaction to Erwinase when it was resumed at week 1 of continuation treatment (Figure 1B). The symptoms included urticaria, bronchospasm, and shortness of breath. He was then rechallenged with intramuscular Elspar (12,500 IU/m² per dose) twice weekly with diphenhydramine (25 mg, PO) and ranitidine (100 mg, PO) premedication one hour before each Elspar administration from week 3 to week 13 of continuation treatment. Elspar was discontinued after 16 doses were given due to extensive venous thrombosis and associated superior vena cava (SVC) syndrome (Supplemental Table 1).

Antibody Testing

A total of 19 serum samples were collected from Patient A from induction through continuation treatment for antibody testing (Table 2). The antibody levels in samples were determined by ELISA as described previously [12]. Two additional antigens (PEG-superoxide dismutase and PEG-catalase) were included in the assay for the detection of anti-polyethylene glycol (PEG) antibodies. None of the samples collected for Patient A tested positive for IgG, IgM, or IgE antibodies against asparaginase (Elspar, Erwinia, and Oncaspar) or PEG. Furthermore, serum samples collected two hours after the first Elspar dose were tested for elevated tryptase levels by ARUP Laboratories (Salt Lake City, Utah). The samples were within the normal concentration range of tryptase, consistent with the absence of a hypersensitivity reaction during the Elspar challenge [13].

Patient B had two serum samples collected during induction immediately following the hypersensitivity reaction (1.6 and 3.5 hours after Oncaspar administration). The samples were tested for IgG, IgM, or IgE antibodies against asparaginase (Elspar, Erwinia, and Oncaspar) and PEG. The patient did not have any detectable levels of anti-asparaginase antibodies, but anti-PEG IgG antibodies were detected (Table 2). No other samples collected for this patient throughout all phases of treatment tested positive for any antibody including anti-PEG antibodies (Table 2). Nevertheless, it is possible that a previous exposure and sensitization to polyethylene glycol may have played a role in the hypersensitivity reaction and antibody response to Oncaspar [14]. Tryptase levels were not measured for Patient B.

Because IgE antibodies may have been bound to mast cells and because its presence in serum can be fleeting, it is possible that IgE was formed in these patients but not detected in the samples we assayed.

Asparaginase activity

Samples from Patient A were collected during induction for asparaginase activity testing, and all samples collected within two weeks of the Oncaspar dose were above 0.1 IU/mL (Table 2). Patient B did not receive a full dose of Oncaspar due to the hypersensitivity reaction, and therefore it is not surprising that the asparaginase activity measured from samples collected during induction were below therapeutic concentrations (Table 2). Only Patient B had a single sample available for asparaginase activity quantification during Erwinase treatment (Table 2), and activity was > 0.1 IU/mL.

Asparaginase activity was determined in several serum samples collected throughout Elspar asparaginase exposure in both patients (Figure 2, Table 2). Asparaginase drug concentrations were modeled using a one-compartment pharmacokinetic model with first-order absorption and Michaelis-Menten elimination [12]. The pharmacokinetic parameters for each patient during Elspar therapy were estimated using NONMEM (version 7.2) [15]. Asparaginase concentrations were above the 0.1 IU/mL threshold in all samples collected within one week of a dose for both patients (Figure 2 and Table 2). The clearance was higher and half-life was shorter for both Patient A and B than were reported for newly diagnosed ALL patients given Elspar, but the treatment doses were high enough to maintain serum asparaginase activity above 0.1 IU/mL (Figure 2, Supplemental Table 2). No CSF asparaginase data are available for either patient during Elspar therapy to evaluate the pharmacodynamics effect of Elspar asparaginase.

Discussion

Successful asparaginase substitutions require that there be no or little cross-reactivity with the offending preparations in regard to clinical reactions and to the neutralization of asparaginase activity. Pretreatment with steroids and antihistamines may mitigate hypersensitivity reactions, but this strategy is not suitable unless neutralizing antibodies are also diminished by pretreatment medication. Therefore, not only is it important for asparaginase treatment strategies to mitigate the severity of the hypersensitivity reaction, but strategies are also required to maintain efficacious concentrations of asparaginase.

Previously, our practice was to discontinue asparaginase treatment if hypersensitivity reactions occur to both Oncaspar and Erwinia asparaginase. However, due to a lack of detection of antibodies to Elspar or Oncaspar, there was no indication that the mediator responsible for the previous reactions would cross-react to Elspar asparaginase. Based on this limited information and due to the early stage in treatment that the patients developed their hypersensitivity reactions, putting them at a higher risk of poor outcome due to low asparaginase exposure [10], we decided to challenge these two patients with Elspar asparaginase.

Elspar challenges were successful in both of these patients after their previous hypersensitivities to other preparations of asparaginase. Although the absence of tryptase in Patient A and the lack of any asparaginase-specific antibodies in all samples tested are consistent with a lack of an immediate hypersensitivity reaction having occurred (as opposed to the pretreatment medications masking the hypersensitivity reaction), the possibility that these patients had hypersensitivity reactions cannot be ruled out. The patients were able to tolerate multiple doses of Elspar, although both eventually had asparaginase discontinued due to other asparaginase-related toxicities. Nevertheless, the Elspar substitutions were able to extend therapy for several weeks, and samples collected during Elspar therapy displayed asparaginase drug concentrations above the minimal threshold required for asparagine depletion [3].

Even though antibodies were not detected in either patient during Elspar therapy, the clearance estimates were higher in both patients compared to *de novo* patients (Supplemental Table 2) [1,16-19]. The pharmacokinetic parameters estimated were more similar to those reported from pediatric patients with relapsed ALL receiving Elspar and negative for anti-asparaginase IgG antibodies (clearance range: 1,768-6,865 mL/day/m²) than to newly-diagnosed ALL patients [12]. Our results suggest that patients with previous allergies to Oncaspar may require higher doses of Elspar asparaginase to achieve serum concentrations comparable to those of non-allergic, *de novo* patients receiving Elspar.

The mechanism responsible for asparaginase-induced hypersensitivity is unknown. Although studies have demonstrated a relationship between Elspar-specific IgG antibodies and reactions to Elspar [20], there are fewer data on mechanisms of reaction to Oncaspar. The cases presented here are the first to show successful challenge with native *E. coli* asparaginase (Elspar) after hypersensitivity reactions to Oncaspar and they support a hypothesis that the mediators of hypersensitivity responsible for Oncaspar allergies may not necessarily cross-react with Elspar asparaginase. These results, based on the two patients included in this case report, suggest that it may be possible to treat patients with Elspar asparaginase after hypersensitivity reactions to Oncaspar asparaginase if there is no evidence of anti-*E. coli* asparaginase antibodies, and that Elspar in such patients can achieve therapeutic drug concentrations in serum.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The study was supported by grants CA 21765, CA 142665, and CA 36401 from the National Cancer Institute, by the American Lebanese Syrian Associated Charities, and by funding from the Sigma-Tau Pharmaceuticals for research on the pharmacology of asparaginase.

References

1. Asselin BL, Whitin JC, Coppola DJ, Rupp IP, Sallan SE, Cohen HJ. Comparative pharmacokinetic studies of three asparaginase preparations. *J Clin Oncol*. 1993; 11:1780–1786. [PubMed: 8355045]
2. Raetz EA, Salzer WL. Tolerability and efficacy of L-asparaginase therapy in pediatric patients with acute lymphoblastic leukemia. *Journal of Pediatric Hematology/Oncology*. 2010; 32(7):554–563. doi:10.1097/MPH.0b013e3181e6f003. [PubMed: 20724951]
3. Riccardi R, Holcenberg JS, Glaubiger DL, Wood JH, Poplack DG. L-asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. *Cancer Res*. 1981; 41:4554–4558. [PubMed: 6895481]
4. Dinndorf PA, Gootenberg J, Cohen MH, Keegan P, Pazdur R. FDA drug approval summary: pegaspargase (oncaspar) for the first-line treatment of children with acute lymphoblastic leukemia (ALL). *The oncologist*. 2007; 12(8):991–998. doi:10.1634/theoncologist.12-8-991. [PubMed: 17766659]
5. Muller HJ, Boos J. Use of L-asparaginase in childhood ALL. *Critical reviews in oncology/hematology*. 1998; 28(2):97–113. [PubMed: 9768345]
6. Woo MH, Hak LJ, Storm MC, Evans WE, Sandlund JT, Rivera GK, Wang B, Pui CH, Relling MV. Anti-asparaginase antibodies following E. coli asparaginase therapy in pediatric acute lymphoblastic leukemia. *Leukemia*. 1998; 12(10):1527–1533. [PubMed: 9766495]
7. Asselin BL. The three asparaginases. *Comparative pharmacology and optimal use in childhood leukemia*. *Adv Exp Med Biol*. 1999; 457:621–629. [PubMed: 10500842]
8. Avramis VI, Avramis EV, Hunter W, Long MC. Immunogenicity of native or pegylated E. coli and *Erwinia* asparaginases assessed by ELISA and surface plasmon resonance (SPR-biacore) assays of IgG antibodies (Ab) in sera from patients with acute lymphoblastic leukemia (ALL). *Anticancer research*. 2009; 29(1):299–302. [PubMed: 19331165]
9. Wang B, Relling MV, Storm MC, Woo MH, Ribeiro R, Pui CH, Hak LJ. Evaluation of immunologic crossreaction of antiasparaginase antibodies in acute lymphoblastic leukemia (ALL) and lymphoma patients. *Leukemia*. 2003; 17(8):1583–1588. [PubMed: 12886246]
10. Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, Hurwitz CA, Moghrabi A, Samson Y, Schorin MA, Arkin S, Declerck L, Cohen HJ, Sallan SE. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood*. 2001; 97(5):1211–1218. [PubMed: 11222362]
11. Pui CH CD, Sandlund JT, Bhojwani D, Evans WE, Relling MV, Jeha S. Treatment of childhood acute lymphoblastic leukemia without cranial irradiation. *Ann Hematol*. 2011; 90(Suppl 1):S61–63.
12. Panetta JC, Gajjar A, Hijiya N, Hak LJ, Cheng C, Liu W, Pui CH, Relling MV. Comparison of native E. coli and PEG asparaginase pharmacokinetics and pharmacodynamics in pediatric acute lymphoblastic leukemia. *Clin Pharmacol Ther*. 2009; 86(6):651–658. doi:10.1038/clpt.2009.162. [PubMed: 19741605]
13. Payne V, Kam PC. Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia*. 2004; 59(7):695–703. doi:10.1111/j.1365-2044.2004.03757.x. [PubMed: 15200544]
14. Richter AW, Akerblom E. Polyethylene glycol reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol modified allergens or placebo, and in healthy blood donors. *Int Arch Allergy Appl Immunol*. 1984; 74(1):36–39. [PubMed: 6706424]
15. Beal, S.; Sheiner, L.; Boeckmann, A. NONMEM user's guides. Icon Development Solutions; Ellicott City, MD, USA: 1989-2011.
16. Avramis VI, Sencer S, Periclou AP, Sather H, Bostrom BC, Cohen LJ, Ettinger AG, Ettinger LJ, Franklin J, Gaynon PS, Hilden JM, Lange B, Majlessipour F, Mathew P, Needle M, Neglia J,

Reaman G, Holcenberg JS, Stork L. A randomized comparison of native *Escherichia coli* asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood*. 2002; 99(6):1986–1994. [PubMed: 11877270]

17. Avramis VI, Panosyan EH. Pharmacokinetic/pharmacodynamic relationships of asparaginase formulations: the past, the present and recommendations for the future. *Clin Pharmacokinet*. 2005; 44(4):367–393. [PubMed: 15828851]
18. Ho DHW, Yap HY, Brown N, Benjamin RS, Friereich EJ, Blumenschein GR, Bodey GP. Clinical Pharmacology of Intramuscularly Administered L-Asparaginase. *J Cancer Res Clin Oncol*. 1981; 21:72–78.
19. Schorin MA, Blattner S, Gelber RD, Tarbell NJ, Donnelly M, Dalton V, Cohen HJ, Sallan SE. Treatment of childhood acute lymphoblastic leukemia: results of Dana-Farber Cancer Institute/Children's Hospital Acute Lymphoblastic Leukemia Consortium Protocol 85-01. *J Clin Oncol*. 1994; 12(4):740–747.
20. Liu C, Kawedia JD, Cheng C, Pei D, Fernandez CA, Cai X, Crews KR, Kaste SC, Panetta JC, Bowman WP, Jeha S, Sandlund JT, Evans WE, Pui CH, Relling MV. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. *Leukemia*. 2012 doi:leu2012102[pii]10.1038/leu.2012.102.

Figure 1. Asparaginase dosing schedule of patients challenged with native *E. coli* asparaginase Two pediatric ALL patients were challenged with native *E. coli* asparaginase (Elspar) after hypersensitivity reactions to PEGylated *E. coli* asparaginase (Oncaspar) and *Erwinia* asparaginase (Erwinase). Planned Oncaspar (○), Erwinia (◇), and Elspar (△) doses relative to the start of induction treatment are shown for Patient A (A) and Patient B (B). All doses that were tolerated by the patients are indicated by filled symbols (●, ◆, and ▲), and asparaginase doses that induced hypersensitivity reactions are illustrated in red (● and ◆). The treatment phases included along the x-axis are induction, consolidation, continuation weeks 1-6 (cont W1-6), reinduction I (Re I), continuation weeks 10-16 (cont W10-16), reinduction II (Re II), and continuation weeks 20-29 (cont W20-29). Therapy for Patient A was interrupted during reinduction II due to severe pancreatitis.

Figure 2. Asparaginase activity modeling for pharmacokinetic parameter estimation

The asparaginase activity of samples collected during continuation treatment from two pediatric ALL patients challenged with native *E. coli* asparaginase (Elspar) was measured (○) and modeled using a one-compartment pharmacokinetic model with first-order absorption and Michaelis-Menten elimination. The solid black line represents the best fit from the pharmacokinetic modeling and the solid red line indicates the 0.1 IU/mL serum concentration threshold required for efficient asparagine depletion.

Table 1

Patient characteristics

Characteristics	Patient A	Patient B
Race	Caucasian	Caucasian
Gender	Female	Male
Age (years)	12	14
Patient medical history	Obesity	Down Syndrome
Treatment arm (risk)	Standard	Standard
Induction dose of Oncaspar	3,000 IU/m ²	3,000 IU/m ² (planned) ^I
Continuation dose of Oncaspar	3,500 IU/m ²	2,500 IU/m ² (planned)
Continuation dose of Erwinase	42,000 IU/m ²	30,000 IU/m ² (planned)
Continuation dose of Elspar	Challenge: 39,000 IU/m ² Reinduction I: 17,500 IU/m ² Reinduction II: 12,500 IU/m ²	12,500 IU/m ²
Route of asparaginase administration	Oncaspar: IV Erwinia: IV Elspar: IV	Oncaspar: IV Erwinia: IV Elspar: IM

^I Oncaspar was replaced with Erwinase (20,000 IU/m²) after hypersensitivity

Table 2

Antibody and asparaginase results

Treatment phase (Asparaginase formulation)	Patient	Antibody Results						Asparaginase Activity	
		Anti-Asparaginase (Elspar, Erwinase, Oncaspar) antibodies				Anti-PEG antibodies			
		IgG (# of samples)	IgE (# of samples)	IgM (# of samples)	IgG (# of samples)	IgE (# of samples)	IgM (# of samples)	Time after dose (# of samples)	Range of activity (IU/mL)
Induction (Oncaspar)	Patient A Patient B	Negative (4) Negative (2)	Negative (4) Negative (2)	Negative (4) Negative (2)	Negative (4) Positive (2)	Negative (4) Negative (2)	Negative (4) Negative (2)	2-40 days (6) 1-4 hours (2)	0.08-2.20 ¹ 0.02-0.05 ²
Continuation Week 1-2 (Erwinase)	Patient A Patient B	Negative (3) Negative (1)	Negative (3) Negative (1)	Negative (3) Negative (1)	Negative (3) Negative (1)	Negative (3) Negative (1)	Negative (3) Negative (1)	NA (0) 4 hours (1)	NA ³ 0.41 ³
Continuation Week 3-18 (Elspar)	Patient A Patient B	Negative (12) Negative (20)	Negative (12) Negative (20)	Negative (12) Negative (20)	Negative (12) Negative (20)	Negative (12) Negative (20)	Negative (12) Negative (20)	1-33 days (12) 1-21 days (20)	0.01-1.69 ⁴ 0.01-1.10 ⁵

¹ After 3,000 IU/m² of Oncaspar;
² After approximately 90 IU/m² of Oncaspar;
³ After 30,000 IU/m² of Erwinase;
⁴ After 39,000 IU/m² to 12,500 IU/m² of Elspar;
⁵ After 12,500 IU/m² of Elspar