

The cross-reactivity of anti-asparaginase antibodies against different L-asparaginase preparations

Beata Zalewska-Szewczyk · Agnieszka Gach ·
Krystyna Wyka · Jerzy Bodalski · Wojciech Młynarski

Received: 6 May 2008 / Accepted: 31 October 2008
© Springer-Verlag 2009

Abstract Repeated administration of L-asparaginase leads to the development of specific antibodies and hypersensitivity reactions. The aim of the study was to evaluate a possible cross-reaction of anti-asparaginase antibodies, developed against the native *E. coli* L-asparaginase (Asparaginase Medac), with other preparations of the enzyme. Sixteen patients with acute lymphoblastic leukemia, in whom in the reinduction phase of treatment hypersensitivity against L-asparaginase was observed and/or the presence of anti-asparaginase antibodies was established were recruited for the present study. Ten out of 16 tested sera showed cross-immunoreactivity to PEG-asparaginase, while no reactivity to L-asparaginase derived from *Erwinia chrysantemi* was observed. Since cross-reacting antibodies were also found in sera of patients with no overt allergic reaction, L-asparaginase may undergo silent inactivation during the reinduction phase of therapy. This finding is of clinical importance with regard to appropriate dosage and necessitates careful enzyme activity monitoring in all patients undergoing repeated treatment with various L-asparaginase preparations.

Keywords Acute lymphoblastic leukemia · Anti-asparaginase antibodies · Cross-reactivity · Hypersensitivity reactions

Introduction

L-asparaginase, an effective antineoplastic agent, has been a mainstay of protocols used in treatment of pediatric acute lymphoblastic leukemia (ALL) for nearly 30 years [1]. The enzyme catalyses the extracellular decay of amino acid L-asparagine to aspartic acid and ammonia [2–4]. Depletion of serum asparagine restricts protein synthesis in sensitive blasts, leads to impairment of cellular function and apoptosis of malignant cells. Although, asparaginase is found in various plant and animal species, only its discovery in microorganism enabled large-scale enzyme production [5, 6].

Nowadays, three formulations of the enzyme are available for therapeutic use. Two native preparations are derived from *Escherichia coli* and *Erwinia chrysantemi*. Both of them are approved for use in the therapy of patients in the front line and at relapse. However, repeated administration of foreign protein leads to the development of specific antibodies and hypersensitivity reactions, which make the continuation of therapy impossible [3, 7–9]. Chemical modification of native *E. coli* enzyme with polyethylene glycol reduces its immunogenicity and markedly prolongs the duration of its action [7, 10, 11]. In patients with clinical hypersensitivity to native enzyme, PEG-asparaginase has been shown to be well-tolerated and effective [12]. Replacement of the preparation which provoked the hypersensitivity, with an alternative form of the enzyme is a valuable therapeutic option. However, in such cases, the question arises whether there is a danger of L-asparaginase cross-inactivation by already existing antibodies developed against the preparation used during induction phase. Thus, the aim of the study was to evaluate the possible cross-reaction of anti-asparaginase antibodies, developed against the native *E. coli* enzyme (Asparaginase

B. Zalewska-Szewczyk (✉) · J. Bodalski · W. Młynarski
Department of Pediatrics, Hematology,
Oncology and Diabetology, 1st Chair of Pediatrics,
Medical University of Łódź, 36/50 Sporna Street,
91-738 Łódź, Poland
e-mail: bszewczyk@toya.net.pl; bszewczyk@alef.umed.lodz.pl

A. Gach · K. Wyka · W. Młynarski
Department of Genetics and Immunopathology,
1st Institute of Pediatrics, Medical University of Łódź,
Łódź, Poland

Medac), with other drug preparations (PEG-asparaginase, Erwinase).

Materials and methods

The investigation was in accordance with the principles of the Declaration of Helsinki, and the appropriate ethical committee approved the protocol and informed consent procedures.

Patient eligibility and treatment schedules

Sixteen patients with ALL treated with *E. coli* L-asparaginase, in whom in the reinduction phase of treatment hypersensitivity against L-asparaginase and/or the presence of anti-asparaginase antibodies was established were recruited for the present study. All patients were treated according to the ALL BFM 95 protocol, which anticipates 8 doses of L-asparaginase 5,000 U/m² in the induction phase (day 12, 15, 18, 21, 24, 27, 30 and 33) and 4 doses of L-asparaginase 10,000 U/m² (day 8, 11, 15 and 18) in the reinduction phase of treatment intravenously given. In the reinduction phase 11 out of 16 patients clinically manifested hypersensitivity against *E. coli* L-asparaginase and were switched to PEG-asparaginase.

Blood samples collection and storage

For the evaluation of the presence and cross-reactivity of anti-asparaginase antibodies blood samples were collected on the 18th day of the reinduction phase. From patients with allergic reaction to *E. coli* L-asparaginase blood samples were collected on the day of presented symptoms, before administration of other drug preparations. Blood samples were collected in 4.9-ml tubes, centrifuged at 2,000g for 10 min, and the serum was stored at −70°C until analysis.

Anti-asparaginase antibodies measurement

The presence of anti-asparaginase antibodies was evaluated using ELISA method, as described previously [13]. Briefly, 96-polystyrene ELISA plates were coated with asparaginase antigen and incubated. Patient plasma was serially added to plates and incubated, thereafter were developed with horseradish peroxidase-conjugated rabbit anti-human IgG monoclonal antibodies. Then the substrate solution was added to each well. Individual wells were read on an ELISA reader set to establish optical density (OD) at wavelength of 450 nm. The count higher than two standard deviation of OD was established as the positive cut down.

Evaluation of cross-reactivity with Western blot analysis

Preparations of *Escherichia coli* L-asparaginase: Asparaginase Medac (Medac GmbH, Germany), Kidrolase (Rhone Poulenc, France), Oncaspar (Medac GmbH, Germany), and *Erwinia chrysanthemi* L-asparaginase: Erwinase (Orphan, UK) were size-fractionated on 12% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in TBS containing 3% bovine serum albumin (BSA) and incubated with the sera diluted 1:300 in 1% BSA 0.05% Tween 20 TBS buffer. After three washes in TBS containing 0.1% Tween 20 membranes were incubated with peroxidase-coupled secondary antibody reactive with human IgG in dilution 1:1,000 (Dako, Denmark). The proteins of interest were visualized with SuperSignal West Pico chemiluminescent substrate (Pierce, USA).

Results

Measured by ELISA all 16 patients had antibodies against *E. coli* L-asparaginase in the reinduction phase of treatment. Eleven children had generalized hypersensitivity reaction in the reinduction therapy that required discontinuation of native *E. coli* asparaginase. Clinical characteristics of the allergic reactions included itching, urticaria, abdominal or chest pain, dyspnea, cough and/or hypotension. In this group of patients 9 of tested sera showed presence of antibodies reactive with native *E. coli* enzyme preparations, Asparaginase Medac and Kidrolase, in Western blot analysis. Six sera were also reactive with PEG-asparaginase. Five children showed no clinical symptoms of hypersensitivity in the reinduction phase, yet 4 of them were positive for anti-asparaginase antibodies reactive with all tested *E. coli* L-asparaginase preparations by Western blotting. None of tested sera cross-reacted with L-asparaginase derived from *Erwinia chrysanthemi* (Fig. 1; Table 1).

We observed the higher titer of anti-asparaginase antibodies estimated by ELISA in the sera which were positive in Western blotting, as compared with those, which did not (average OD 0.64 vs. 0.27); however, the difference was not statistically significant ($P = 0.21$ using non-parametric *U* Mann-Whitney test).

Discussion

L-asparaginase is an integral part of the modern day chemotherapy protocols of ALL. Owing to myelosuppressive nature and rare adverse late-effects in children it is considered to be an ideal agent for combination therapy

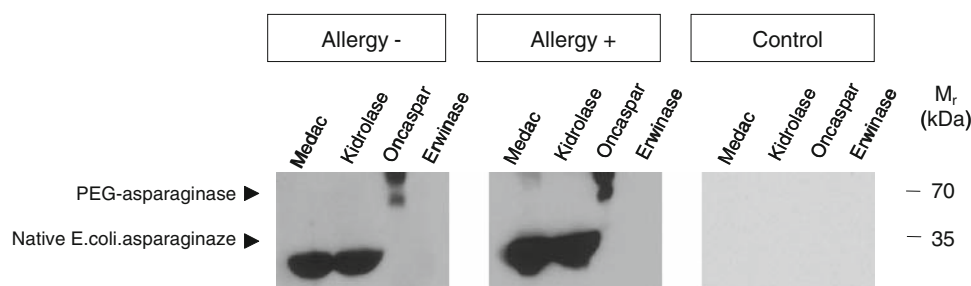


Fig. 1 Western blot analysis of sera immunoreactivity to several L-asparaginase preparations. The evaluation was performed using pooled sera from patients with clinical signs of hypersensitivity and from individuals without hypersensitivity during the reinduction

Table 1 The cross-reactivity between anti-asparaginase antibodies against native *E. coli* asparaginase (Asparaginase Medac) and other enzyme preparation

Clinical signs of allergy	Number of analyzed sera	Cross-reaction (the number of reacting sera)			
		Medac	Kidrolase	Oncaspar	Erwinia
Present	5	4	4	4	0
Absent	11	9	9	6	0

protocol for childhood leukemia. However, clinical hypersensitivity, which develops in about two-thirds of patients treated with native enzyme preparations, limits its subsequent use [5, 14]. In this study 11 children showed allergic reactions to *E. coli* L-asparaginase in the reinduction phase of treatment, while in 5 patients no hypersensitivity was observed. Generation of antibodies reactive with L-asparaginase attenuates pharmacological effect of the drug [5, 11] and is associated with the higher risk of relapse in high-risk ALL [15].

In case of allergic reactions to native *E. coli* L-asparaginase patients are usually switched to either pegylated form of the enzyme or asparaginase derived from *Erwinia chrysantemi*. We aimed to compare cross-reactivity of antibodies developed against native *E. coli* asparaginase to other drug formulations. Using Western blot analysis we found that majority of tested sera were cross-reactive with PEG-asparaginase, but none with *Erwinia* enzyme. This finding is consistent with other report where cross-reactivity was evaluated with ELISA method [16]. ELISA seems to be more sensitive for detection of anti-asparaginase antibodies and should be recommended for clinical screening. However, there is likely that, during preparation of the PEG-asparaginase for ELISA some epitopes may be shown, which were not present in native PEG-asparaginase. Thus, in our case Western blot approach gave additional evidence for cross-reactivity since the size of the band corresponds to native PEG-asparaginase.

phase of ALL treatment. Sera obtained from healthy volunteers, who did not receive, L-asparaginase were used as a negative control. A smear, which is observed at the top of PEG-asparaginase band is probably related to degraded preparation

On the other hand, high immunogenicity of native *E. coli* enzyme can be greatly diminished by covalent attachment of polyethylene glycol. PEGylation has also been found to markedly prolong L-asparaginase half-life, thus eliminates the problem of frequent medication. Clinical studies show that PEG-asparaginase half-life in patients with allergic reaction to native *E. coli* enzyme can be markedly shortened compared to patients receiving modified enzyme in the induction phase, 1.8 and 5.7 days, respectively [10]. Muller et al. [17] monitored activity of PEG-asparaginase in reinduction treatment in 70 children treated according to BFM protocol, who during the induction phase received native *E. coli* L-asparaginase. The authors reported that after 2 weeks of PEG-asparaginase application nearly one-third of patients had lower than desired serum enzyme activity, while no clinically manifested allergy was noted. Since it is widely postulated that the decrease in enzyme activity is antibody-related phenomenon, our observation suggests that the cross-reactivity could explain unexpectedly decreased enzyme activity in these patients.

Lack of reactivity of tested sera to Erwinase in Western blot experiments can be explained by significant difference in the amino acid composition between *E. coli* and *Erwinia chrysantemi* preparations. Although Erwinase is a therapeutic option for patients with allergic reactions to native *E. coli* enzyme it has many disadvantages, including high immunogenicity similar to native *E. coli* enzyme [18] and very-short plasma half-life [10]. In patients requiring discontinuation of native *E. coli* asparaginase in the reinduction phase expected half-lives of PEG-asparaginase and Erwinase would be 1.8 and 0.65 days, respectively [10]. Thus, in spite of observed cross-reactivity PEG-asparaginase is a valuable preparation for patients hypersensitive to native drug, however, shortened half-life time should be considered during treatment in reinduction phase.

Western blot analysis enables visualizing reactivity of anti- *E. coli* asparaginase antibodies with other enzyme

preparations. Three of the tested sera failed to show immunoreactivity in Western blotting while measured by ELISA all samples had antibodies against *E. coli* L-asparaginase. Two of these sera had low titers of anti-asparaginase antibodies in the ELISA measurement. Thus, Western blot seems to be less-sensitive method compared to ELISA. Moreover, ELISA evaluation was made soon after sample collection whereas Western blot analysis was performed using long-term stored and previously defrozen samples, thus protein degradation may be responsible for some of the observed discrepancy.

Conclusion

Our results indicate cross-reactivity between antibodies directed to native *E. coli* L-asparaginase and its pegylated form, but not Erwinia derived enzyme. Moreover, we detected presence of cross-reacting antibodies in sera of patients with no overt allergic reaction. Thus, even in the absence of clinical hypersensitivity L-asparaginase anticancer efficacy may be reduced in the reinduction therapy. This is of clinical importance with regard to appropriate dose and schedule in the reinduction phase of treatment. Our finding emphasizes necessity of careful enzyme activity monitoring in all patients undergoing repeated courses of L-asparaginase treatment in order to achieve continuous asparagine depletion. It is especially important in the case of use of pegylated form of enzyme, after the occurrence of hypersensitivity reaction against native *E. coli* L-asparaginase.

Acknowledgments Supported by National Health Committee, grant No 3 P05E 030 22 and No 2P05E 020 29.

Conflict of interest statement The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

1. Narta UK, Kanwar SS, Azmi W (2007) Pharmacological and clinical evaluation of L-asparaginase in the treatment of leukemia. *Crit Rev Oncol Hematol* 61:208–221
2. Duval M, Suci S, Ferster A, Rialland X, Nelken B, Lutz P, Benoit Y, Robert A, Manel AM, Vilmer E, Otten J, Philippe N (2002) Comparison of *Escherichia coli*-asparaginase with *Erwinia*-asparaginase in the treatment of acute lymphoblastic leukemia: results of a randomized European Organisation for Research and Treatment of Cancer–Children’s Leukemia Group phase 3 trial. *Blood* 99:2734–2739
3. Müller HJ, Boos J (1998) Use of L-asparaginase in childhood ALL. *Crit Rev Oncol Hematol* 28:97–113
4. Pui CH (1995) Childhood leukemia. *N Engl J Med* 332:1618–1630
5. 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, Neqlia J, Reaman G, Holcenberg JS, Stork L (2002) 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* 99:1986–1994
6. Broome JD (1968) Factors which may influence the effectiveness of L-asparaginase as tumor inhibitors. *Br J Cancer* 22:595–602
7. Abuchowski A, Kazo GM, Verhoest CR Jr, Van Es T, Kafkevitz D, Nuci ML, Viau AT, Davis FF (1984) Cancer therapy with chemically modified enzymes. I Antitumor properties of polyethylene glycol-asparaginase conjugates. *Cancer Biochem Biophys* 7:175–186
8. Cheung NK, Chau IY, Coccia PF (1986) Antibody response to *Escherichia coli* L-asparaginase: prognostic significance and clinical utility of antibody measurement. *Am J Pediatr Hematol Oncol* 8:99–104
9. Woo MH, Hak LJ, Storm MC, Evans WE, Sandlund JT, Rivera GK, Wang P, Pui CH, Relling MV (1998) Anti-asparaginase antibodies following *E. coli* asparaginase therapy in pediatric acute lymphoblastic leukemia. *Leukemia* 12:1527–1533
10. Asselin BL, Whithin JC, Coppola DJ, Rupp IP, Sallan SE, Cohen HJ (1993) Comparative pharmacokinetic studies of three asparaginase preparation. *J Clin Oncol* 11:1780–1786
11. Kamisaki Y, Wada H, Yagura T, Matsushimi A, Inada Y (1981) Reduction in immunogenicity and clearance rate of *Escherichia coli* L-asparaginase by modification with monomethoxypolyethylene glycol. *J Pharmacol Exp Ther* 216:410–414
12. Yashimoto T, Nishimura H, Saito Y (1986) Characterization of PEG modified L-asparaginase from *E. coli* and its application to the therapy of leukemia. *Jpn J Cancer Res* 77:1264–1271
13. Zalewska-Szewczyk B, Andrzejewski W, Bodalski J (2004) Development of anti-asparaginase antibodies in childhood lymphoblastic leukemia. *Pediatr Blood Cancer* 43:600–602
14. Ettinger LJ, Kurtzberg J, Voute PA (1995) An open-label, multicenter study of polyethylene glycol L-asparaginase for the treatment of acute lymphoblastic leukemia. *Cancer* 75:1176–1181
15. Panosyan EH, Seibel NL, Martin-Aragon S, Gaynon PS, Avramis IA, Sather H, Franklin J, Nachman J, Ettinger LJ, Steinherz P, Cohen LJ, Siegel SE, Avramis VI (2004) Children’s Cancer Group Study Asparaginase antibody and asparaginase activity in children with higher-risk acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 26:217–226
16. Wang B, Relling MV, Storm MC, Woo MH, Ribeiro R, Pui CH, Hak LJ (2003) Evaluation of immunologic crossreaction of anti-asparaginase antibodies in acute lymphoblastic leukemia (ALL) and lymphoma patients. *Leukemia* 17:1583–1588
17. Müller HJ, Löning L, Horn A, Schwabe D, Gunkel M, Schrappe M, von Schütz V, Henze G, Casimiro da Palma J, Ritter J, Pinheiro JP, Winkelhorst M, Boos J (2000) Pegylated asparaginase (Oncaspar™) in children with ALL: drug monitoring in reinduction according to the ALL/NHL-BFM 95 protocols. *Br J Haematol* 110:379–384
18. Albertsen BK, Schröder H, Jakobsen P, Avramis VI, Müller HJ, Schmiegelow K, Carlsen NT (2002) Antibody formation during intravenous and intramuscular therapy with Erwinia asparaginase. *Med Pediatr Oncol* 38:310–316