

BRIEF REPORT

Development of Anti-Asparaginase Antibodies in Childhood Acute Lymphoblastic Leukemia

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L-asparaginase is a basic agent of antileukemic therapy, but allergic reactions against the drug and the development of anti-asparaginase antibodies are significant side-effects. The aim of our study was to estimate the presence of anti-asparaginase antibodies and to correlate the results with clinically apparent allergic reactions and with L-asparaginase activity during the treatment. We examined 13 children with newly diagnosed acute lymphoblastic leukemia (ALL), treated according to the Polish Pediatric Leukemia/Lymphoma Group protocol, based on ALL-BFM 95. Enzyme activity was estimated in serum samples, collected before each L-asparaginase administration, using the photometrical method. Anti-asparaginase antibody concentration was measured by enzyme-linked immunosorbent

assay (ELISA) at the two time points: at the last day of L-asparaginase treatment in the induction and the reinduction phase. The mean L-asparaginase activity was 273 IU/L in the induction phase; no anti-asparaginase antibodies in this phase of treatment were found. The mean L-asparaginase activity was 425 IU/L in the reinduction phase of treatment; in five children anti-asparaginase antibodies were detected. In four of these five children low L-asparaginase activity and/or allergic reactions against L-asparaginase were noted. Our observations suggest a correlation between the presence of anti-asparaginase antibodies and L-asparaginase activity in childhood ALL. *Pediatr Blood Cancer* 2004;43: 600–602. © 2004 Wiley-Liss, Inc.

Key words: antibodies; children; hypersensitivity; L-asparaginase

L-asparaginase is a basic agent of antileukemic therapy, used in the front-line treatment of acute lymphoblastic leukemia (ALL) [1]. L-asparaginase is a bacterial protein and when given to humans may result in the development of antibodies, which can cause allergic reactions against L-asparaginase and influence on the enzyme's activity. Allergic reactions against L-asparaginase can limit the use of the drug; about 10% of patients do not complete the planned course of L-asparaginase because of hypersensitivity, even when the preparation of the enzyme is changed to a different product [2]. The frequency and severity of allergic reactions depend on several factors such as the type of the enzyme, the route of drug administration, and the time point of treatment [2–4]. Some authors have suggested that there is a correlation between anti-asparaginase antibodies production and clinical manifestation of hypersensitivity [5]. However, it is still not clear whether there is any correlation between the development of anti-asparaginase antibodies and L-asparaginase activity during the treatment.

The aim of our study was to estimate the presence of anti-asparaginase antibodies (IgG class) and to correlate the results with clinically apparent allergic reactions and with L-asparaginase activity in children with ALL. We examined 13 children (5 boys and 8 girls), aged 3–15 years (mean 4.8 year) with new diagnosed ALL, who were treated according the Polish Pediatric Leukemia/

Lymphoma Group protocol, based on ALL-BFM 95 protocol. Patients received eight (5,000 IU/m²) doses of L-asparaginase every 3 days in the induction phase and four (10,000 UI/m²) doses every 3 days in the reinduction phase. *E. coli* asparaginase (Medac) was used. All patients were receiving glucocorticoids (prednisone in induction phase or dexamethasone in reinduction phase), anthracyclines (daunorubicin in induction phase and doxorubicin in reinduction phase), and vincristine. L-asparaginase was given as an 1-hr intravenous infusion. Whenever any allergic reaction occurred, the *E. coli* L-asparaginase type was changed to *Erwinia* L-asparaginase or PEG-asparaginase. We collected blood samples before L-asparaginase administration to determinate drug activity during treatment. L-asparaginase activity was measured using a photometric method, according to the technique described by Boos et al. [6].

The measurement of anti-asparaginase antibodies (IgG class) was performed at two time points: on the last day of

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L-asparaginase treatment in the induction and in the reinduction phase. Anti-asparaginase concentrations were measured by enzyme-linked immunosorbent assay (ELISA). *E. coli* asparaginase was diluted in 0.05 M carbonate–bicarbonate buffer, pH 9.4. 96-Polystyrene ELISA plates (Poly-Sorp Nunc Immuno Plate, Nalge Nunc International, Roskilde, Denmark) were coated with 100 µl of asparaginase antigen and incubated at 2–8°C overnight. Plates were blocked at room temperature for at least 90 min with 0.1% PBS–BSA blocking solution. The wells were drained and washed with 0.05% PBS–Tween buffer three times. Patient's serum was serially diluted to 1:200; 1:400; 1:800; 1:1,600; 1:3,200; 1:6,400; 1:12,800 with PBS–Tween buffer and 100 µl of each solution was added to plates and incubated at room temperature for 1 hr. Controls included uncoated wells (background control), antigen-coted wells without addition of primary antibodies (control of non-specific binding of anti-human antibodies) and coated wells without patients' serum. Wells were then drained and washed with PBS–Tween three times and developed with 100 µl of horseradish peroxidase-conjugated rabbit anti-human IgG (Dako Cytomation, Copenhagen, Denmark) diluted at 1:10,000 with PBS–Tween. After incubation in room temperature, the plates were washed three times with PBS–Tween. One hundred microliters of substrate solution (20 mg of *ortho*-phenyldiamine–HCl, hydrogen peroxide) (Sigma-Aldrich, St. Louis, MO, USA) was added to each well. After 30 min of incubation at room temperature in darkness, the reaction was stopped by addition of citric acid. Individual well were quantitated using an ELISA reader (Benchmark, Bio-Rad, Hercules, CA, USA) at 450 nm.

We registered all allergic reaction during L-asparaginase treatment and classified them according to the WHO-scale of allergy degree. No allergic reaction in

the induction phase was noted. All children received L-asparaginase at a dose of 5,000 IU/m². The mean L-asparaginase activity was 273 UI/L (SD 157.5). On the last day of the induction, no anti-asparaginase antibodies in the IgG class were found.

In the reinduction phase, a dose of 10,000 IU/m² L-asparaginase was used. The mean L-asparaginase activity was 425 IU/L (SD 297). In this phase of treatment, eight children did not develop the anti-asparaginase antibodies or presented any clinical signs of allergy during the treatment. The mean L-asparaginase activity in these children was 467 IU/L (SD 293).

In five children, anti-asparaginase antibodies were detected and their titer ranged from 1/200 to 1/6,400 (Table I). Only two of these patients did not show clinical signs of allergy and had L-asparaginase activity above then 100 IU/L. In three children with anti-asparaginase antibodies detected, the enzyme activity in serum was lower than the limit of detection. Two of these children showed generalized signs of allergy (dyspnea, skin rash, abdominal and muscle pain), after the third and fourth dose of L-asparaginase, respectively. In one of them the unexpected low L-asparaginase activity preceded the clinical manifestation of allergy.

DISCUSSION

L-asparaginase is not expressed in humans. Commercial production uses several bacterial strains such as *Escherichia coli* and *Erwinia chrysanthemi* [2]. Allergy against L-asparaginase and development of anti-asparaginase antibodies are significant side effects. Woo et al. [7] demonstrated that hypersensitivity to L-asparaginase or the development of antibodies to L-asparaginase may not impact the outcome of childhood ALL. Other data suggest that the development of anti-asparaginase antibodies and/

TABLE I. Asparaginase Activity, Anti-Asparaginase Antibodies, and Presence of Allergy in Analyzed Children, During the Second Exposure on the Drug

Patient	Mean L-asparaginase activity (IU/L)	Presence of anti-asparaginase antibodies	Clinical sign of allergy
1	987	Not detected	No allergy
2	547	1/800	No allergy
3	554	Not detected	No allergy
4	205	Not detected	No allergy
5	404	Not detected	No allergy
6	739	Not detected	No allergy
7	218	Not detected	No allergy
8	579	Not detected	No allergy
9	<20	1/6,400	Urticaria, back pain, hypotonia, WHO 4
10	423	Not detected	No allergy
11	121	1/200	No allergy
12	<20	1/800	Urticaria, dyspnoe, WHO 2
13	<20	1/1,600	Skin rash, abdominal pain, vomiting, WHO 2

or allergic reactions might influence the enzyme activity or its half-life [4]. Albertsen et al. [3] analyzed the results obtained in 39 children with newly diagnosed ALL who were treated with *Erwinia*-asparaginase, given intramuscularly or intravenously. In this study, in the induction phase of treatment, no anti-asparaginase antibodies were detected. During the second exposure, 1/8 patients treated intravenously and 3/11 patients treated intramuscularly developed anti-asparaginase antibodies. It is important to note that low enzyme activity was observed in the same patients who developed antibodies. However, low enzyme activity could be explained because of a very short half-life of *Erwinia*-asparaginase, which was used in those children [8]. None of those patients manifested any symptoms of allergy.

Our observations confirm that the development of anti-asparaginase antibodies could be responsible for the decrease of the L-asparaginase activity. We could confirm the data of Woo et al. [5], who have shown that the presence of anti-asparaginase antibodies correlated with occurrence of allergic reactions. However, they did not report if the presence of these antibodies influenced the enzyme's activity. We observed the presence of anti-asparaginase antibodies in 5/13 children on the last day of the second exposure to the drug. We found that children who developed anti-asparaginase antibodies had low asparaginase activity during treatment. Most data support the role of IgG antibodies development, especially the IgG₃ subtype, in allergic reactions to L-asparaginase. Specific IgM antibodies against L-asparaginase were also detected. Although the clinical course of allergy could

indicate the type 1 of hypersensitivity, IgE antibodies not to play an important role in allergy against L-asparaginase [2]. More data are needed to estimate the importance of the presence of anti-asparaginase antibodies in the childhood ALL.

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