

Antibody Formation During Intravenous and Intramuscular Therapy With Erwinia Asparaginase

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Background. Determination of the frequency of antibody formation during first and second exposure to Erwinia asparaginase after i.v. and i.m. administration. **Procedure.** Thirty-nine children with newly diagnosed acute lymphoblastic leukemia (ALL) were included in this prospective study. Antibodies were determined (ELISA method) in plasma from these patients on specific days during and after therapy with 30,000 IU/m² i.v. or i.m. every day for ten days during the induction phase (first exposure). For 19 children, antibodies were measured in plasma during and after the re-induction phase (second exposure) following treatment with 30,000 IU/m² i.v. or i.m. twice a week for two weeks (Mondays and Thursdays). On the same days of therapy, enzyme activity (spectrophotometric method)

and the concentration of asparagine (HPLC) was determined. **Results.** During the first exposure, none of the patients developed anti-Erwinia asparaginase antibodies. During the second exposure, one patient (1 of 8 patients) treated intravenously developed antibodies, which were associated with disappearance of enzyme activity and reappearance of asparagine. Three of eleven patients developed antibodies of pharmacokinetic importance after i.m. therapy. None of the children had any clinical symptoms of hypersensitivity. **Conclusions.** The formation of antibodies and subsequently altered pharmacokinetics of Erwinia asparaginase seemed to be of importance only during a second period of asparaginase therapy. Med Pediatr Oncol 2002;38:310–316. © 2002 Wiley-Liss, Inc.

Key words: childhood acute lymphoblastic leukemia; Erwinia asparaginase; asparaginase antibodies; asparaginase pharmacokinetics

INTRODUCTION

Asparaginase is a well-established part of therapy of acute lymphoblastic leukemia (ALL) and lymphomas. The enzyme hydrolyzes the non essential amino acid, asparagine, into aspartic acid and ammonia extracellularly. Some leukemic cells are restricted in upregulation of their asparagine synthetase [1], and therefore, they rely on exogenous supplies. During asparaginase therapy, the systemic circulation is depleted of asparagine, resulting in inhibition of protein synthesis in the leukemic cells, which leads to cell death. The pharmacological end-point of therapy is asparagine depletion in plasma. Riccardi et al. [2] were the first to show that enzyme activities above 100 IU/L ensure complete asparagine depletion from the body fluid compartments.

Asparaginase preparations are derived from bacterial sources (*E. coli* and *Erwinia chrysanthemi*), and therefore, they are immunogenic. The primary limitation to the use of asparaginase is clinical hypersensitivity.

Allergic reactions and the development of antibodies are the side effects of greatest importance in the treatment with asparaginase. Hypersensitivity reactions in all the various manifestations are reported to occur at rates between 20 and 35% [3–7]. Hypersensitivity reactions

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can occur from any dose of asparaginase, including the first one, but they are more common the greater the number of previous doses administered. These reactions usually necessitate discontinuation of asparaginase therapy, and maybe, subsequent substitution with a different preparation.

The association between hypersensitivity reactions, anti-asparaginase antibodies, and altered pharmacokinetics has been reported from many studies about *E. coli* preparations [3,8–11]. The presence of antibodies does not necessarily result in an allergic reaction, but is correlated with rapid asparaginase clearance, a term called “silent antibodies” [11], which represents a mechanism of drug resistance.

An *E. coli*-derived preparation is part of most treatment protocols worldwide, and Erwinia asparaginase or PEG-asparaginase (polyethylene glycol conjugated *E. coli* enzyme) are used in case of allergic reactions. These last two mentioned preparations are regarded to be less immunogenic compared to the *E. coli* preparations.

The mode of administration may also be of importance for the risk of occurrence of allergic reactions. Rizzari et al. [12] reported that systemic allergic reactions occurred with higher frequency after a second exposure if Erwinia asparaginase was given i.v. compared to i.m. (7/12 vs. 2/145). A higher incidence of hypersensitivity reactions after i.v. administration was also reported by Evans et al. [7].

Administration of a reduced total dose of asparaginase or the presence of neutralizing antibodies result in insufficient treatment, and may leave these patients with a worse prognosis.

In the NOPHO-protocol (the Nordic Society for Pediatric Haematology and Oncology) Erwinia asparaginase has been part of the treatment of ALL since 1992, and has been administered in the same dose i.v. and i.m. both during the induction and the re-induction phases. In the following is a presentation of the first data on the frequency of antibody formation against Erwinia asparaginase during induction therapy and after administration a second time during re-induction therapy, correlated to plasma enzyme activities and asparagine depletion.

MATERIALS AND METHODS

From March 1997 to October 1999, 39 children with newly diagnosed ALL were included in our study (mean age 5.8 years (range 1.2–14.1 years); gender: 18 girls and 21 boys). During this period, all patients with newly diagnosed ALL were eligible to enter the study. Three centers participated in the study. In two centers, i.m. administration of asparaginase was used and in the third, i.v. administration was used. They all received conventional asparaginase therapy according to the NOPHO-92 ALL-protocol (protocol initiated in 1992).

Based on clinical characteristics at diagnosis, patients were divided into treatment groups. Patients who received normal intensive therapy were treated with asparaginase once during the induction phase, and the rest of the patients received asparaginase a second time during the re-induction phase.

Plasma samples were analyzed from all 39 patients during induction therapy. Twelve patients received i.v. therapy. Among the 27 patients in the i.m. group, were three patients who had their first dose i.v. and a fourth patient who had the first four doses i.v. The change of mode of administration for all four patients was due to gastrointestinal symptoms. These four patients were included in the i.m. group anyway as they received the majority of doses intramuscularly.

Nineteen of the 39 patients (8 in the i.v. group and 11 in the i.m. group) also received re-induction therapy.

All patients received Erwinia asparaginase (Erwinase; Ipsen-Speywood Pharmaceuticals Ltd, Maidenhead, UK) 30,000 IU/m² intravenously or intramuscularly every day for 10 days during the induction phase from Day 37 until Day 46 (in this report Day 0–9). During the first 36 days, they all received i.v. vincristine and doxorubicine, oral prednisolone, and intrathecal methotrexate. During asparaginase treatment, prednisolone was tapered from 60 mg/m²/day to 0.

All patients, but the patients who received normal intensive therapy were treated with Erwinia asparaginase 30,000 IU/m² i.v. or i.m. twice a week (Mondays and Thursdays) for two weeks during the re-induction phase (protocol Day 169, 172, 176, and 179 (intermediate risk-group) or 232, 235, 239, and 242 (high risk-group); in this report Day 0, 3, 7, and 10). During this period, they also received oral dexamethasone (10 mg/m²/day), i.v. vincristine and daunorubicin (Day 169 or 232 and 176 or 239) and intrathecal methotrexate on Day 169 (232).

Sample Collection

Blood samples were drawn into heparinized glass tubes and centrifuged at 4°C. Plasma samples were as soon as possible stored at –70°C until the time of analysis. Samples from other centers were sent on dry ice. When samples are kept on ice, the activity of the enzyme is zero.

During the *induction phase*, blood samples were withdrawn on Day 0, 8, and 27 (18 days after the last asparaginase dose) for determination of anti-Erwinia asparaginase antibodies. Enzyme activities were measured on Day 0, 4, and 8. After i.v. administration, enzyme activity was determined before infusion, at infusion stop (infusion over 3 hr), 5 and 24 hr after the beginning of infusion. After i.m. administration, enzyme activity was determined before application, ½, 2, and 24 hr after administration.

During the *re-induction phase*, blood samples were withdrawn on Day 0, 10, and 21 (11 days after the last asparaginase dose) for determination of anti-Erwinia asparaginase antibodies. For two patients, it was also possible to evaluate the level of antibodies 18 weeks after re-induction therapy. Enzyme activity was measured on Day 0, 10, and 21 during re-induction therapy. After i.v. administration, enzyme activity was measured before application, at infusion stop (infusion over 3 hr) and 5 hr after the beginning of infusion on Day 0 and 10. After i.m. administration enzyme activity was determined before, ½ and 2 hr after administration on Day 0 and 10.

Determination of Anti-Erwinia Asparaginase Antibodies

Anti-asparaginase antibodies (IgG type) were detected using a semiquantitative ELISA method with horseradish peroxidase conjugated anti-human immunoglobulin [9].

The intraday CV value was 7%, evaluated by measuring one standard concentration (25 U/ml) 32 times on one day. The interday CV value was 10%, evaluated by measuring a pool of a known concentration (25 U/ml) in triplicate 10 times over a period of 1 month. The interday CV at 3,125 U/ml (minimum detectable concentration) was 24%.

Samples with O.D.'s above that corresponding to that of 3,125 U/ml were considered to be positive for antibodies.

Asparaginase Activity

The concentrations of asparaginase were measured using a spectrophotometric method, determining enzyme activity by the use of Nessler's reagent [13]. The intraday CV value was estimated by measuring one standard concentration (400 IU/L) 35 times on one day and was 2.7%. The interday CV value was evaluated from triplicates (analysis of the same sample 20 times over 2 months) and was found to be 5.6%. The interday CV at 100 IU/L (minimum detectable concentration) was 29.4%.

Trough levels were defined as the asparaginase activities determined immediately before the next administration of Erwinia asparaginase.

Amino Acid Analysis

The amino acid analysis was performed by HPLC [14] with a limit of quantification for asparagine of 0.2 µM. Only samples with enzyme activities ≤ 100 IU/L were tested for asparagine, as enzyme activities above 100 IU/L have been shown to secure complete asparagine depletion. These measurements were performed at the laboratory at the Department of Pediatric Haematology-Oncology, University of Münster, Germany.

The depletion of asparagine was graded according to Boos et al. [14] with some alterations: complete depletion, ≤ 0.2 µM; nearly complete depletion, > 0.2–≤ 0.5 µM; moderate reduction, > 0.5–≤ 1 µM; slight reduction, > 1–≤ 25 µM; no reduction, > 25 µM; asparagine, normal range: 40–80 µM.

Statistics

Comparison of frequencies of patients who developed antibodies of pharmacokinetic importance (disappearance of plasma enzyme activity (activity below 100 IU/L) and reappearance of asparagine (above 0.2 µM) in plasma) after i.v. and i.m. administration after a second exposure to Erwinia asparaginase was performed by Fisher's exact test.

RESULTS

Induction Therapy

One patient in the i.m. group had measurable antibodies before therapy; 4.0 U/ml on Day 0, and 6.5 U/ml on Day 8. One patient in the i.v. group also had measurable antibodies from the start; 11.9 U/ml on Day 0, 9.2 U/ml on Day 8, and 32.8 U/ml on Day 27. These two patients were naive to asparaginase. The antibodies did not have any influence on enzyme activity as all enzyme activities during therapy were above 100 IU/L, and may be due to lack of sufficient specificity of the method. These two patients did not receive re-induction therapy.

The remaining 37 patients had antibody levels below the detection limit of the method. Nineteen (5 in the i.v. group and 14 in the i.m. group) of the 39 patients had a blood sample withdrawn on Day 27 for antibody determination, and thus had three antibody determinations. The remaining 20 patients had antibodies measured twice during induction therapy.

None of the patients had antibodies of pharmacokinetic importance (the presence of antibodies and the disappearance of enzyme activity) during the first exposure to Erwinia asparaginase.

Re-Induction Therapy

Anti-Erwinia asparaginase antibodies, enzyme activities, and corresponding serum asparagine for enzyme activities below 100 IU/L for all patients during re-induction therapy on protocol Day 0, 10, and 21 after i.v. and i.m. administration are listed in Tables I and II.

Trough enzyme activities below 100 IU/L 3 or 4 days after the last dose (administered twice a week for 2 weeks) correspond well to enzyme activities calculated from the pharmacokinetic parameters of Erwinia asparaginase (half-life ($T_{1/2}$) 6.4 hr (range 3.7–9.3), absorption rate constant (k_a) 0.81 Days⁻¹ (range 0.63–0.99), bioavailability after i.m. administration (F) 27% (range 11–61%))

TABLE I. Anti-Erwinia Asparaginase Antibodies, Enzyme Activities, and Corresponding Plasma Asparagine for Enzyme Activities Below 100 IU/L for all Patients During Re-Induction Therapy After i.v. Administration

Patient	Day	Antibodies (U/ml)	Enzyme activity (IU/L)	Asparagine (μ M) ^a
1	10	< MDC	4446 ^b /16545 ^c /1677 ^d	
1	21	< MDC	< 100	≤ 0.2
2	0	< MDC	< 100 ^b	
2	10	< MDC	< 100 ^b /13318 ^c	≤ 0.2
2	21	< MDC	< 100	≤ 0.2
3	0	< MDC	< 100 ^b /28018 ^c	
3	10	< MDC	< 100 ^b /20728 ^c	2.12
3	21	< MDC	< 100	> 25
4	10	< MDC	< 100 ^b /13181 ^c	≤ 0.2
4	21	< MDC	< 100	≤ 0.2
5 ^e	0	3.6	< 100 ^b /22583 ^c	
5 ^e	10	846.4	< 100 ^b / < 100 ^c	> 25
6	0	< MDC	< 100 ^b /27826 ^c	
6	10	< MDC	< 100 ^b	> 25
6	21	< MDC	< 100	> 25
7	0	4.0	< 100 ^b /29075 ^c	
7	10	3.5	< 100 ^b /49719 ^c /22075 ^d	≤ 0.2
7	21	3.5	< 100	1.6
8	10	20.4	381 ^b /23980 ^c	
8	21	18.6	< 100	≤ 0.2

^aAsparagine not determined on Day 0.

^bBefore therapy (Day 0)/trough enzyme activity (Day 10).

^cAt infusion stop (infusion over 3 hr).

^dFive hours after the beginning of infusion.

^eAll does during induction therapy and the two first doses during re-induction therapy were given i.v., and the last two doses during re-induction therapy were given i.m. Change of mode of administration was due to gastrointestinal symptoms.

<MDC: below minimum detectable concentration.

[13]. For the patients with measurable antibodies, the detection of enzyme activities below 100 IU/L shortly after administration (i.v. group; at infusion stop and 5 hr after the beginning of infusion, and i.m. group; ½ and 2 hr after administration) must be caused by neutralizing antibodies.

Among the i.v. treated patients, one patient (no. 5) had a very high increase in the concentration of antibodies during re-induction therapy, resulting in disappearance of enzyme activity and reappearance of asparagine on Day 10. Unfortunately, no blood sample was withdrawn on Day 21. Patient no. 7 and 8 had measurable (above the limit of detection) concentrations of antibodies, but these antibodies were not neutralizing (enzyme activities above 100 IU/L).

Among the patients receiving i.m. therapy (Table II), three patients (no. 12, 13, and 18) had an increase in the concentration of antibodies during re-induction treatment combined with enzyme activities below 100 IU/L, and reappearance of asparagine. Patient no. 10, 11, and 19 had measurable concentration of antibodies, which seemed not to inactivate or block the function of the enzyme, as some enzyme activities above 100 IU/L were found. Thus, 3 out of 11 patients from the i.m. group had antibodies, which resulted in insufficient asparagine deple-

tion. For all three patients, there was *no* enzyme activity in *any* of the three samples measured on Day 10. Two of these three patients (patient no. 12 and 13) also had antibodies measured on Day 21, and the antibodies were still elevated at this time point. Patient no. 14 had an antibody concentration of 4.3 U/ml on Day 21 (11 days after the last dose) and no antibodies were found in the previous samples.

The incidences of formation of neutralizing antibodies after i.v. and i.m. administration was not statistically significant (P -value = 0.60).

Four patients developed antibodies of importance, and two of them had no measurable antibodies on Day 0 (before the first dose during re-induction). The antibodies were detected on Day 10. Two patients (no. 5 and 12) had measurable antibodies from Day 0 during re-induction. During induction therapy, these patients had no antibodies.

The tendency to develop antibodies could not be correlated with high enzyme activities. Patient no. 12 and 18 had antibodies determined 18 weeks after re-induction therapy with *Erwinia* asparaginase. Patient no. 12 still had measurable antibodies (23.3 U/ml), whereas patient no. 18 did not.

None of the four patients, who developed antibodies had any clinical symptoms of hypersensitivity. Among

TABLE II. Anti-Erwinia Asparaginase Antibodies, Enzyme Activities, and Corresponding Plasma Asparagine for Enzyme Activities Below 100 IU/L for All Patients During Re-Induction Therapy After i.m. Administration

Patient	Day	Antibodies (U/ml)	Enzyme activity (IU/L)	Asparagine (μ M) ^a
9	0	< MDC	< 100 ^b / $\leq$ 100 ^c /1174 ^d	
9	10	< MDC	< 100 ^b /673 ^c /1928 ^d	$\leq$ 0.2
10 ^e	0	9.2	< 100 ^b / $\leq$ 100 ^c /1022 ^d	
10 ^e	10	7.6	< 100 ^b /253 ^c /2236 ^d	$\leq$ 0.2
11	0	3.4	< 100 ^b / $\leq$ 100 ^c / $\leq$ 100 ^d	
11	10	3.4	< 100 ^b / $\leq$ 100 ^c /230 ^d	$\leq$ 0.2
12	0	11.6	< 100 ^b / $\leq$ 100 ^c /100 ^d	
12	10	48.4	< 100 ^b / $\leq$ 100 ^c / $\leq$ 100 ^d	> 25
12	21	62.8	< 100	> 25
12	Week 18 ^f	23.3	—	
13	0	< MDC	< 100 ^b / $\leq$ 100 ^c /292 ^d	
13	10	72.9	< 100 ^b / $\leq$ 100 ^c / $\leq$ 100 ^d	0.21
13	21	16.2	< 100	> 25
14	0	< MDC	< 100 ^b /1751 ^d	
14	10	< MDC	< 100 ^b /2794 ^c /8452 ^d	$\leq$ 0.2
14	21	4.3	< 100	> 25
15	0	< MDC	< 100 ^b /125 ^c /1232 ^d	
15	10	< MDC	< 100 ^b / $\leq$ 100 ^c /285 ^d	$\leq$ 0.2
15	21	< MDC	< 100	> 25
16	0	< MDC	< 100 ^b / $\leq$ 100 ^c /262 ^d	
16	10	< MDC	< 100 ^b /410 ^c /1869 ^d	0.5
16	21	< MDC	< 100	1.6
17	0	< MDC	< 100 ^b / $\leq$ 100 ^c /274 ^d	
17	10	< MDC	< 100 ^b / $\leq$ 100 ^c /305 ^d	$\leq$ 0.2
17	21	< MDC	< 100	2.2
18	0	< MDC	< 100 ^b /744 ^d	
18	10	48.4	< 100 ^b / $\leq$ 100 ^c / $\leq$ 100 ^d	> 25
18	Week 18 ^f	< MDC	—	
19 ^g	0	6.9	< 100 ^b	
19 ^g	10	5.3	134 ^b	$\leq$ 0.2

^aAsparagine not determined on Day 0.^bBefore therapy (Day 0)/trough enzyme activity (Day 10).^cThirty minutes (1/2 hr) after administration.^dTwo hours after administration.^eNo blood samples during induction therapy.^fEighteen weeks after the last asparaginase dose.^gFirst dose during induction therapy was given by the i.v. route.

< MDC: below minimum detectable concentration.

the other patients, there were no symptoms of allergic reaction either.

DISCUSSION

During asparaginase therapy, the development of antibodies is a side effect of major concern. Two studies [7,15] have reported that no allergic reactions were observed until the second exposure to asparaginase, but in one of these studies [7], plasma levels of antibodies were not determined, so the frequency of silent antibodies was not evaluated. Woo et al. [15] found that post-induction anti-*E. coli* asparaginase antibody levels (Day 29 of treatment) were higher in reacting than in non-reacting patients. In another study, Woo et al. [16] reported that 6 out of 154 patients had an allergic reaction

during induction therapy with *E. coli* asparaginase, of whom 4 had a high antibody titer on Day 29. In the present study, none of the 39 patients developed antibodies during the induction phase. The patients who developed a high antibody titer during the re-induction phase had post-induction antibody level (Day 27 of treatment) below the limit of detection like the rest of the patients. Day 27, the day of determination of antibodies, is almost the same as in the Woo et al. study [15], so this does not explain the difference. The patients in the Woo et al. study [15] received a different asparaginase preparation (*E. coli*) in a different schedule (three times weekly i.m. for a total of nine doses over 3 weeks) compared to the present study.

In our study, two patients had antibodies at study entry, although these children were naive to asparaginase. This is a known phenomenon (Avramis, personal

communication), and the antibodies may or may not be of clinical importance.

In our study, during re-induction therapy, 1 of 8 patients in the i.v. group and 3 of 11 patients in the i.m. group developed high titer antibodies, which resulted in disappearance of enzyme activity in all blood samples analyzed on the same day as the high antibody concentration was detected. The patients did not have any clinical symptoms of hypersensitivity. This phenomenon is termed as "silent antibodies" or "silent hypersensitivity" [11]. In the studies done by Woo et al. [15,16], plasma enzyme activity and the concentration of asparagine were not determined, so the extent of neutralizing antibodies was not evaluated.

The low trough plasma enzyme activities found during the re-induction phase in the present study can be explained by the pharmacokinetics of *Erwinia* asparaginase [13]. For four patients with measurable antibodies (patient no. 5, 12, 13, and 18), there was *no* measurable enzyme activity in *any* of the blood samples at the time of high titer antibodies. The absence of enzyme activity is caused by neutralizing antibodies.

The conclusion of our study that the incidence of neutralizing antibodies is independent of the route of administration is to some extent hampered by the relatively low number of investigated patients.

The increased antibody concentration was present on Day 0 for 2 out of 4 children during re-induction therapy, whereas the increase for the remaining 2 children appeared later after one or more asparaginase doses, so antibody titer determination at the beginning of the re-induction phase could not be used as a method for screening patients who might not benefit from *Erwinia* asparaginase re-induction therapy.

In our study, it was not possible to conclude, whether the incidence of high titer antibodies had any prognostic importance. Woo et al. [16] reported no difference in EFS (event-free survival) among patients who did and did not have antibodies or did and did not develop allergic reactions. Larson et al. [17] found that asparaginase allergy was not prognostic among adults with ALL. Neither the concentration of antibodies, enzyme nor asparagine were measured in this last study. Asselin et al. [11] found that patients with low antibody titers cleared asparaginase slowly and had a significantly higher response rate, and that the absence of clinical hypersensitivity did not necessarily correlate with low antibody titers, which was also found in the present study.

In the setting of the NOPHO-92 protocol, the formation of antibodies and subsequently altered pharmacokinetics of asparaginase seemed to be of importance only for the patients who were exposed to asparaginase a second time.

Drug monitoring during asparaginase therapy remains extremely important in order to secure the patients the

optimal treatment. Evaluation of enzyme activities and antibodies together may reveal whether low plasma enzyme activities are due to insufficient dosing of the drug or due to development of neutralizing antibodies. The results of drug monitoring as described above may also give us the information we need to conclude which way of administration minimizes the risk of developing allergic reactions as well as neutralizing antibodies.

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