

Pharmacokinetics of native *Escherichia coli* asparaginase (Asparaginase medac) and hypersensitivity reactions in ALL-BFM 95 reinduction treatment

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Summary. Repeated asparaginase treatment has been associated with hypersensitivity reactions against the bacterial macromolecule in a considerable number of patients. Immunological reactions may range from anaphylaxis without impairment of serum asparaginase activity to a very fast decline in enzyme activity without any clinical symptoms. Previous investigations on a limited number of patients have shown high interindividual variability of asparaginase activity time courses and hypersensitivity reactions in about 30% of patients during reinduction treatment. Therefore, monitoring of reinduction treatment was performed prospectively in 76 children with newly diagnosed acute lymphoblastic leukaemia (ALL). According to the ALL-Berlin–Frankfurt–Münster (BFM) 95 protocol, 10 000 U/m² body surface area of native *Escherichia coli*

asparaginase (Asparaginase medac) was given on d 8, 11, 15 and 18. In 45/76 children, trough and peak activities were determined with every dose, and also on d 4 and d 11 after the last administration. Data on asparaginase activity were not available from the remaining 31 patients, but information with regard to hypersensitivity reactions only was given. Eighteen out of 76 patients (24%) suffered a clinical hypersensitivity reaction; however, no silent inactivation was observed. Activity in the therapeutic range of greater than 100 U/l for at least 14 d was determined in 43 of the 45 patients who were analysed for enzyme activity.

Keywords: *E. coli* asparaginase, pharmacokinetics, drug monitoring, reinduction, acute lymphoblastic leukaemia.

The efficacy of L-asparaginase in induction and intensification treatment of acute lymphoblastic leukaemia (ALL) and some non-Hodgkin lymphomas (NHL) has been demonstrated in a number of studies, including controlled clinical trials (Jones *et al.*, 1977; Sallan *et al.*, 1983; Desai *et al.*, 1997). Thus, asparaginase has been included in at least the induction therapy in nearly all paediatric and adult ALL treatment protocols. As asparaginase causes depletion of the

asparagine blood pool, blast cells sensitive to treatment with asparaginase are restricted in their protein biosynthesis and nucleotide (DNA and RNA) synthesis, leading to apoptosis and subsequent cell death (Sobin & Kidd, 1965; Becker & Broome, 1967, 1969; Cooney *et al.*, 1970).

In addition to blasts, normal cells in a variety of organ systems with high metabolic turnover are also restricted in their protein synthesis, entailing impairment of liver and pancreatic function, and restriction of coagulation factor synthesis (Durden *et al.*, 1983; Gugliotta *et al.*, 1991; Cheung *et al.*, 1994; Nowak-Göttl *et al.*, 1996). Additionally, immunological reactions against the bacterial macromolecule are of great concern (Killander *et al.*, 1976; Billett *et al.*,

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1992) and hypersensitivity reactions, ranging from anaphylaxis with regular activity time courses to fast decline in serum asparaginase activity without any clinical sign of hypersensitivity, can be observed (Fabry *et al.*, 1985; Kurtzberg *et al.*, 1993).

Hypersensitivity reactions have been seen particularly with re-exposure to the same drug after a period of therapy without asparaginase, as is the case during reinduction treatment (Dellinger & Miale, 1976; Boos *et al.*, 1996). We have performed investigations with different drugs in induction and reinduction treatment in the past. Owing to limited cross immunogenicity, allergic reactions should be less prevalent. While in the regular ALL/NHL-BFM (Berlin–Frankfurt–Münster) protocols 10 000 U/m² body surface area (BSA) of the native *Escherichia coli* enzyme was given twice weekly for 2 weeks, this treatment was changed to *Erwinia* asparaginase and the polyethylene glycol (PEG)-modified *E. coli* enzyme in two different drug monitoring programmes (Vieira Pinheiro *et al.*, 1999; Müller *et al.*, 2000). With both of these treatment modalities a marked decrease in allergic reactions was seen. Using *Erwinia* asparaginase, however, a considerable number of patients had activities outside the therapeutic range after 2 d, whereas PEG asparaginase (Oncaspar) resulted in a very fast decline of activity in one third of the patients. Because of these observations, we initiated a drug monitoring programme of *E. coli* asparaginase reinduction treatment in a limited number of patients, who were treated with the same preparation previously used during induction.

PATIENTS AND METHODS

Between December 1998 and February 2000, 76 children (47 male; 29 female) with newly diagnosed ALL from eight centres of the ALL-BFM study group (Aarau, Berlin-Buch, Gießen, Hannover, München, Stuttgart, Tübingen, Ulm) were enrolled in a drug monitoring programme. The age range was 1¹¹/₁₂–18⁴/₁₂ years (mean 6⁷/₁₂ years, median

4⁹/₁₂ years). All children were started on reinduction treatment according to the ALL-BFM 95 protocol with native *E. coli* asparaginase (Asparaginase medac). Plasma samples for determination of *E. coli* asparaginase activity were available from 45 patients, whereas in a further 31 patients only information with regard to hypersensitivity reactions was obtained.

Reinduction treatment. On d 8, 11, 15 and 18 of the treatment plan (ALL-BFM 95), 10 000 U/m² BSA native *E. coli* asparaginase (Asparaginase medac) were given as a 2-h intravenous infusion. The reinduction chemotherapy further included daily dexamethasone (DEXA 10 mg/m² BSA, d 1–31) as well as vincristine (VCR 1.5 mg/m² BSA) and doxorubicin (ADR 30 mg/m² BSA) on d 8, 15, 22 and 29 (Reiter *et al.*, 1994; Schrappe *et al.*, 1994).

Sample collection. For laboratory analysis, blood samples were to be collected before the start of each drug administration after the infusion had been finished (trough and peak activities) and after the last administration on d 22 and 29 of the protocol. Immediately after collection, the samples were centrifuged and the serum was analysed without prior freezing (unchanged stability at room temperature was demonstrated for up to 7 d, unpublished observations).

Determination of asparaginase activity. Asparaginase activity was measured photometrically at 450 nm in a standardized asparagine-rich buffer system assaying the ammonia released after reaction with Nessler's reagent. The assay had a limit of quantification of 20 U/l. Details of the asparaginase assay have been published previously (Boos *et al.*, 1996; Werber *et al.*, 1997). For calculation and graphic presentation of the data, activities below the limit of quantification were set at zero. In Figure 1 only, the semilogarithmic scale of the y-axis meant that these limits were set at 10 U/l.

Samples for determination of trough and peak activities. Owing to clinical reasons, asparaginase administrations were shifted to days earlier or later than predicted in the treatment plan in a number of patients. Thus, data of samples taken on

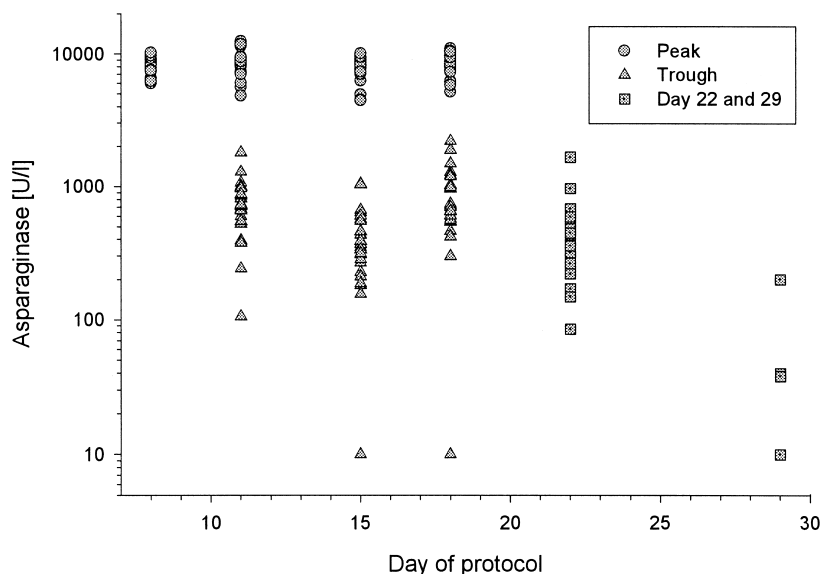


Fig 1. Asparaginase serum peak and trough activities of patients treated at the exact time points defined by the ALL-BFM protocol. Activities under the limit of quantification were set to 10 U/l.

Table I. Number of patients with hypersensitivity reactions.

Patient data	Total	Allergic reaction
Drug monitoring	47	3/47
Clinical information only	29	15/29
Total	76	18/76

d 10–12, 14–16 and 17–19 were used for calculation of trough and peak activities. In each case, however, the time intervals between the four administrations were in strict accordance with the protocol (3 d between the first and the second, and the third and the fourth administration; and 4 d between the second and the third administration, as defined by the BFM protocol). For presentation of asparaginase activities measured in samples from protocol d 22 and 29, only samples from patients who were treated in strict accordance with the protocol were used (Fig 1).

RESULTS

Hypersensitivity reactions during reinduction treatment with asparaginase

Data concerning hypersensitivity reactions were available from all 76 patients and serum samples for determination of *E. coli* asparaginase activity were taken from 45 of the 76 patients. Samples were sent after changing treatment to another drug because of hypersensitivity reactions in an additional 2/76 patients. Therefore, samples for drug monitoring were available from 47 patients in total.

In the group of 47 patients with drug monitoring data, three patients had an allergic reaction. Because two of these three patients showed an allergic reaction immediately after starting the first asparaginase infusion on d 8, samples for drug monitoring were available only after the treatment was shifted to another drug. In one patient, ErwinaseTM was used as an alternative drug at a dose of 20 000 U/m² on an alternate day basis (Monday, Wednesdays and Fridays) for eight administrations with trough and peak activities in the expected range (> 100 U/l after 2 d and about 50 U/l after 3 d), whereas, in the other patient, treatment was changed

to OncasparTM 1000 U/m². Owing to a shortened activity time course after OncasparTM, this patient was then treated further with ErwinaseTM 20 000 U/m². As a hypersensitivity reaction occurred during the first ErwinaseTM administration in this patient, asparaginase treatment was discontinued. In the third patient, an allergic reaction was seen during the first administration of Asparaginase medac, but the trough activity after 3 d was in the therapeutic target range greater than 100 U/l. Because of the clinical reaction, however, therapy was changed to ErwinaseTM. No further information about this patient was available. Thus, serum samples for determination of *E. coli* asparaginase activity were available from 45 patients.

No samples for determination of asparaginase activity were sent from the remaining 29 patients, but information about the clinical course was analysed. In 15/29 of these patients, an allergic reaction was seen during treatment with Asparaginase medac for reinduction. Because treatment was continued with another drug, no samples for *E. coli* asparaginase measurement were sent. Overall, hypersensitivity reactions were observed in 18/76 (24%) of the patients (Table I).

Asparaginase medac serum activity

From the 45 patients with drug monitoring data after administration of Asparaginase medac, samples for the determination of peak activity were available from 38, 39, 35 and 39 of the children after the first, second, third and fourth administration respectively. At these time points, the mean peak activities were 7613 U/l, 8369 U/l, 7850 U/l and 7512 U/l. The mean trough activities after the second, third and fourth administration were 664 U/l, 446 U/l and 704 U/l respectively. In the 27 samples available from d 22, mean asparaginase activity was 439 U/l, whereas on d 29, mean activity in 20 samples was 18 U/l. Details are shown in Table II.

Toxicity

Details of toxicity other than hypersensitivity were not collected for this investigation.

DISCUSSION

Repeated treatment with asparaginase is limited by clinically

Table II. Plasma asparaginase activity in patients with samples for drug monitoring of *E. coli* asparaginase.

Pharmacokinetic target/ time after administration	Mean (SD) (U/l)	Median (range) (U/l)	Patients activity < 100 U/l	Samples/ Patients
Peak d 8	7613 (1491)	7861 (4,302–10 215)	0	38/45
Trough d 11 ± 1	664 (380)	655 (106–1806)	0	40/45
Peak d 11 ± 1	8369 (1694)	8506 (3,981–12 426)	0	39/45
Trough d 15 ± 1	446 (259)	370 (< 20–1050)	2	37/45
Peak d 15 ± 1	7850 (1378)	8050 (4,428–10 049)	0	35/45
Trough d 18 ± 1	704 (460)	607 (< 20–2198)	2	40/45
Peak d 18 ± 1	7512 (1639)	7882 (4,699–10 979)	0	39/45
d 22	439 (333)	401 (< 20–1661)	2	27/45
d 29	18 (46)	< 20 (< 20–200)	19	20/45

relevant hypersensitivity and immunologically based fast decline in serum activity (Dellinger & Miale, 1976; Asselin *et al*, 1993; Ahlke *et al*, 1997). As asparaginases from different bacterial strains and one modified by polyethylene glycol with only limited cross-immunogenicity are available, continuation of treatment is often possible by changing the drug (Ohnuma *et al*, 1972; Billett *et al*, 1992). Our own previous investigations on the pharmacokinetic profiles of different asparaginase preparations have shown that, for comparable treatment intensity, adjustment of schedule and dose is necessary if one drug is substituted by another (Boos *et al*, 1996). To routinely switch all patients to asparaginase from *Erwinia chrysanthemi* in reinduction, after induction treatment according to the ALL/NHL-BFM protocols with the native *E. coli* enzyme, to achieve acceptable treatment intensity it was necessary to administer 9 doses of 20 000 U/m² BSA of the *Erwinia* enzyme over 3 weeks on an alternate day regimen (Monday, Wednesday and Friday) instead of 4 doses of 10 000 U/m² BSA of *E. coli* asparaginase given over 2 weeks. During this investigation, only one clinical allergic reaction was seen in the 21 patients included in the drug monitoring programme (Vieira Pinheiro *et al*, 1999).

Because of the great number of doses required with the *Erwinia* enzyme, polyethylene glycol-conjugated *E. coli* asparaginase (PEG asparaginase) was used in reinduction in a limited number of patients, as the markedly prolonged half-life of this drug requires only one administration. Drug monitoring data on this preparation have shown that one group of patients had an activity time course as predicted, with serum activities of ≥ 100 U/l for at least 2 weeks, but the remaining one third of the patients showed a fast decline in asparaginase activity. No clinical sign of hypersensitivity reaction was seen in the patients treated with PEG-ASNase (Müller *et al*, 2000).

As no systematically documented information for regular reinduction treatment using the native *E. coli* asparaginase (Asparaginase medac) in induction and reinduction (according to the BFM protocols) was available, we initiated a drug monitoring programme. The current investigation was performed to obtain further information on the pharmacokinetics of native *E. coli* asparaginase when the enzyme is used repeatedly, and to evaluate the extent of hypersensitivity reactions entailed by this treatment. No systematic documentation of toxicity other than hypersensitivity reactions was intended. Although samples for measurement of *E. coli* asparaginase activity were sent from only 45 of the 76 patients included, information concerning allergic reactions were available from all patients. Because it was seen that about 30% of patients treated with native *E. coli* asparaginase in induction and again in reinduction suffered a clinical hypersensitivity reaction (Boos *et al*, 1996), the proportion of 24% (18/76 patients) affected in our study was in the expected range. In one girl with an allergic reaction during the first administration of *E. coli* asparaginase in reinduction, subsequent treatment with PEG-ASNase was associated with a rapid decline in activity, whereas a further shift to *Erwinia* asparaginase led to another clinical manifest allergic reaction which necessitated termination of asparaginase treatment.

Riccardi *et al* (1981) have shown that *E. coli* asparaginase activity in the range of about 100 U/l is sufficient for asparagine depletion in serum and cerebrospinal fluid (CSF). Our own data have confirmed these results (Boos *et al*, 1996). We thus assumed complete asparagine depletion in serum samples with activity in this range.

Three patients had trough activities lower than the limit of quantification (< 20 U/l) at some time point, but in all these patients trough activities reached the expected range

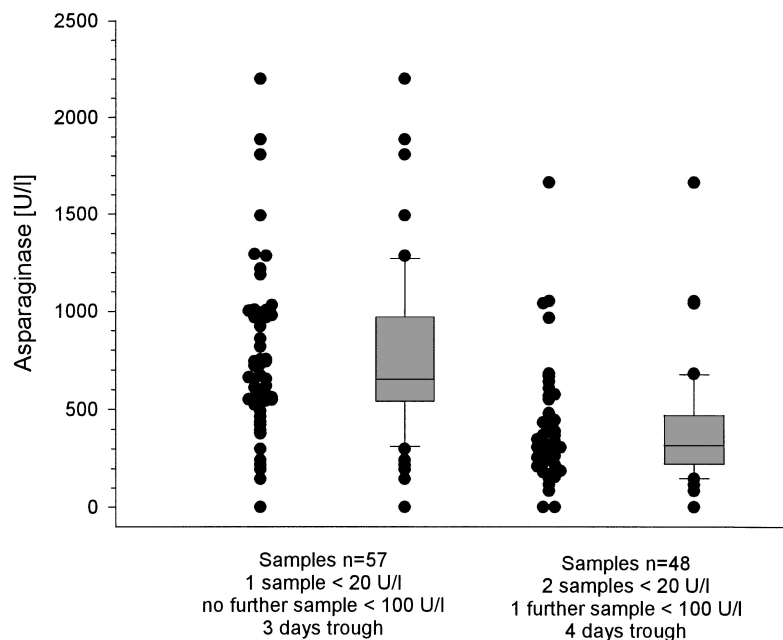


Fig 2. Trough activities 3 and 4 d after administration of *E. coli* asparaginase. The median, 10th, 25th, 75th and 90th percentiles are shown as vertical boxes with error bars.

of > 100 U/l again, after further doses. This phenomenon had not occurred in the past during routine drug monitoring in hundreds of samples and may be related to errors in sampling. Therefore, we identified no patient with so-called 'silent inactivation'. As asparaginase should be given at intervals of 3 and 4 d in the ALL/NHL-BFM protocols, trough activity data were separated according to the time elapsed from the last administration. After 3 d, in only one of the patients described above was an activity of < 20 U/l determined at a single time point. No further patient with activity less than 100 U/l was seen. Four days after the previous dose, two samples from two of the patients with temporary trough activities under the limit of quantification were identified with activities < 100 U/l in addition to one sample from another patient with asparaginase activity of 85 U/l. In all other patients, the activity was in the therapeutic range (Fig 2).

To determine the time interval with asparaginase activity > 100 U/l after the last administration, samples from d 22 and 29 of the protocol (4 and 11 d after last dose) were measured. On d 22, only one of the samples showed no activity and, in another, the activity was < 100 U/l. On d 29, only 5 out of 20 patients had quantifiable activity, including one with activity > 100 U/l.

These data show that, using 4 doses of 10 000 U/m² *E. coli* asparaginase, nearly all patients without a clinical hypersensitivity reaction who had samples available for drug monitoring reached activities in the therapeutic range for about 2 weeks. There were only three patients with trough activities < 100 U/l at some time point, but all of these had activities in the therapeutic range during the following administrations. In general, our monitoring data of all asparaginase preparations used in reinduction have shown that acceptable treatment intensity is attainable with all drugs. However, whereas with 10 000 U/m² of the historical *E. coli* drug Crasnitin, 5000 U/m² Asparaginase medac and 1000 U/m² Oncaspar 'silent inactivation' seems to be the predominant kind of hypersensitive reaction, necessitating strict drug monitoring, with Asparaginase medac 10 000 U/m² used in induction or in reinduction, only clinically manifest allergic reactions but no immunologically mediated fast decline in activity were seen (Boos et al, 1996; Ahlke et al, 1997; Müller et al, 2000). Therefore, for identification of 'silent inactivation', the former drugs and doses should be used in reinduction under drug monitoring only, whereas with repeated use of 10 000 U/m² Asparaginase medac one has to consider the risk of clinically relevant hypersensitivity reactions, including possible anaphylactic shock.

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