Cancer Chemother Pharmacol (2002) 49: 149–154
DOI 10.1007/s00280-001-0391-5

ORIGINAL ARTICLE

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PEG-asparaginase (Oncaspar) 2500 U/m^2 BSA in reinduction and relapse treatment in the ALL/NHL-BFM protocols

Received: 16 June 2001 / Accepted: 8 October 2001 / Published online: 16 November 2001
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Abstract *Purpose*: As previous data had shown that only two-thirds of patients had the predicted activity time courses when PEG-asparaginase 1000 U/m^2 was used in reinduction after native *E. coli* asparaginase in induction treatment of acute lymphoblastic leukaemia (ALL), drug monitoring was performed with the use of a higher dose. *Methods*: Because one-third of patients had insufficient serum asparaginase activity time courses after a single dose of 1000 U/m^2 PEG-asparaginase during reinduction treatment, a dose of 2500 U/m^2 PEG-asparaginase, which is the approved dosage in Germany, was used in 39 reinduction and 20 relapse patients to determine whether prolongation of the activity time course may be possible with this higher dose, and to look for significant differences between reinduction and relapse patients. *Results*: After 1, 2 and 3 weeks, the mean activities were 1113 ± 699 U/l, 231 ± 259 U/l, and 13 ± 35 U/l in the reinduction patients, and 1078 ± 649 U/l, 165 ± 195 U/l and 19 ± 28 U/l in the relapse patients, respectively. There were a considerable number of patients with a substantially shortened activity time course in both groups. In 10 of 39 reinduction patients and in 7 of 24 doses during relapse treatment, only activities < 100 U/l were found after 1 week with a further fast decline. No statistically significant differences between the two patient groups could be shown at any time-point. *Conclusions*: Comparison of these data with activities after 1000 U/m^2 PEG-asparaginase showed no prolongation of the time with activity in the therapeutic range with the higher dose. Therefore, for a longer duration of therapeutic activity, administration of further doses is mandatory.

Keywords PEG asparaginase · Pharmacokinetics · Reinduction · Relapse · Acute lymphoblastic leukaemia

This work was supported by the German Federal Department of Research and Technology (no. 01 EC 9401) and by Medac GmbH, Hamburg, Germany.

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Introduction

Since therapeutic activity of L-asparaginase in the treatment of malignant haematological diseases was observed in the middle of the last century, the enzyme has been an essential part of nearly all regimens for the therapy of acute lymphoblastic leukaemia (ALL) and of some non-Hodgkin's lymphomas (NHL) [18, 19]. Because asparaginase hydrolyses the non-essential amino acid asparagine into aspartic acid and ammonia,

therapeutic doses of the enzyme deplete the serum asparagine pool, which under physiological conditions is in the range of about 40 to 80 μmol/l. Upregulation of asparagine synthetase activity is thought to be restricted in blasts sensitive to asparaginase, leading to impairment of protein biosynthesis, DNA and RNA synthesis and finally to apoptosis and cell death [3, 4, 10, 13, 22].

Because asparaginases are isolated from microorganisms and an increase in immunogenicity can be observed with increasing taxonomic distance between organisms, and the molecular mass and complexity of the protein, immunological reactions are of great concern in the treatment of humans with asparaginase [6, 7]. The whole spectrum of hypersensitivity reactions may be seen from fast decline of enzyme activity without any clinical sign of immunological reaction to anaphylactic shock with a regular activity time course [1, 8, 9]. After reaction against one of the preparations, continuation of treatment is often possible by changing the drug [5, 14]. Investigations using intravenous administration have shown, however, that changing the drug from the *E. coli* to the *Erwinia* enzyme necessitates the administration of more than twice the dose for a comparable treatment intensity [20].

Therefore, investigations with PEG-asparaginase (Oncaspar) using a single dose of 1000 U/m^2 body surface area (BSA) were performed with drug monitoring in a limited number of patients during reinduction treatment in the ALL/NHL-BFM 95 protocols. Of 66 patients without a hypersensitivity reaction against the native or the pegylated *E. coli* enzyme observed during this study, two-thirds showed predicted activity time courses with serum asparaginase activities of ≥100 U/l for at least 14 days, and the remaining one-third showed a fast decline in activity [11]. Because these shortened activity time courses suggested insufficient treatment intensity in a considerable number of patients, a dose of 2500 U/m^2 BSA, as approved in Germany and the USA for PEG-asparaginase on the basis of the same data, was used in a limited number of patients with and without an anamnestic allergic reaction against the native enzyme, to determine whether it would be possible to obtain the predicted pharmacokinetic treatment intensity in a greater proportion of patients by dose escalation.

Patients and methods

Between November 1997 and July 2000, 59 children (29 male, 30 female) undergoing reinduction therapy for ALL ($n=37$) or NHL ($n=2$), or treatment for ALL relapse ($n=20$) were enrolled in a drug monitoring program. Reinduction treatment was performed in four centres of the ALL-BFM study group (Essen, Frankfurt, Münster, Vienna), and patients with relapse were treated in nine centres of the ALL-BFM study group (Berlin, Frankfurt, Hannover, Heidelberg, Homburg, Kiel, Münster, Neunkirchen, Tübingen) and one patient was treated in each of three clinics of the COALL group (Bielefeld, Düsseldorf, Hamburg).

Information about immunological reactions associated with pretreatment were not available from all patients, but in four of the relapse patients an allergic reaction to native *E. coli* asparaginase was reported during reinduction treatment. All of these patients were changed to *Erwinia* enzyme without further complications. All children with ALL ($n=57$) or NHL ($n=2$) started treatment according to the ALL/NHL-BFM 95/2000 and ALL-REZ BFM 96 protocols, respectively, and all children except one were treated with 2500 U/m^2 BSA PEG-asparaginase. Because of a BSA of less than 0.6 m^2, with approval of the German authority, this child was treated with a dose of 82.5 U/kg body weight. During the drug monitoring program, ALL treatment was changed from the 95 to the 2000 protocol, Therefore most of the children were treated during reinduction in so-called 'protocol II', but two patients were treated in accordance with 'protocol III', which now in addition is part of the new protocol ALL-BFM 2000.

The age range was 2 years 1 month to 17 years 3 months (mean 6 years 3 months, median 4 years 8 months) in the reinduction group, and 3 years 9 months to 16 years 1 month (mean 8 years 8 months, median 8 years 2 months) in the relapse group. Four patients in the relapse group received a second administration of an identical dose.

Reinduction treatment protocol II

On day 8 of the treatment plan, 2500 U/m^2 BSA PEG-asparaginase (Oncaspar, pegylated *E. coli* asparaginase from a bacterial strain identical to the Asparaginase Medac preparation, Kyowa Hakko) was given as a 2-h intravenous infusion. This administration replaced four doses of 10,000 U/m^2 BSA *E. coli* asparaginase on days 8, 11, 15 and 18 of the reinduction treatment as defined by the ALL/NHL-BFM 95 protocol. In addition, the reinduction chemotherapy included a combination of daily dexamethasone (10 mg/m^2, days 1 to 31), and vincristine (1.5 mg/m^2) and doxorubicin (30 mg/m^2) on days 8, 15, 22 and 29 [15, 17].

Reinduction treatment protocol III

On day 1 of the treatment plan, 2500 U/m^2 BSA PEG-asparaginase (Oncaspar) was given as a 2-h intravenous infusion. This administration replaced four doses of 10,000 U/m^2 BSA *E. coli* asparaginase on days 1, 4, 8 and 11 of the reinduction treatment as defined by the ALL-BFM 2000 protocol. In addition, the reinduction chemotherapy included a combination of daily dexamethasone (10 mg/m^2, days 1 to 15 with a gradual dose reduction to day 24), and vincristine (1.5 mg/m^2) and doxorubicin (30 mg/m^2) on days 1 and 8.

Relapse treatment

Treatment according to the ALL-REZ BFM 96 protocol consists of repeated administration of multiagent chemotherapy blocks with asparaginase. In the first block (F1), after a cytoreductive prephase, one dose of native *E. coli* asparaginase (Asparaginase Medac) 10,000 U/m^2 BSA as defined by the protocol was used, and in the second block (F2) this was replaced by one dose of 2500 U/m^2 BSA PEG-asparaginase (Oncaspar) given as a 2-h intravenous infusion. The chemotherapy during the F2 block further included daily dexamethasone (20 mg/m^2, days 1 to 5), vincristine (1.5 mg/m^2, day 1), cytarabine (3 g/m^2 twice daily, days 1 and 2), and intrathecal methotrexate, cytarabine and prednisolone on day 5.

Desired asparaginase serum activities

A serum *E. coli* asparaginase activity of 100 U/l has been suggested by Riccardi et al. [16] to be sufficient for adequate asparagine depletion in plasma and cerebrospinal fluid. Therefore, as only two-thirds of the patients treated with 1000 U/m^2 Oncaspar during reinduction had shown this activity for about 14 days in the past [11], a dose of 2500 U/m^2 Oncaspar was given in order to increase the time during which asparaginase activity was in this range.

Sample collection

Blood samples for determination of asparaginase activity were collected every 3rd or 4th day as part of standard clinical blood sampling. Monitoring was performed until serum asparaginase activity had dropped below the limit of quantification (LOQ) (< 20 U/l). Immediately after withdrawal, the samples were centrifuged and the serum was sent for measurement without freezing (stability at room temperature was proven for up to 7 days; unpublished data).

Since blood samples for the assessment of enzyme activity 1, 2 and 3 weeks after administration were not available for the same days in every patient, the data from days 6 to 8, 13 to 15, and 20 to 22 were pooled for the time-points 1 week, 2 weeks and 3 weeks, respectively. If there was more than one sample for weekly determination of enzyme activity from the same patient, only one of the results was selected randomly.

Determination of asparaginase activity

Asparaginase activity was determined in a standard rich buffer system measuring the ammonia released photometrically at 450 nm after reaction with Nessler's reagent. The assay had an inter- and intra-assay reproducibility with a coefficient of variation of < 15% down to the LOQ of 20 U/l. The details of this assay have been published elsewhere [6, 21]. For graphical presentation of the data, activities less than 20 U/l were set to one-tenth of the LOQ (2 U/l).

Determination of amino acids

Systematic measurement of amino acids was not included in the drug monitoring program because the multicenter design of this investigation would not allow special preparation of blood samples which is necessary for the determination of amino acids.

Calculation of individual activity time courses

To determine the population-based pharmacokinetics, individual activity time courses were calculated using the computer program NONMEM, version V, level 1.0 [2] with a nonlinear one-compartment pharmacokinetic model (Michaelis-Menten) without saturable elimination and with conditional estimation. With the conditional estimation method, estimates of the population parameters and simultaneously the random interindividual effects are derived. This nonlinear model provided the best fit among different pharmacokinetic models examined (unpublished data). The estimates from this model for patients treated with PEG-asparaginase 1000 U/m^2 BSA are presented. Comparison of the measured and model-derived asparaginase activities showed a very good fit with an intersect of 0.532 and a slope of 0.988 (r^2 0.945).

Statistics

To compare the serum asparaginase activity of patients during reinduction or relapse treatment 1, 2 and 3 weeks after administration of the drug, the Mann-Whitney Rank Sum Test was used. Statistical analysis was performed using Sigmastat 2.03 software (Jandel Scientific).

Results

Patients undergoing reinduction treatment according to the ALL/NHL-BFM protocols

Measurement of asparaginase activity 1 week after administration was possible in 37 samples from 39 patients. In the two patients without samples after 1 week, the following samples withdrawn some days later showed no asparaginase activity. Therefore, the time-point at which activity was below the therapeutic range could not be determined in these two patients. For determination of enzyme activity 2 weeks after administration, 34 samples from 39 patients were available, including 13 patients without quantifiable activity in samples from an earlier time-point than 2 weeks. In 4 of 39 patients the time-point with activity below the therapeutic range could not be determined, because of missing samples after determination of activity in the therapeutic range at an earlier time-point, whereas in another patient without a 2-week sample, activity was ≥100 U/l at a later time-point. In 2 of 34 of the samples the activity was 56 U/l and 85 U/l, respectively, and therefore below the target limit of ≥100 U/l. The other 19 patients had activities in the therapeutic range, and 3 weeks after administration 29 samples were available for measurement from the 39 patients. These results are summarized in Table 1. No hypersensitivity reactions were reported in the whole group of patients.

Patients undergoing treatment according to the ALL-REZ BFM 96 protocol

For determination of asparaginase activity 1 week after administration, 17 samples from 24 administrations in 20 patients were available. In four of seven administrations without samples after 1 week, activity was above the desired limit of 100 U/l at a later time-point, whereas in two of the remaining administrations no information for the rest of the activity time-course was available at all, and in another one, activity was 49 U/l after 18 days. Therefore, the time-point with activity below the therapeutic range could not be determined in these activity time courses. One patient had an activity of 95 U/l, and the remaining 13 of 17 administrations showed activities above the target limit of 100 U/l. In four patients, two identical doses were given in different treatment blocks. In all of these patients a complete data set was available from one administration only, showing a regular activity time course. The limited data from the second administration, however, suggested a comparable activity time course after both administrations in all four patients.

Asparaginase activity 2 weeks after administration could be determined in samples from 24 administrations in 11 patients, including four of five samples without quantifiable activity which were measured at an earlier time-point than 2 weeks. In 12 of the 24 administrations the time-point with activity below the desired range could not be determined because of missing samples after determination of activity in the therapeutic range at an earlier time-point. In one administration without samples after 2 weeks, activity of more than 100 U/l was measured at a later time-point. After 3 weeks samples from 24 administrations in 11 patients were available for measurement including seven of seven samples without

Table 1 Plasma asparaginase activity (U/l) in patients during reinduction and relapse treatment according to the ALL-BFM 95/2000 and ALL-REZ BFM 96 protocols, respectively. Differences between the data from reinduction and relapse treatments were not statistically significant at any time-point. Because in relapse some of the patients were treated with more than one dose, the reference point is the number of administrations and not the number of patients in this group

Time after administration	Reinduction			Relapse		
	Median (range)	Patients activity < 20 U/l	Samples/patients	Median (range)	Patients activity < 20 U/l	Samples/doses
Total group						
Day 7 ± 1	1383 (< 20–1997)	8	37/39	1331 (< 20-1985)	3	17/24
Day 14 ± 1	222 (< 20–929)	13	34/39	69 (< 20-454)	5	11/24
Day 21 ± 1	< 20 (< 20–184)	21	29/39	< 20 (< 20–66)	7	11/24
Activity ≥100 U/l day 14						
Day 7 ± 1	1629 (1079–1997)		20/20	1499 (1124–1985)		6/6
Day 14 ± 1	341 (172–929)		19/20	391 (131–454)		5/6
Day 21 ± 1	21 (< 20–184)		14/20	52 (< 20–66)		4/6
Patients with activity ≥100 U/l						
On day 7 ± 1	1577 (377–1997)		29/29	1383 (903–1985)		13/17
On day 14 ± 1	341 (172–929)		19/20	391 (131–454)		5/6
On day 21 ± 1	184		1/1			0/0

quantifiable activity which was measured at an earlier time-point than 3 weeks. These results are also shown in Table 1. Hypersensitivity reactions were not reported.

Statistical analysis

Median serum activities of patients treated with asparaginase during reinduction or relapse determined 1, 2 and 3 weeks after drug administration showed no statistically significant differences at any time-point.

Toxicity

Toxicity was not intended to be documented during the drug monitoring.

Discussion

Asparaginase treatment is associated with special problems, unique in the spectrum of substances used in cancer treatment. As the asparaginases common in clinical practice are macromolecules obtained from different microorganisms, one of the major side effects is hypersensitivity reactions against the foreign proteins. These reactions are reported to occur in about 25% to 30% of patients, but the rate may be much higher if the same drug is used repeatedly [7]. However, because of limited cross-immunogenicity of enzymes from different biological sources, continuation of treatment is often possible by changing the drug [5, 14]. For comparable treatment intensity, adjustment of dose and schedule is necessary because of the different pharmacokinetic and pharmacodynamic properties of the asparaginases [1, 6]. As the native *E. coli* enzyme is the drug of choice in most first-line treatments in Germany, hypersensitivity reactions against this drug are common. Because studies by our group have shown that substitution by the *Erwinia chrysanthemi* enzyme necessitates a considerable increase in dose and in the number of administrations [20], and with the pegylated *E. coli* asparaginase at a dose of 1000 U/m^2 BSA asparagine depletion over the envisaged period of time occurs in about two-thirds of patients [11], an investigation with 2500 U/m^2 of PEG-asparaginase in reinduction and relapse treatment was performed.

Population-based investigation of the pharmacokinetics of different PEG-asparaginase doses have shown that the activity time courses in human serum do not fit a linear pharmacokinetic model, but a Michaelis-Menten kinetic, since a faster drop of activity is seen with higher serum activities (unpublished data; [11]). Therefore, we did not expect that increasing the dose from 1000 to 2500 U/m^2 BSA would result in serum activities in the therapeutic range of ≥100 U/l for about twice the time, but we did expect to be able to determine whether prolongation of the activity time course is possible by dose escalation. As drug monitoring with 1000 U/m^2 PEG-asparaginase has shown a marked interindividual variability in the activity time courses [11], this was also expected in a comparable manner with the higher dose. Comparison of the drug monitoring data from the two doses, as shown in Fig. 1, demonstrates that with 2500 U/m^2 peak activities were about twice those seen with 1000 U/m^2, but there was no prolongation of the time with serum activities of ≥100 U/l with comparable interindividual variability. In the context of these findings, it is remarkable that data available from reinduction drug monitoring using four times the dose (10,000 U/m^2) of native *E. coli* asparaginase in 76 patients show no silent inactivation, but clinical hypersensitivity reactions in 24% of the patients [12]. It seems that with PEG-asparaginase silent inactivation may be the predominant type of hypersensitivity reaction,

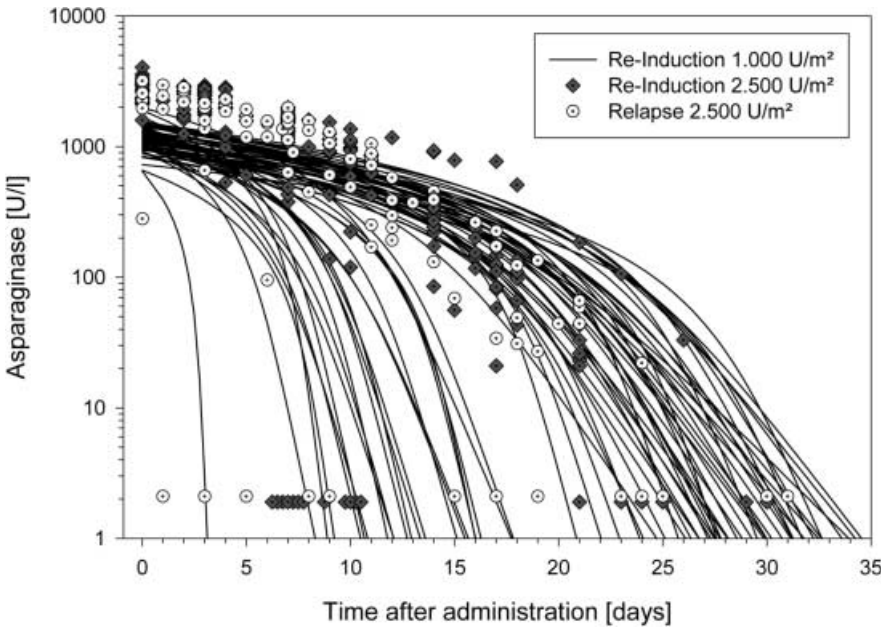

Fig. 1 Activity time courses of patients treated with PEG-asparaginase 1000 U/m^2 BSA in the background, together with symbols indicating measured activities under treatment with PEG-asparaginase 2500 U/m^2 BSA during reinduction and relapse. Activities below the LOQ are set 2 U/l

necessitating strict drug monitoring, whereas with the native *E. coli* enzyme mainly clinical reactions are seen.

Integration of patients treated in front-line and in relapse with 2500 U/m^2 PEG-asparaginase gave us the chance to look for differences in the activity time courses in relation to the extent of pretreatment with asparaginase. As can be seen in Fig. 1, there were some relapse patients with no demonstrable activity as early as a few days after administration of the drug, but there were also ALL patients undergoing initial treatment without activity after less than 1 week. No statistically significant differences between the median activities in patients in reinduction and in relapse was seen 1, 2 and 3 weeks after drug administration. Therefore, an influence of the extent of pretreatment with asparaginase could not be demonstrated in these patients. Four of the relapse patients were changed to the *Erwinia* enzyme during reinduction treatment because of an allergic reaction to the native *E. coli* preparation. Although limited by the small number of patients, there is no indication of a shortened activity time course in these patients. In another five patients, asparaginase reinduction treatment was performed with the same pegylated *E. coli* enzyme that was used for relapse treatment in this investigation. Even though one of the patients showed no measurable serum asparaginase activity the day after drug administration, a shortened activity time course cannot be assumed in the remaining four patients. The value of this information is uncertain, however, because of the small number of samples in these patients.

Measurement of asparagine was not carried out because the multicenter design of the drug monitoring program would not allow special preparation of blood samples immediately after withdrawal, which is necessary for the determination of amino acids in serum [6]. It has been shown by Riccardi et al., however, that plasma *E. coli* asparaginase activities about 100 U/l are sufficient for complete asparagine depletion in serum as well as CSF [16]. Because this finding has been confirmed repeatedly, an activity of ≥100 U/l was taken as the lower therapeutic range in this investigation [6, 20].

The findings showed that escalation of PEG-asparaginase dose in general was not associated with prolongation of the time interval with activity in the therapeutic range. However, although observed in a limited number of patients only, neither allergic reactions against the native preparation of the identical enzyme nor pretreatment with PEG-asparaginase appeared to lead to a shortened activity time course in most patients. This may give the opportunity for repeated doses, if a prolonged time interval with activity in the therapeutic range is desired. Because the number of patients treated in this way is very limited, ongoing clinical studies will provide further data on the pharmacokinetics with repeated use of PEG-asparaginase. Since there are a considerable number of patients with shortened activity time courses without clinical signs of hypersensitivity, treatment with PEG-asparaginase should in general be performed with drug monitoring. With the background of pharmacokinetic data now available, the number of specimens may be reduced to two or three samples withdrawn 5 to 7 days and 12 to 14 days after administration of the drug.

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