MONITORING THE ASPARAGINASE ACTIVITY AND ASPARAGINE LEVELS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA TREATED WITH DIFFERENT ASPARAGINASE PREPARATIONS

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A b s t r a c t

The objective of the study was to monitor the asparaginase activity and level of serum asparagine in children with acute lymphoblastic leukaemia and non-Hodgkin's lymphoma during the induction and re-induction stage of the ALL/NHL-BFM 95 protocol after the administration of two different preparations of asparaginase.

During induction 11 children, who had been treated with 5,000 IU/m^2 of *Escherichia Coli* (Kidrolase) 8 times in 3-day intervals, were examined. During re-induction 7 children received a dose of 10,000 IU/m^2 Kidrolase 4 times in 3 and/or 4-day intervals. Due to allergic reaction to Kidrolase, the preparation from *Erwinia chrysantemi* (Erwinase) was administered during re-induction to four children in the same dose. Therapy with Kidrolase was found to be sufficient during induction and re-induction, while Erwinase therapy during re-induction is less effective.

L-asparaginase is an important component of acute lymphoblastic leukaemia and non-Hodgkin's lymphoma treatment; the various asparaginase preparations are simply not interchangeable. The present data indicate that it is necessary to specify the optimal dose and dosing pattern of Erwinase when substituted by Kidrolase.

K e y w o r d s

Child, Leukaemia, Pharmacokinetics, Asparaginase, Asparagine

INTRODUCTION

In the treatment of childhood lymphoblastic leukaemia, L-asparaginase is an essential element with proven therapeutic efficacy, which catalyses the hydrolysis of the generally non-essential amino acid L-asparagine to L-aspartic acid and ammonia (*1*). As lymphoblastic leukaemic blast cells express only limited amounts of enzyme asparagine synthetase they are dependent on the availability of extra-cellular asparagine. Asparaginase treatment causes a decrease in the asparagine serum concentration. Leukaemic blasts are thus restricted in their protein synthesis

following exposure to asparaginase; in consequence the cells not expressing sufficient amounts of asparagine synthetase are killed (*2, 3, 4, 5*). Glutamine is a second substrate for asparaginase and changes in glutamine metabolism have been considered as contributing to asparaginase-associated side effects (*5, 6*).

Asparaginase causes a wide spectrum of side effects, such as hepatic dysfunction, pancreatitis, severe allergic reactions and many others (*7, 8, 9, 10, 11*). Most of the toxic effects are related either to the inhibition of protein synthesis as a consequence of asparagine depletion or to the immunological reactions to bacterial protein (*6, 12*). In addition, anti-asparaginase antibody formation may lead to a decrease in plasma asparaginase activity without clinical evidence of hypersensitivity. This so-called "silent" inactivation may result in diminished efficacy of the asparaginase therapy (*13*).

Biologically, the enzyme L-asparaginase is derived from either *Escherichia coli* or *Erwinia chrysantemi*, each preparation having different chemical and immunological properties (*14, 15*). *Erwinia* asparaginase is considered to be comparably less toxic and is frequently employed in the event of allergic reactions to *Escherichia coli* asparaginase (*16, 17*). Although *Erwinia* asparaginase has a shorter half-life than *Escherichia coli* asparaginase, preparations are usually interchanged without modifying the dosage or the application interval (*18*).

Considering the above described differences we decided to compare the pharmacokinetic properties of two different asparaginase preparations, which are used to treat acute lymphoblastic leukaemia and non-Hodgkin's lymphoma of children receiving treatment according to the ALL-BFM 95 or NHL-BFM 95 treatment protocols. The objective of the present study was to monitor the activity of asparaginase and the level of asparagine in the serum.

MATERIAL AND METHODS

Between July 2000 and June 2001, 22 children (11 female; 11 male) received treatment according to ALL-BFM 95 or NHL-BFM 95 treatment protocols for acute lymphoblastic leukaemia or non-Hodgkin's lymphoma, and underwent routine monitoring during induction (protocol I) and re-induction (protocol II). Therapy was started with the *Escherichia coli* preparation – Kidrolase, Roger Bellon. Erwinase (Speywood) was applied when allergic reactions to *Escherichia coli* preparations were observed.

INDUCTION – PROTOCOL I.

During induction, 11 children, who had been treated with 5,000 IU/m^2 of *E. coli* (Kidrolase) 8 times in 3-day intervals starting on day 12 of therapy, were examined. The chemotherapeutic regime included prednisone (PRED 60 mg/m^2) on days 1–36, weekly applications of vincristine (VCR 1.5 mg/m^2) and daunorubicin (DNR 30 mg/m^2) on days 8 and 15 for the SR group and on days 8, 15, 22 and 29 for the MR group, as well as intra-thecal methotrexate (MTX) on days 1, 12 and 33.

RE-INDUCTION – PROTOCOL I.

During re-induction 7 children received a dose of 10,000 IU/m^2 Kidrolase 4 times in 3 and/or 4-day intervals. Due to allergic reaction to Kidrolase the preparation from *E. chrysantemi* (Erwinase) was administered during re-induction to 4 children in the same dose. The chemotherapeutic regime

included dexamethasone (DEXA 10 mg/m^2) on days 1–30, vincristine (VCR 1.5 mg/m^2) and doxorubicin (ADR 30 mg/m^2) on days 8, 15, 22 and 29.

Sample Collection

Blood samples were withdrawn 1–2 h prior to each asparaginase infusion during routine pre-treatment laboratory tests. The interval between collections of the samples was 3 days except on day 15 of reinduction therapy, when the interval was 4 days.

The samples were immediately centrifuged and divided in half. One part (400 μl serum) was de-proteinised by adding 10% of 100 ul sulphosalicylic acid (w/w) and deep frozen for storage until amino acid analysis. The other part was immediately frozen and used to determine the asparaginase activity.

High-performance liquid chromatography

The HP 1100 chromatographic system from Hewlett Packard (Waldbronn, Germany) consisted of a vacuum degasser (model G1322A), binary pump (model G1312A), an autosampler (model G1313A), a column thermostat (model G1316A) and a diode array detector (model G1315A). The liquid chromatographic system was controlled by ChemStation software, Rev A 07.01.

A Zorbax Exlipse AAA column (150x4.6 mm I.D., 3.5μm particle size) was obtained from Agilent Technologies (Waldbronn, Germany) and a guard cartridge Meta Guard (4.6 mm I.D., Inertsil ODS-3.5 μm particle size) was obtained from Metachem Technologies (Torrance, CA, USA). The mobile phase consisted of a solvent A: 40 mM NaH$_2$PO$_4$ (adjusted to pH 7.7 with 10 M NaOH) and a solvent B: ACN:MeOH:water (45:45:10, v/v/v). The flow rate was 2.0 ml.min^{-1} and temperature of the column oven was set at 40 °C. The column eluate was monitored with a diode array detector at 338 nm.

L-Asparaginase activity

A mixture of 100 ul serum and 400 ul 0.044 mM L-asparagine buffer solution was incubated at 37°C for exactly 45 min. After addition of 250 ul trichloracetic acid 24.5% (w/w) and centrifugation, 250 ul of the supernatant was added to the Nesslers solution (2000 ul water plus 250 ul Nesslers reagent). The optical density at 450 nm was compared with the ammoniumÐsulphate-Nessler-stan dard curve based on daily calibration at concentrations of 0.05, 0.1, 0.25, 0.5, 1.0 and 2.5 mM. The units of enzyme activity were defined as micromoles of ammonia released per minute. Asparaginase standard curves between 10 and 10000 U/l in human-standard-plasma resulted in an inter- and intra-assay reproducibility with coefficient variations of < 15% down to 20 U/l. The detection limit was 20 U/l. Because of the spontaneous release of ammonia in the plasma, lower activities could not be accurately quantified. Details of the asparaginase assay and *in vitro* comparisons of different asparaginase preparations have been published previously (*19*).

Statistics

In the present study we used statistical methods described by Snedecor and Cochran and own programmes edited in MS Visual Basic and MS Office 2000. Evaluations of the contrasts between the means were based on standard procedures (*20*).

RESULTS

Induction – Protocol I.

The average activity of asparaginase after the administration of Kidrolase during induction (protocol I) monitored in 65 samples (11 patients) was 80.270±63.184 IU/l (average ± S.E.). *Table 1* shows that in 5 monitorings in 3 patients the activity of asparaginase during induction (protocol I) was below the detection limit, i.e. 20 IU/l. In 64% of the samples the activity of asparaginase ranged between

Distribution of enzyme activities and L-asparagine levels in serum from patients under treatment according to protocol I a II of the ALL/NHL-BFM 95

	Asparaginase	Protocol I	Protocol II	
		Kidrolase	Kidrolase	Erwinase
		% samples(n)	% samples(n)	% samples(n)
Asparaginase activity				
≤ 20 IU/l		7,69 (5)	3.74 (1)	20,00 (3)
>20-≤100 IU/l		64,6 (42)	62.96 (17)	60,00 (9)
>100 IU/l		27,7 (18)	33.30 (9)	20,00 (3)
Total		100,00 (65)	100,00 (27)	100,00 (15)
L-asparagine				
≤0.1 μM/l		89,56 (60)	92.59 (25)	80,00 (12)
>0.1-≤0.5 μM/l				
>0.5≤1 μM/l				
>1 μM/l		10,44(7)	7.41 (2)	20,00 (3)
Total		100,00 (67)	100,00 (27)	100,00 (15)

20 and 100 IU/l, and in 18 monitorings, i.e. in nearly 28%, the activity was above 100 IU/l.

Comparisons with asparagine levels given in *Table 1* show complete depletion of asparagine in the serum during induction (protocol I) in nearly 90% of all the analysed samples, while the level of asparagine was above the limit of detection only in 7 monitorings.

The average level of asparagine in the serum according to protocol I prior to the application of infusion containing Kidrolase was 14.938 μM/l; after the application of Kidrolase the level of asparagine in the serum decreased significantly within the 3rd day of therapy ($P < 0.05$) and continued to decrease in the individual days until termination of therapy.

Re-Induction – Protocol II.

During re-induction we examined 11 children between 3 to 13 years of age. Seven patients were treated with a preparation from *Escherichia coli* – Kidrolase. Due to an allergic reaction of 4 children, Kidrolase treatment had to be substituted by the preparation from Erwinia chrysantemi – Erwinase. A standard therapeutic dose of

10,000 IU/m^2 of both preparations was administered. The average asparaginase activity during re-induction (protocol II) after the application of Kidrolase was 100,860±19,074 IU/l (average ± S.E.). Asparaginase activity was below the limit of detection, i.e. below 20 IU/l, only in one case (3.74%) in one patient. In 17 measurements, i.e. in 62.96% of the samples, asparaginase activity ranged between 20 and 100 IU/l. In 9 measurements, i.e. in 33.3% of the samples, the activity of asparaginase was higher than 100 IU/l (*Table 1*).

After the administration of Erwinase during re-induction (protocol II) the average asparaginase activity was 70.13±79.444 IU/l (average ± S.E.). In two patients and 3 monitorings the activity of asparaginase was below the limit of detection (20 IU/l). In 9 monitorings (60% of the samples) in 2 patients the activity of asparaginase ranged between 20 and 100 IU/l. In 3 monitorings (30% of the samples) the level was above 100 IU/l (*Table 1*).

During re-induction, complete depletion of asparagine in the serum occurred after the administration of Kidrolase in 25 monitorings, i.e. in 92.6% of the samples (*Table 1*). In 2 monitorings in 2 patients the level of asparagine was higher than 1 μM/l. Before treatment the level of asparagine was 13.806 μM/l and after the administration of Kidrolase during re-induction (protocol II) the level of asparagine decreased significantly in all monitorings (P < 0.05).

Complete depletion of asparagine in the serum was reported in 80% of the analysed samples, i.e. in 12 monitorings, after treatment with the preparation from Erwinia chrysantemi – Erwinase (*Table 1*). In one patient in 3 monitorings the levels were above 1 μM/l. Before treatment the level of asparagine was 12.589 μM/l. After the administration of Erwinase during the re-induction (protocol II) the difference compared to day 0 of treatment was statistically significant only on day 3 of therapy.

DISCUSSION

A number of published studies confirmed different pharmacokinetic properties of *Escherichia coli* and Erwinia asparaginase and even individual pharmacokinetic differences among the respective preparations of *Escherichia coli* asparaginase (*18, 21, 22*). Erwinia asparaginase has a shorter half-life (ca 0.6 d) than *E. coli* asparaginase (1.3 d) (*18*). However, during re-induction (protocol II ALL- BFM 95), both *E. coli* and Erwinia asparaginase were administered in the same dose, i.e. 10000 IU/m^2. Comparative pharmacokinetic studies showed that not only the dose, time interval and simultaneously applied cytostatic could significantly affect the biological effect of L-asparaginase, but also the applied preparation as such (*23, 2, 21, 24*). These data indicate that substitution of one preparation by another in the same dose and interval is not always effective.

Our patients received Kidrolase in the case of *E. coli* asparaginase or a preparation from Erwinia chrysantemi – Erwinase; therefore our results, particularly in the case of *E. coli* asparaginase, cannot be fully compared with previous studies.

The average activity of *E. coli* asparaginase in induction (protocol I) and re-induction (protocol II) was 80.27 and 100.86 IU/l, respectively. The activity of Erwinia asparaginase was 70.113 IU/l. The activity of asparaginase in the patients fluctuated considerably; this result was probably due not only to the individual variability but also because the group of patients was small.

Table 1 shows the division of samples of enzymatic activity below 100 IU/l and the percent of all samples where complete depletion of asparagine occurred during induction and re-induction after the administration of *E. coli* and Erwinia asparaginase according to protocol ALL-BFM 95. In the early 1980's *Riccardi et al.* reported that complete depletion of asparagine in the serum and in the cerebrospinal fluid was achieved in patients with asparaginase activity of at least 100 IU/l (*25*). In 1996 *Boos et al.* discovered that an asparaginase activity of 74 IU/l in patients treated with Crasnitin preparations (*E. coli* asparaginasa) during induction also resulted in satisfactory depletion of asparagine in a high number of patients. In the present study we found that the majority of monitored samples of an enzymatic activity < 100 IU/l also resulted in complete depletion of asparagine in the serum (*Table 1*) and is in accordance with data presented in other publications (*21, 26, 27, 28*).

Furthermore we proved that depletion of asparagine in the serum after administration of Kidrolase during induction was complete in 90% of monitorings and during re-induction in nearly 92,6%. The depletion of asparagine after the administration of Erwinase was complete only in 80 %. Our results indicate that the dosage pattern for Kidrolase was more effective and led to satisfactory depletion of asparagine in the serum during the induction and re-induction stages according to the ALL-BFM 95 protocol. Erwinia asparaginase treatment during re-induction was less effective (*Table 1*). We therefore assume that a 3 to 4-day interval between the individual Erwinase applications is too long. *Boos et al.* (1996) reached the same conclusions; they discovered that during re-induction the same dose of Erwinia asparaginase administered in the same intervals, resulted in levels of asparaginase activity near to the quantification limit, i.e. ca 20 IU/l (*21*), which corresponded with the unsatisfactory depletion of asparagine in the serum, i.e. only about 26%, compared to the results of the present study.

An allergic reaction to intravenous application of *Escherichia coli* asparaginase was observed in 4 of our patients out of the total number of 22, i.e. in 18.2 %. None of the patients suffered allergic reactions after the administration of Erwinia asparaginase. Many studies mentioned that there is an association between the hypersensitive reaction, asparaginase antibodies and alternative pharmacokinetics (*7, 29*). The presence of antibodies need not lead to clinical symptoms of allergic reactions, but correlates with a rapid reduction in enzymatic activity. This phenomenon is called "silent inactivation" (*13, 29*).

In the present study, we found a systematic decrease in the activity of asparaginase in one patient during induction followed by unsatisfactory depletion of asparagine

in the serum and no clinical symptoms of allergic reaction. In another patient with an allergic reaction after the first administration of Kidrolase during re-induction, the following Erwinia asparaginase treatment was associated with decreased asparaginase activity and unsatisfactory depletion of asparagine in the serum. In the case of this patient we could not exclude the presence of cross-reactive antibodies.

In conclusion we can say that there are many types of L-asparaginase preparations of different efficiency. Further studies should be focused on the determination of the optimal dosage and uniform dosage pattern for the respective preparations.

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MONITOROVÁNÍ AKTIVITY ASPARAGINASY A HLADIN ASPARAGINU U DĚTÍ
S AKUTNÍ LYMFOBLASTICKOU LEUKEMIÍ LÉČENÝCH RŮZNÝMI PREPARÁTY
ASPARAGINASY

S o u h r n

Cílem práce bylo stanovení aktivity asparaginasy a hladiny asparaginu v séru u dětí s akutní lymfoblastickou leukemií nebo non Hodgkinským lymfomem během indukční a reindukční fáze protokolu ALL/NHL-BFM 95 po podání dvou různých preparátů asparaginasy.

Během indukce bylo vyšetřeno 11 dětí. Všichni pacienti byli léčeni preparáty *E. coli* asparaginasy (Kidrolase) v dávce 5.000 IU/m^2 celkem 8x ve třídenních intervalech. Během reindukce bylo léčeno preparáty *E. coli* asparaginasy celkem 7 dětí. Infuze byly aplikovány ve tří nebo čtyřdenních intervalech v dávce 10 000 IU/m^2. Preparát z *Erwinia chrysantemi* (Erwinase) byl aplikován pro alergickou reakci na *E. coli* asparaginasu 4 dětem ve stejné dávce. Terapie preparátem Kidrolase byla dostačující v průběhu indukce i reindukce zatímco terapie Erwinasou byla méně efektivní.

L-asparaginasa je důležitou součástí léčby akutní lymfoblastické leukemie a non Hodgkinova lymfomu. Různé preparáty asparaginasy nejsou jednoduše zaměnitelné. Naše data naznačují, že je nutné specifikovat dávku a dávkovací interval pro Erwinasu při alergické reakci na Kidrolasu.

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