Monitoring of Erwinia asparaginase therapy in childhood ALL in the Nordic countries

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Aims Evaluation of L-asparaginase therapy in the NOPHO-92 ALL-protocol (treatment protocol of acute lymphoblastic leukaemia of the Nordic Society of Paediatric Haematology and Oncology, initiated in 1992) after intravenous and intramuscular administration of Erwinia asparaginase during induction and re-induction therapy.

Methods Forty children with newly diagnosed acute lymphoblastic leukaemia received Erwinia asparaginase (30 000 IU/m² i.v. or i.m.) during induction therapy (every day for 10 days), and 19 children received Erwinia asparaginase (30 000 IU/m² i.v. or i.m.) during re-induction therapy (twice a week for 2 weeks). Within the treatment periods asparaginase trough activity (using a spectrophotometric assay) was determined on specific days. The goal of therapy is complete L-asparagine depletion, which asparaginase activities above 100 IU l⁻¹ have been shown to ensure. Therefore determination of L-asparagine (using a h.p.l.c. method) was performed only in plasma samples with asparaginase activities below 100 IU l⁻¹.

Results During induction therapy 92.2% of the trough enzyme activities were above 500 IU l⁻¹ for the i.v.-treated patients, and 92.4% of the trough enzyme activities were above 500 IU l⁻¹ for the i.m.-treated patients. During re-induction therapy 64.7% of the trough enzyme activities were below 100 IU l⁻¹ in the i.v.-treated group, and 73.3% of the trough enzyme activities were below 100 IU l⁻¹ in the i.m.-treated group. For trough enzyme activities below 100 IU l⁻¹ L-asparagine depletion was complete in two thirds of the samples.

Conclusions In the NOPHO-92 ALL-protocol L-asparaginase treatment during induction therapy was unnecessarily intense, but during the re-induction phase it appeared inadequate.

Keywords: Erwinia asparaginase, front-line therapy, i.m. administration, i.v. administration, L-asparagine depletion, second exposure, trough enzyme activities

Introduction

L-Asparaginase has been used to treat acute lymphoblastic leukaemia (ALL) for more than 30 years. The enzyme hydrolyses L-asparagine into L-aspartic acid and ammonia extracellularly, and thus depletes the systemic circulation of L-asparagine. Asparagine synthetase activity in some malignant lymphoblasts is low, and they rely on

an exogenous supply of the amino acid [1–3], otherwise their protein synthesis would be halted, leading to cell death. The pharmacological end-point of therapy is L-asparagine depletion in plasma. Riccardi *et al.* [4] showed that enzyme activities above 100 IU l⁻¹ ensure complete L-asparagine depletion from body fluid compartments. Enzyme activities below 100 IU l⁻¹ may also result in L-asparagine depletion in some patients [5, 6].

The native forms of L-asparaginase used in clinical practice derive either from *Erwinia chrysanthemi* or from various strains of *Escherichia coli* (*E. coli*). These preparations have different biochemical, pharmacokinetic and immunological properties [7–9]. In most ALL-protocols

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an *E. coli* preparation is used, with the *Erwinia chrysanthemi* preparation being reserved for patients suffering hypersensitivity reactions to the *E. coli* preparation. Information on front-line therapy with Erwinia asparaginase is limited, but recently Rizzari *et al.* [5] published data on trough plasma enzyme activity, plasma and CSF L-asparagine concentrations during front-line therapy with Erwinia asparaginase but only during the induction phase. In the Nordic Countries Erwinia asparaginase has been part of the ALL-protocol since 1992, where it replaced Crasnitin (an *E. coli* preparation made by the Bayer Company). In the NOPHO-protocol [10], high-risk patients were exposed to Erwinia asparaginase a second time during re-induction therapy.

The present study has been undertaken to investigate if plasma trough enzyme activities and the extent of depletion of L-asparagine differ after i.v. and i.m. administration of Erwinia asparaginase in the setting of the NOPHO-92 ALL-protocol, during both the induction and re-induction phases.

Methods

Forty children with newly diagnosed ALL were included in the study (age 1–14 years). They all received conventional L-asparaginase therapy according to the NOPHO-92 ALL-protocol. 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 intravenous vincristine and doxorubicin, oral prednisolone, and intrathecal methotrexate. During L-asparaginase treatment prednisolone was tapered (from 60 to 0 mg m⁻² day⁻¹ with a total dose of approximately 2500 mg m⁻² from day 1 to day 46).

The higher risk patients also received Erwinia asparaginase 30 000 IU m⁻² intravenously or intramuscularly twice a week (Mondays and Thursdays) for 2 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 days 0, 3, 7 and 10). During this period they also received oral dexamethasone, intravenous vincristine and daunorubicin (days 169 or 232 and 176 or 239) and intrathecal methotrexate on day 169 (232).

Sample collection

At specific times during therapy blood samples were withdrawn into heparinized glass tubes and centrifuged at 4° C. The plasma samples were stored at –70° C until the time of analysis. Samples from other centres were sent and

received packed on dry ice. During the induction phase blood samples were withdrawn before asparaginase injection on days 1, 4, 5, 8, and 9, and one final blood sample was taken on day 13. During the re-induction phase blood samples were withdrawn before asparaginase injection on days 3, 7 and 10. One plasma sample was also obtained on day 14.

Quantification of asparaginase activity using Nessler's reagent

The plasma concentrations of asparaginase were measured by a spectrophotometric method using Nessler's reagent [11]. The activity of the enzyme is expressed in international units per litre (IU l⁻¹). One international unit is defined as the amount of enzyme required to catalyse the formation of 1 µmol of ammonia from asparagine in 1 min at 37° C. The intraday CV (coefficient of variation) value was estimated by measuring one standard concentration (400 IU/l) 35 times on one day and was 2.7%. The interday CV was evaluated from triplicate analysis of the same sample 20 times over 2 months and was found to be 5.6%. The interday CV at 100 IU l⁻¹ was 29.4%, which is set as the minimum detectable concentration in this study. The working range of activity was 100–6000 IU l⁻¹. Trough plasma concentrations were defined as the asparaginase activities immediately before the next administration of Erwinia asparaginase.

Determination of L-asparagine

All samples with enzyme activities ≤100 IU l⁻¹ were analysed for L-asparagine by reverse phase h.p.l.c. [6].

The limit of quantification of asparagine in human plasma was 0.2 µM. In aqueous solution the intraday CV was <7.5%, and the interday CV was <10% over the whole linear range. The coefficient of variation (day-to-day reproducibility) for L-asparagine at the limit of quantification (0.2 µM) in plasma was 14.7%.

Assessment of extent of depletion of L-asparaginase in plasma

The depletion of L-asparagine was graded mainly according to Boos *et al.* [6]: complete depletion ≤0.2 µM; almost complete depletion >0.2–≤0.5 µM; moderate reduction >0.5–≤1 µM; slight reduction >1–≤25 µM; no reduction >25 µM.

Results

Trough enzyme activities

During induction therapy following i.v. treatment trough asparaginase activity was (mean ± s.d.) 2360 ±

1580 IU l⁻¹, range 198–6000 IU l⁻¹ (51 samples from 15 children), and following i.m. treatment it was 1710 ± 1025 IU l⁻¹, range 100–5022 IU l⁻¹ (93 samples from 25 children). This difference was statistically significant ($P < 0.05$, Mann–Whitney Rank Sum Test; 95% confidence intervals on the difference 218, 1079). In both groups the trough enzyme activities were greatly above the recommended activity of 100 IU l⁻¹, shown to deplete L-asparagine completely [4, 6, 12–14].

During re-induction therapy, the mean ± s.d. trough activity of asparaginase in the i.v. group was 110 ± 190 IU l⁻¹, range 0–688 IU l⁻¹ (17 samples from 8 children), and in the i.m. group it was 83 ± 189 IU l⁻¹, range 0–768 (30 samples from 12 patients). This difference was not statistically significant ($P > 0.05$, Mann–Whitney Rank Sum Test; 95% confidence intervals on the difference –89, 143).

The distribution of trough enzyme activities during the induction and re-induction phase is shown in Figure 1.

Figure 1 Distribution of trough enzyme activities during induction and re-induction therapy after intravenous (i.v.) and intramuscular (i.m.) administration of *Erwinia* asparaginase. (1: <100 IU l⁻¹, 2: >100–≤500 IU l⁻¹, 3: >500–≤5,000 IU l⁻¹, 4: >5,000 IU l⁻¹).

Distribution of L-asparagine concentrations in plasma for enzyme activities below 100 IU l⁻¹

Trough asparaginase activity was determined in 191 samples after both i.m. and i.v. administration during induction and re-induction therapy. In 35 (18%) of these samples enzyme activity was below 100 IU l⁻¹ although there was complete L-asparaginase depletion in two thirds of these samples (Table 1).

Duration of the total depletion of L-asparagine from plasma

Twenty-two patients (16 in the i.m. group and 6 in the i.v. group) had the concentration of asparaginase measured 4 days after the last dose during induction therapy, and 9 patients (6 in the i.m. group and 3 in the i.v. group) 4 days after the last dose during re-induction therapy.

Complete L-asparagine depletion was defined as (1) a trough enzyme activity of >100 IU l⁻¹, or (2) a L-asparagine concentration of <0.2 µM.

During induction therapy L-asparagine depletion was complete in both the i.v. and i.m. groups. L-asparagine depletion was still complete in 20 out of 22 patients 4 days after the last dose. The remaining two patients were in the i.m. group.

The majority of patients did not achieve complete L-asparagine depletion during re-induction therapy (day 0 to day 10). For 5 of the 7 patients who had L-asparaginase measured 4 days after the last dose during re-induction therapy, it had reappeared in the plasma. Two patients (one from each group) still had complete L-asparagine depletion but an enzyme activity below 100 IU l⁻¹ 4 days after the last dose.

Discussion

The dosing regimen for *Erwinia* asparaginase in the NOPHO-92 protocol was based upon the dose of Crasnitin in the previous protocol (10,000 IU/m² i.v. every day for 10 days during induction therapy, and twice

Table 1 Distribution of L-asparagine concentrations in plasma for enzyme activities below 100 IU l⁻¹.

L-asparagine concentration	% samples (n)	Number of patients
Complete depletion ≤0.2 µM*	67.6 (23)	17
Almost complete depletion >0.2–≤0.5 µM	8.8 (3)	3
Moderate reduction >0.5–≤1 µM	–	–
Slight reduction >1–≤25 µM	5.9 (2)	1
No reduction >25 µM	17.6 (6)	4
Total	100 (34) [#]	19

*limit of detection.

[#]In 35 plasma samples the enzyme activity was <100 IU l⁻¹. In one case there was not enough material for determination of the concentration of L-asparagine.

a week during re-induction therapy) and on the shorter half-life of the former, 6.4 h [11] compared with the latter, 18 h [7].

The dosage and frequency of administration of Erwinia asparaginase in the NOPHO-protocol differ significantly from that in other protocols [5, 6, 8, 12, 13]. The dose is three times larger, during induction therapy the drug is administered daily, and during the re-induction phase it is administered twice weekly for 2 weeks. In the NOPHO-protocol the frequency of dosing during the induction phase is similar to that in the early ALL-protocols. Daily administration of asparaginase was considered to induce fewer allergic reactions compared with schedules involving asparaginase free intervals, since daily administration of large quantities of an antigen may overwhelm the immune response. As the results within NOPHO have improved significantly over the years and as the incidence of allergic reactions during asparaginase therapy has been low, dosing regimens have remained unchanged.

In the present study trough enzyme activities during the induction phase were very high, but during the re-induction phase they were very low (Figure 1). The latter findings are in accordance with those of Rizzari *et al.* [5], after both i.v. and i.m. treatment with asparaginase. In our study the minimum detectable concentration was 100 IU l^{-1} , and when no asparaginase was detected, its concentration was set at 0 IU l^{-1} . In the study of Rizzari *et al.* [5] the minimum detectable concentration was 50 IU l^{-1} , which probably accounts for the lower mean concentrations found in our study. When the mean trough concentration was recalculated after exclusion of activities below 100 IU l^{-1} , it was approximately three times higher.

Rizzari *et al.* [5] found that in patients treated with i.v. or i.m. Erwinia asparaginase 56% and 33% of samples, respectively, had mean trough concentrations $\geq 100 \text{ IU l}^{-1}$ compared with 35.3% and 26.7% (re-induction therapy) in the our study. The dose in the NOPHO setting was three times larger than that used by Rizzari *et al.* [5], but asparaginase was administered on Mondays and Thursdays, resulting in 3 and 4 day intervals between trough concentrations, and thus lower mean trough enzyme activities.

There was a significant difference between mean trough enzyme activities after i.v. and i.m. administration during the induction phase. However, during re-induction therapy no significant difference in mean trough enzyme activities between the two modes of administration was found. Trough enzyme activities showed larger intra- and especially interindividual variation, which makes drug monitoring advisable.

The reason for the high trough activities during the induction phase and the low trough enzyme activities

during the re-induction phase is that the same dose is given daily during induction therapy but only twice a week during re-induction therapy. The low trough enzyme activities during re-induction correspond well with enzyme activities calculated from the pharmacokinetic parameters of Erwinia asparaginase [11].

For trough enzyme activities below 100 IU l^{-1} L-asparagine depletion was still complete in two-thirds of all samples. In the study of Rizzari *et al.* [5] L-asparagine depletion was complete in all samples, although 30.5% of all samples had enzyme activity $< 50 \text{ IU l}^{-1}$. For many patients the required activity of asparaginase for achieving complete L-asparagine depletion probably is lower than 100 IU l^{-1} , but this limit seems reasonable in order to ensure that the great majority of patients were L-asparagine depleted during therapy. Induction therapy seems to have ensured complete L-asparagine depletion for at least 15 days, irrespective of the mode of administration. However, re-induction therapy seems to have been inadequate.

As a consequence of this work, a pilot study with asparaginase Medac (an *E. coli* preparation) was performed at our institution in Aarhus. This preparation has a much longer half-life (23 h), which offers less frequent administration and/or the administration of a lower dose. Asparaginase Medac will be introduced in the new NOPHO protocol in the summer of 2001.

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