Cancer Chemother Pharmacol (2001) 48: 77–82
DOI 10.1007/s002800100286

ORIGINAL ARTICLE

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Pharmacokinetics of *Erwinia* asparaginase after intravenous and intramuscular administration

Received: 29 August 2000 / Accepted: 23 February 2001 / Published online: 9 May 2001
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Abstract *Purpose*: To describe the pharmacokinetics of *Erwinia* asparaginase (ASNase) after intravenous (i.v.) and intramuscular (i.m.) administration. *Methods*: A group of 29 children with newly diagnosed acute lymphoblastic leukemia (ALL) received *Erwinia* ASNase 30,000 IU/m^2 every day for 10 days during multiagent induction therapy. Of these patients, 13 received i.v. therapy and 16 received i.m. therapy. During the reinduction phase the patients received *Erwinia* ASNase 30,000 IU/m^2 twice a week for 2 weeks (Mondays and Thursdays) (8 patients in the i.v.-treated group and 11 patients in the i.m.-treated group). ASNase activity (spectrophotometric assay) was measured in plasma samples obtained from the patients at various times during therapy. *Results*: The estimated half-life was 6.4 ± 0.5 h ($n = 13$), the absorption rate after i.m. administration was found to limit elimination. The apparent volume of distribution corresponded well with the volume of plasma. The estimated clearance suggested that *Erwinia* ASNase is a low-clearance drug. Bioavailability after i.m. administration was (mean ± SEM) $27.0 \pm 4.5\%$ (range 11–61%; $n = 12$). *Conclusions*: In this study the pharmacokinetic parameters after i.v. and i.m. administration of *Erwinia* ASNase were determined based on a substantial number of patients. The present findings emphasize the importance of conducting proper pharmacokinetic studies before a new drug or a new preparation of a drug is introduced in a different schedule.

Keywords *Erwinia* ASNase · Elimination rate constant · Absorption rate constant · Volume of distribution · Bioavailability

Introduction

Since 1992 *Erwinia* ASNase has been used in the treatment of acute lymphoblastic leukemia (ALL) in childhood in the Nordic countries. L-Asparaginase (ASNase) hydrolyses the amino acid L-asparagine to aspartic acid and ammonia, thus depleting plasma of L-asparagine. L-ASNase is used in the treatment of ALL and lymphomas, as some malignant lymphoblasts are restricted in their synthesis of L-asparagine and therefore rely on an exogenous supply [3, 15]. As a result of L-asparagine depletion, protein synthesis is inhibited and the cells die.

The pharmacokinetics of ASNase have been most extensively studied for the *E. coli* preparations [1, 5, 6, 12, 14], the primary source of enzyme in most protocols, whereas the available information about the pharmacokinetics of *Erwinia* ASNase is sparse [1, 14]. Some kinetic studies have shown that *E. coli* ASNase obeys first-order kinetics [10, 12] but others have not [6, 12]. The half-life of *Erwinia* ASNase has been poorly described after i.v. administration [14], but well described after i.m. administration [1]. Information about clearance, volume of distribution, bioavailability and the absorption rate constant after i.m. administration of *Erwinia* ASNase is

This work was supported by The Aarhus University Research Foundation; The Institute for Clinical Experimental Research, University of Aarhus; The Danish Cancer Society; The M. Brogaard & Wife Foundation; The Gerda and Aage Haensch Foundation; The Beckett Foundation; The Danish Children's Cancer Foundation; and The Anders Hasselbalch Foundation

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not available in the literature. Studies comparing the pharmacokinetics of *Erwinia* ASNase after i.v. and i.m. administration have not been performed.

This report describes the pharmacokinetics of *Erwinia* ASNase 30,000 IU/m^2 given every day for 10 days during the induction phase, and 30,000 IU/m^2 given twice a week (Mondays and Thursdays) for 2 weeks during the reinduction phase for the higher risk patients. The drug was administered either by the i.v. route ($n = 13$) or the i.m. route ($n = 16$).

Materials and methods

Patients

The study included 29 children with newly diagnosed ALL (1.5 to 14 years of age). They all received conventional L-ASNase therapy according to the NOPHO-92 ALL protocol (The Nordic Society for Pediatric Hematology and Oncology, protocol initiated 1992). Some patients received *Erwinia* ASNase (Erwinase; Ipsen-Speywood Pharmaceuticals, Maidenhead, UK) 30,000 IU/m^2 i.v. (infusion over 3 h) and others received i.m. therapy every day for 10 days during the induction phase from day 37 until day 46 (in this report day 0 to day 9). During the first 36 days they all received i.v. vincristine and doxorubicin, oral prednisolone, and intrathecal methotrexate. During L-ASNase treatment prednisolone was tapered as the only other drug (total dose of approximately 2500 mg/m^2).

The higher risk patients also received *Erwinia* ASNase 30,000 IU/m^2 i.v. (infusion over 3 h) or i.m. twice a week (Mondays and Thursdays) for 2 weeks during the reinduction phase, i.e. protocol days 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 these periods they also received oral dexamethasone, i.v. 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 samples were stored at –70°C. Plasma samples from other centers were sent on dry ice.

During the induction phase blood samples were withdrawn as follows. For the 13 patients receiving i.v. therapy, blood samples were obtained on day 0 before ASNase administration, immediately after infusion had ended, and 5 and 24 h after the beginning of infusion of the first dose. On days 4 and 8 blood samples were taken before administration, and 5 and 24 h after the beginning of infusion. Finally, a blood sample was taken on day 13. From the 16 patients receiving i.m. therapy, blood samples were obtained on day 0 before ASNase administration, 0.5, 2 and 24 h after the first dose. On days 4 and 8 blood samples were taken before administration, and 2 and 24 h after. A blood sample was also taken on day 13.

During the reinduction phase blood samples were drawn on all days of therapy, which were days 0, 3, 7 and 10. After i.v. administration blood samples were taken before, immediately after infusion had ended, and 5 h from the start of infusion. A blood sample was also obtained on day 14. After i.m. administration blood samples were obtained before and 0.5 and 2 h after administration. A blood sample was also drawn on day 14.

Quantification of the enzyme activity using Nessler's reagent

The concentration of ASNase in plasma samples was measured using a spectrophotometric method, determining enzyme activity by the use of Nessler's reagent. Standard samples (6000, 4000, 2000, 1000, 500, 200 and 100 IU/l), a control, and plasma samples were diluted 1:5 in 0.05 *M* Tris-HCl (pH 8.5) to obtain a measurable working range (100–6000 IU/l) in which the concentrations in most of the samples were expected to lie, and 40 µl of the diluted samples, 110 µl 0.05 *M* Tris-HCl (pH 8.5) and 50 µl 0.04 *M* L-asparagine buffer solution were incubated for 30 min at 37°C. After the addition of 50 µl 15% trichloroacetic acid and centrifugation, 200 µl of the supernatant was added to 650 µl of redistilled autoclaved water. Finally, 100 µl of Nessler's reagent was added. The samples were allowed to stand for 15 min at room temperature before the optical density (OD) at 405 nm (triplicates in microplates, and read using an ELISA reader) was measured. Standard curves were constructed, plotting OD at 405 nm versus known asparaginase activities (IU/l) using polynomial regression (degree – 2) analysis. Samples with ODs above that corresponding to 6000 IU/l were reanalyzed using a standard curve with standard concentrations of 50,000, 40,000, 30,000, 20,000, 10,000 and 5,000 IU/l. These samples were diluted 1:20 in 0.05 *M* Tris-HCl.

The intraday CV, estimated by measuring one standard concentration (400 IU/l) 35 times on 1 day, was 2.7%. The interday CV, evaluated from triplicates (analysis of the same sample 20 times over 2 months), was found to be 5.6%. The interday CV at 100 IU/l (minimum detectable concentration) was 29.4%.

Calculations

All the pharmacokinetic parameters were estimated from plasma concentration-time data during induction therapy. During the reinduction phase data were insufficient to allow any estimations. On the assumption of a one-compartment model and first-order kinetics, the pharmacokinetics of *Erwinia* ASNase were evaluated after i.v. and i.m. administration.

Intravenous administration

The elimination rate constants (ke) for the 13 patients undergoing i.v. therapy were estimated from postinfusion concentrations-time data from one to three administrations during the induction phase. On day 0 (three postinfusion concentration-time points) ke was estimated by linear regression analysis. By linear regression analysis ke was also estimated from data from days 4 and 8 (two measurements each day). The mean ke [$ke_{mean} = (ke_{day\,0} + ke_{day\,4+8})/2$] was used to estimate C(T) (the plasma concentration at infusion stop) for the infusions on day 0 and day 4 + 8 by linear regression analysis. The plasma concentration C(t) during an infusion is given by [4]:

$$C(t) = \frac{\text{Dose}}{Vd * ke * T} * \left(1 - e^{-ke*t}\right) \quad (1)$$

where T is the infusion time. C(T), the concentration at the end of infusion, is given by:

$$C(T) = \frac{\text{Dose}}{Vd * ke * T} * \left(1 - e^{-ke*T}\right) \quad (2)$$

When infusion has ceased the concentration C(t) decreases according to the equation:

$$C(t) = C(T) * \left(e^{-ke*(t-T)}\right) \quad (3)$$

In the NOPHO-92 protocol the i.v. infusions during the induction phase were given over 3 h every day for 10 days. Therefore the trough concentration (C_{trough}) prior to each sequential administration, and the continued elimination of C_{trough} during the infusion time had to be included in the calculations. During the first i.v. infusion C(T) (the concentration at the time of infusion stop) was estimated by linear regression analysis. During later i.v. infusions (day 4 + 8) the $C(T)_{read}$ (given by the linear regression analysis) was adjusted for previous trough concentrations as follows:

$$C(T) = C(T)_{read} - C_{trough} * e^{-ke,mean*T} \quad (4)$$

Semilogarithmic plots of postinfusion concentration-time data were used to estimate ke when data were only available on either day 4 or day 8, in addition to day 0.

$C(T)_{mean}$ $[(C(T)_{day\ 0} + C(T)_{day\ 4+8})/2]$ was used to calculate the volume of distribution by rearrangement of Eq. 2:

$$Vd = \frac{Dose}{C(T) * ke * T} * \left(1 - e^{-ke*T}\right) \quad (5)$$

Clearance is given by:

$$Cl = ke * Vd \quad (6)$$

The elimination half-life, T1/2, was then calculated from:

$$T^1/_2 = \frac{\ln 2}{ke_{mean}} \quad (7)$$

The equations describing the concentration-time profiles during the induction phase (i.e. during infusion and after infusion) are as follows.

During infusion (t = 0→3 h):

$$C(t) = \frac{Dose}{Vd * ke * T} * \left(1 - e^{-ke*t}\right) + C_{trough} * e^{-ke*t} \quad (8)$$

After infusion (t = 3→24 h):

$$C(t) = C(T) * \left(e^{-ke*(t-T)}\right) + C_{trough} * e^{-ke*(t+T)} \quad (9)$$

For the definition of C(T) see Eq. 2.

In order to simulate the concentration-time profiles of plasma enzyme activity during 10 days of i.v. therapy with *Erwinia* ASNase, the calculated ke (mean value), Vd (mean value), and the C_{trough} calculated from Eq. 4 (t = 24 h) after the first dose were inserted into the equations. It was assumed that a steady-state trough concentration had been reached by the time of administration of the second dose (24 h is approximately 4×T1/2). During reinduction therapy the C_{trough} is approximately zero.

Intramuscular administration

On the assumption of a one-compartment model and first-order absorption and elimination kinetics, the plasma concentration after a single extravascular administration as function of time is given by the Bateman function:

$$C(t) = \frac{F * ka * dose}{Vd * (ka - ke)} * \left(e^{-ke*t} - e^{-ka*t}\right) \quad (10)$$

where F is bioavailability, i.e. the fraction of the administered dose reaching the systemic circulation. The mean values of ke and Vd from the i.v. data were inserted into this equation.

An apparent elimination rate constant (k_{app}) based on plasma concentration-time data obtained after the last dose allowed us to use non-linear regression analysis (the Bateman function) on five concentration-time points (0, 0.5, 2, 24 and 96 h) in order to estimate the absorption rate constant, ka, and a rather rough estimation of the bioavailability F after i.m. administration.

Simulation of plasma enzyme activity each day during 10 days of i.m. *Erwinia* ASNase therapy as function of time was performed by insertion of the estimated parameters into the following equation:

$$C(t) = \frac{F * ka * dose}{Vd * (ka - ke)} * \left(e^{-ke*t} - e^{-ka*t}\right) + C_{trough} * e^{-kapp*t} \quad (11)$$

Results

Intravenous administration

The half-life of *Erwinia* ASNase was found to be (mean ± SEM) 6.4 ± 0.5 h (range 3.7–9.3 h) based on data from the 13 children who received i.v. therapy. The estimated half-lives showed minor intraindividual variation, but major interindividual variation (Table 1). Because samples missing the half-life could only be estimated from data after one or two infusions for some patients. The volume of distribution, Vd, was (mean ± SEM) 1.35 ± 0.09 l/m^2 (range 0.82–1.72 l/m^2). When multiplying by the body surface area for each patient, the volume of distribution correlated well with plasma volume, assuming that the plasma volume was 4% of the body weight. Clearance was calculated to be (mean ± SEM) 0.16 ± 0.02 l/m^2 per h (range 0.09–0.32 l/m^2 per h). The concentration-time profiles of plasma enzyme activity during 10 days of i.v. therapy with *Erwinia* ASNase were simulated using Eqs. 8 and 9

Table 1 Estimated pharmacokinetic parameters for 13 children after administration of intravenous *Erwinia* ASNase during the induction phase (one, two or three infusions monitored per patient)

Patient no.	No. of infusions	Age (years)	Weight (kg)	Surface area (m^2)	$C(T)_{mean}$ (IU/l ×1000)[a]	ke (h^{-1})	T1/2 (h)	Vd (l/m^2)	Vd (l)	Plasma volume (l)[b]	Cl (l/m^2/h)
1	2	6	21	0.84	25 (22/28)	0.09	7.9	1.05	0.88	0.84	0.09
2	3	3.5	16	0.64	16 (17/16)	0.15	4.6	1.49	0.95	0.64	0.23
3	2	6.5	22	0.98	18 (20/16)	0.08	9.0	1.50	1.47	0.88	0.11
4[c]	2	1.5	13	0.50	15	0.15	4.7	1.58	0.79	0.52	0.23
5	1	4	17	0.70	19	0.12	5.9	1.30	0.91	0.68	0.15
6	1	12	40	1.25	20	0.09	7.7	1.30	1.63	1.60	0.12
7	2	9	29	0.95	16 (15/18)	0.09	7.7	1.60	1.52	1.16	0.14
8	2	12	40	1.25	17 (18/16)	0.07	9.3	1.58	1.98	1.60	0.12
9	2	12	48	1.53	30 (25/35)	0.13	5.2	0.82	1.25	1.92	0.11
10[c]	2	3	14	0.61	14	0.19	3.7	1.72	1.05	0.56	0.32
11	1	6.5	22	0.98	18	0.09	7.3	1.66	1.62	0.88	0.15
12	1	3	14	0.61	24	0.13	5.5	1.10	0.67	0.56	0.14
13	3	6	24	0.88	29 (32/25)	0.16	4.3	0.83	0.73	0.94	0.13
Mean ± SEM					0.12 ± 0.01	6.4 ± 0.5	1.35 ± 0.09	1.19 ± 0.12	0.98 ± 0.13	0.16 ± 0.02	

[a] Numbers in parentheses are the C(T) values on day 0, day 4, day 8 or day 4 + 8

[b] Assumed to be 4% of body weight

[c] Infusions on days 4 and 8, therefore only one C(T) from the linear regression analysis on the plasma concentration-time data from these 2 days is given

(Fig. 1). The same two equations were used to simulate concentration-time profiles during reinduction therapy (Fig. 2), where the patients received the same dose as during induction therapy, but only on days 0, 3, 7 and 10.

Intramuscular administration

The apparent elimination rate constant (k_{app}), which could be estimated from the data from only seven patients, was found to be (mean ± SEM) 0.81 ± 0.05 day^{-1} (range 0.77–0.99 day^{-1}). Comparison of this rate constant with the elimination rate constant from the i.v. data (which was 0.12 ± 0.01 h^{-1}, 2.88 day^{-1}) suggests a slow absorption phase. Absorption rate limits elimination, so that the decline in the plasma concentration reflects absorption rather than elimination. For some patients ka could not be estimated, and the concentration-time profiles after a single i.m. injection for these patients were constructed using the mean value for ka, 0.81 day^{-1}, for the other patients. Bioavailability after i.m. administration was determined for 12 patients using the Bateman function. The results are given in Table 2.

Simulation of plasma enzyme activity each day during 10 days of i.m, *Erwinia* ASNase therapy as function of time was done by insertion of the estimated parameters into Eq. 11 (Figs. 3 and 4). In Fig. 4 (reinduction) the line C = 100 IU/l is shown and represents the lowest value in the measurable working range for the method, and all activities < 100 IU/l were set to be 0 IU/l.

Discussion

E. coli-derived ASNase preparations are the primary source of enzyme in most treatment protocols of ALL worldwide, and only in cases of hypersensitivity reactions to these preparations is *Erwinia* ASNase or PEG ASNase administered. The available information about the pharmacokinetics of *Erwinia* ASNase is therefore sparse. The preparation was introduced into the NOPHO protocol in 1992 without any further investigations. Over-treatment with ASNase may cause side effects (coagulopathy, pancreatitis, hepatotoxicity [8, 13] and probably hypersensitivity reactions [7, 9]), and under-treatment (enzyme activities below 100 IU/l) may result in insufficient L-asparagine depletion and thus insufficient treatment [2, 11, 14].

In the present study the pharmacokinetics of *Erwinia* ASNase were evaluated in a substantial number of patients from whom only a few plasma samples could be drawn. The description of the pharmacokinetics after i.v. administration is based on the assumption of a one-compartment model and first-order kinetics. In order to perform optimal pharmacokinetic studies, blood samples should be drawn regularly during at least two

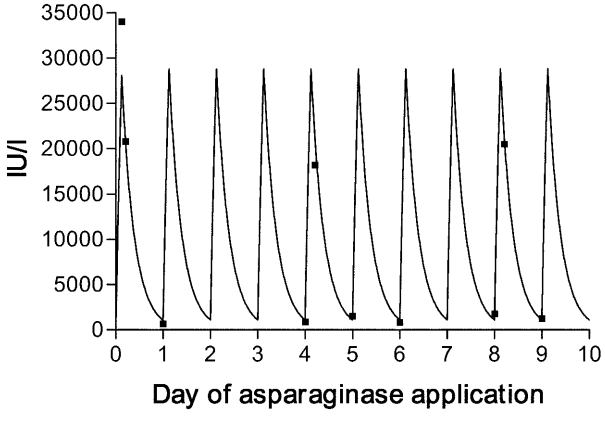

Fig. 1 Simulation of plasma enzyme activity after repeated i.v. administration during the induction phase (patient no. 13)

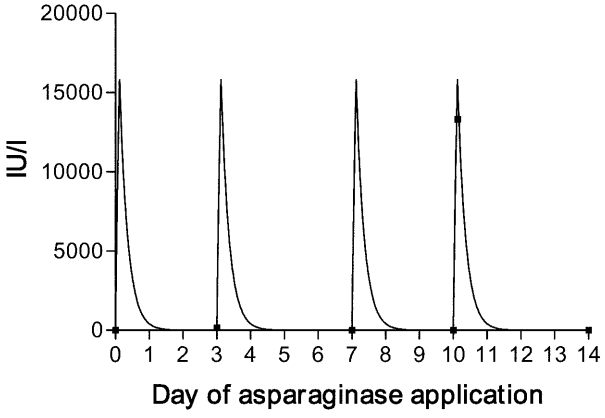

Fig. 2 Simulation of plasma enzyme activity after repeated i.v. administration during the reinduction phase (patient no. 2)

Table 2 Estimated pharmacokinetic parameters after i.m. administration of *Erwinia* ASNase on day 0 during the induction phase (one administration monitored per patient) (k_{app} apparent elimination rate constant)

Patient no.	k_{app} (day^{-1})	ka (day^{-1})	F (%) (bioavailability)
14	–	0.81[a]	28
15	–	0.81[a]	11
16	–	0.81[a]	21
17	0.63	0.63	–[b]
18	0.87	0.87	61
19	0.77	0.77	28
20	–	0.81[a]	46
21	–	0.81[a]	19
22	–	0.81[a]	11
23	–	0.81[a]	18
24	0.92	0.92	–[b]
25	–	0.81[a]	14
26	0.99	0.99	44
27	0.69	0.69	–[b]
28	0.82	0.82	–[b]
29	–	0.81[a]	23
Mean ± SEM	0.81 ± 0.05	–	27 ± 4.5

[a]Mean value of the estimated apparent elimination rate constant
[b]Insufficient samples for non-linear regression analysis

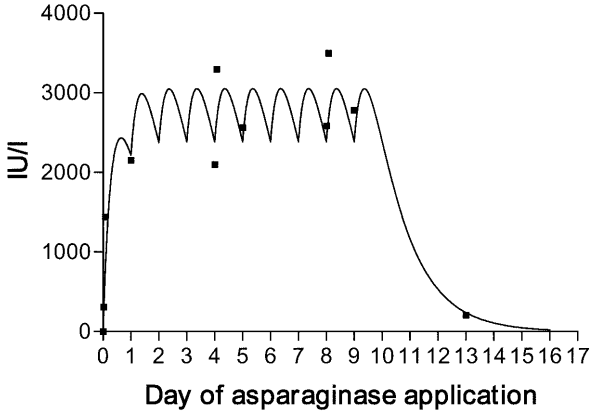

Fig. 3 Simulation of plasma enzyme activity after repeated i.m. administration during the induction phase (patient no. 5)

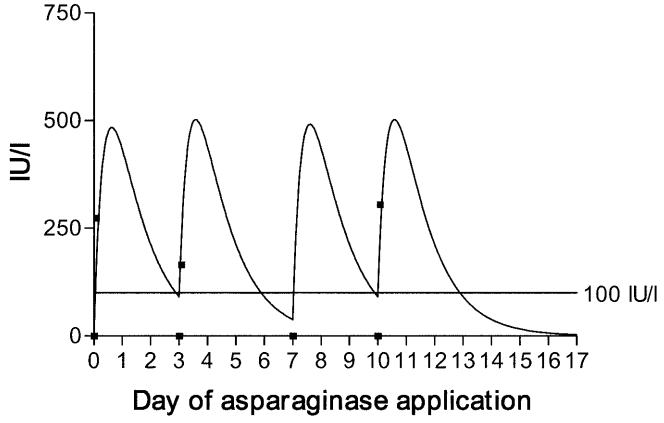

Fig. 4 Simulation of plasma enzyme activity after repeated i.m. administration during the reinduction phase (patient no. 9)

half-lives from each patient. In the present study, however, it was not possible ethically to have more blood samples withdrawn from the children. The estimated half-life based on data from 13 children who received i.v. therapy was 6.4 ± 0.5 h, which was comparable with the half-life found by Werber [14] of 7.2 h (mean from two patients). The half-life after i.m. administration has been reported to be longer (15.5 h, ten patients) [1]. In the present study the elimination half-life was assumed to be the same regardless of the mode of administration, but when the enzyme is administered i.m. (or s.c.), the absorption phase might influence the apparent rate of elimination. The absorption rate after i.m. administration was found to limit elimination, which seems plausible as ASNase is a large protein, and therefore is absorbed slowly. The volume of distribution was found to correspond well with the plasma volume, which also has been found after i.v. administration of *E. coli* ASNase [5, 6].

The simulations of plasma enzyme activity as a function of time (Figs. 1, 2, 3 and 4) are attempts to describe the expected enzyme activity during therapy based on the estimated pharmacokinetic parameters, and demonstrate that the dose given during the induction phase resulted in extremely high plasma concentrations after both i.v. and i.m. administration, and that the dose and schedule during substantial periods of the reinduction phase resulted in absent (i.v. group) or low (i.m. group) enzyme activities. Riccardi et al. [11] have shown that plasma enzyme activities above 100 IU/l ensure L-asparagine depletion from the body fluid compartments. So in the present setting, the patients were over-treated during induction therapy (Figs. 1 and 3) and under-treated during reinduction therapy (Figs. 2 and 4).

In conclusion, the pharmacokinetics of *Erwinia* ASNase after i.v. and i.m. administration were determined, adding to the available information. Parameters, such as the half-life and the volume of distribution, were confirmed in our study. The present study demonstrated the importance of conducting pharmacokinetic studies before a new drug or a new preparation of a drug is introduced in a different schedule in order to secure the patients optimal treatment.

Acknowledgements We thank Karen Busch for excellent technical assistance. We also thank the nursing staff at the Department of Pediatric Oncology at Skejby Hospital, Aarhus, Denmark, at Odense Hospital, Odense, Denmark, and at Rigshospitalet, Copenhagen, Denmark, for collecting blood samples from the patients.

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