High Incidence of Symptomatic Hyperammonemia in Children with Acute Lymphoblastic Leukemia Receiving Pegylated Asparaginase

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Received: 27 February 2012 / Revised: 14 May 2012 / Accepted: 16 May 2012 / Published online: 1 July 2012
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Abstract Asparaginase is a mainstay of treatment of childhood acute lymphoblastic leukemia. Pegylation of asparaginase extends its biological half-life and has been introduced in the newest treatment protocols aiming to further increase treatment success. Hyperammonemia is a recognized side effect of asparaginase treatment, but little is known about its incidence and clinical relevance. Alerted by a patient with severe hyperammonemia after introduction of the new acute lymphoblastic leukemia protocol, we analyzed blood samples and clinical data of eight consecutive patients receiving pegylated asparaginase (PEG-asparaginase) during their treatment of acute lymphoblastic leukemia. All patients showed hyperammonemia ($>50 \mu\text{mol/L}$) and seven patients (88 %) showed ammonia concentrations $>100 \mu\text{mol/L}$. Maximum ammonia concentrations ranged from 89 to $400 \mu\text{mol/L}$. Symptoms varied from mild anorexia and nausea to headache, vomiting, dizziness, and lethargy and led to early interruption of PEG-asparaginase in three

patients. No evidence of urea cycle malfunction was found, so overproduction of ammonia through hydrolysis of plasma asparagine and glutamine seems to be the main cause. Interestingly, ammonia concentrations correlated with triglyceride values ($r = 0.68$, $p < 0.0001$), suggesting increased overall toxicity.

The prolonged half-life of PEG-asparaginase may be responsible for the high incidence of hyperammonemia and warrants future studies to define optimal dosing schedules based on ammonia concentrations and individual asparagine and asparaginase measurements.

Introduction

In the past four decades, overall survival of childhood acute lymphoblastic leukemia (ALL) has improved dramatically from 34 % in the early 1970s to 86 % in the year 2004 (Kamps et al. 2010). Addition of asparaginase to treatment protocols has contributed significantly to this improved outcome (Muller and Boos 1998).

Asparaginase depletes plasma of the nonessential amino acid asparagine by hydrolyzing it into aspartic acid and ammonia. Since leukemic cells possess insufficient asparagine synthetase activity, an intracellular deficiency of asparagine leads to inhibition of protein synthesis and subsequent cell death (Prager and Bachynsky 1968).

Three different preparations of asparaginase are used in current ALL treatment protocols: native *Escherichia coli* L-asparaginase, *Erwinia chrysanthemi* L-asparaginase, and pegylated asparaginase (PEG-asparaginase). Pegylation of L-asparaginase reduces immunogenic potential and extends the half-life of the enzyme activity from 1.3 days to 5.7 days (Asselin et al. 1993).

Communicated by: Claude Bachmann

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DCOG - INTENSIFICATION/CONTINUATION MR PATIENTS part 1

Fig. 1 Schematic drawing of the Dutch Childhood Oncology Group intensification/continuation of medium-risk patients. This part of therapy starts 20 weeks after diagnosis. *DEXA* dexamethasone, *VCR* vincristine, *DOX* doxorubicine, *MTX* methotrexate, *PEG-ASP* PEG-asparaginase;

6-MP 6 mercaptopurine, *ARA-C* cytarabine, *DAF* di-adreson-F, *PO* per os, *IV* intravenously, *push* rapid injection; *1 h* infusion during 1 h, *ITH* intrathecal

Despite its proven efficacy, several side effects of asparaginase treatment have been reported: hypersensitivity reactions, disturbed liver functions, coagulation disorders, pancreatitis, hyperlipidemia, and silent inactivation by antibodies (Muller and Boos 1998).

Hyperammonemia due to asparaginase therapy has been described in a number of case reports (Leonard and Kay 1986; Alvarez and Zimmerman 2000; Laterza et al. 2003; Jorck et al. 2011), but little is known about its incidence, causative mechanism, and clinical relevance. The influence of the prolonged half-life of PEG-asparaginase on the duration and severity of hyperammonemia is unknown as well. Therefore, we studied ammonia concentrations and other metabolic parameters in pediatric ALL patients receiving PEG-asparaginase and observed hyperammonemia ($>50 \mu\text{mol/L}$) in all patients and ammonia concentrations $>100 \mu\text{mol/L}$ in seven out of eight patients.

Design and Methods

After presentation of the index case (patient A), we analyzed ammonia concentrations in seven consecutive patients treated in our hospital and assigned to the medium risk group of the Dutch Childhood Oncology Group (DCOG) ALL 10 protocol during their treatment with PEG-asparaginase. Measurements were conducted between 2007 and 2009.

The DCOG ALL 10 protocol is a protocol based on the pediatric ALL protocol of the German Berlin-Frankfurt-

Münster (BFM) Group, consisting of induction, high-dose methotrexate, followed by minimal residual disease-based assignment to standard, medium, or high-risk intensification and continuation treatment. The outline of the intensification/continuation treatment for medium-risk-group patients is shown in Fig. 1.

Ammonia concentrations were analyzed before the first administration of PEG-asparaginase, at least once, 1 week after PEG-asparaginase when also dexamethasone was administered in the previous week (week 2, 8, 14, 20, or 26) as well as 1 week after PEG-administration when no dexamethasone was administered (week 4, 6, 10, 12, 16, 18, 22, 24, 28, or 30). Hyperammonemia was defined as ammonia concentrations $>50 \mu\text{mol/L}$ and clinical significant hyperammonemia as ammonia concentrations $>100 \mu\text{mol/L}$ (Laterza et al. 2003; Cohn and Roth 2004; Steiner et al. 2007). At the above-mentioned time points, we also measured liver enzymes (alanine transaminase, aspartate transaminase, gamma glutamyl transferase, and alkaline phosphatase), bilirubin, total amylase, lipase, triglycerides, and cholesterol. In case of elevated ammonia concentrations or symptoms or signs suggestive of hyperammonemia, repeated ammonia measurements were performed. Blood samples were transported to the lab, centrifuged in the cold, and immediately stored at -20°C to deactivate the effect of PEG-asparaginase. Blood samples for ammonia analysis were generally taken 3–4 h after the last meal and analyzed by standardized enzymatic-photometric assay using a DxC 800 analyzer (Beckman Coulter diagnostics division, Brea, CA, USA).

To rule out secondary abnormalities such as urea cycle disorders, organic acidurias or fatty acid oxidation disorders, plasma amino acids, acylcarnitine concentrations, and, in six patients, urine orotic acid excretion were measured. In order to deactivate the PEG asparaginase in vitro, the blood sample tubes for amino acids and ammonia were immediately put in ice water, rapidly transported to the lab, centrifuged in the cold and the plasma deproteinized before storing the supernatant at -20°C until analysis.

Symptoms and signs associated with hyperammonemia, like headache, lethargy, nausea, vomiting, dizziness, and neurological symptoms were documented in the patient files. A waiver of the requirement for informed consent was granted by the Institutional Review Board of the University Medical Center Utrecht, which reviewed the study.

Results and Discussion

Patient characteristics and results are shown in Tables 1 and 2. Hyperammonemia was detected in all patients and ammonia concentrations $>100\text{ }\mu\text{mol/L}$ in seven out of eight consecutive patients with acute ALL during treatment with PEG-asparaginase. All patients with clinically significant hyperammonemia displayed symptoms. Although generally mild, symptoms were severe in three patients (A, C, and D), leading to hospital admission and discontinuation of PEG-asparaginase therapy.

Hyperammonemia after asparaginase therapy was first reported by Leonard and Kay in 1986 (Leonard and Kay 1986), followed by a number of other cases (Alvarez and Zimmerman 2000; Laterza et al. 2003; Jorck et al. 2011). Although the exact incidence of symptomatic hyperammonemia after asparaginase therapy is unknown, the small number of cases reported suggests a low frequency. Indeed, symptomatic hyperammonemia is not mentioned in recent overviews of toxicity of asparaginase treatment (Muller and Boos 1998; Earl 2009; Rytting 2010). In contrast, our observations suggest that symptomatic hyperammonemia is the rule rather than the exception after PEG-asparaginase administration.

This discrepancy may in part be a consequence of the tendency to analyze ammonia levels only in case of severe symptoms suggestive of hyperammonemia. In support, the only study that measured ammonia concentrations regardless of symptoms observed a similar high frequency of hyperammonemia: 7 out of 10 patients described by Steiner et al. reached ammonia concentrations $>100\text{ }\mu\text{mol/L}$ 1 day after L-asparaginase administration (Steiner et al. 2007). However, these patients all remained without symptoms, contrasting

with the overt symptoms in our patients. One could argue that most symptoms in our patients were nonspecific and could be attributable, at least in part, to other chemotherapeutics being administered. However, patients themselves reported these symptoms to be more severe than they experienced during other parts of leukemia treatment. Moreover, all symptoms disappeared after cessation of PEG-asparaginase administration. We speculate that the higher incidence of clinical symptoms of hyperammonemia in this study is secondary to the use of PEG-asparaginase. Due to the prolonged half-life of PEG-asparaginase, ammonia concentrations may not return to normal before the next dose is administered and ammonia toxicity may accumulate. As shown in Fig. 2, patients B, D, and F show a pattern consistent with this hypothesis. The linear correlation between the number of received doses of PEG-asparaginase and ammonia concentrations (Fig. 3) also supports this hypothesis. The half-life of PEG-asparaginase is likely to be longer than the above-mentioned 5.7 days, since asparagine was still depleted 3–4 weeks after the last dose of PEG-asparaginase in three out of three patients with available data. Interestingly, ammonia concentrations correlated with triglyceride values ($r = 0.68$, $p < 0.0001$), suggesting increased overall toxicity.

Little is known about the exact mechanism through which asparaginase causes hyperammonemia. Normally, ammonia is removed rapidly from the circulation by being incorporated to glutamine in brain and muscle after which it is converted to urea via the urea cycle in the liver (Lockwood et al. 1979). Most likely, the altered equilibrium between glutamine and glutamate in the patients studied, favoring glutamate, contributes to the expansion of the ammonia pool. Given the plasma concentrations of asparagine and glutamine, the production of ammonia may well exceed the detoxification capacity in the liver. Indeed, analysis of plasma amino acid concentrations showed a complete depletion of asparagine and a marked reduction in glutamine concentration. The concentrations of aspartate (range: 21–50 $\mu\text{mol/L}$, reference values: 3–15 $\mu\text{mol/L}$, five out of six patients) and glutamate (range: 169–733 $\mu\text{mol/L}$, reference values: 17–69 $\mu\text{mol/L}$, six out of six patients) were strongly increased. Plasma concentrations of all urea cycle intermediates and excretion of orotic acid remained normal during therapy in all samples analyzed, arguing against malfunction of the urea cycle. Malnutrition during chemotherapy and proteolysis may induce catabolism, theoretically further increasing the ammonia load. However, we found no deficits of other amino acids in plasma, as would be expected in malnourished patients. Moreover, in case of weight loss during chemotherapy, tube feeding is instituted instantly in our hospital. Plasma acylcarnitine concentrations were also

Table 1 Patient characteristics and description of toxicity. *M/F* male or female, *ALL* Acute Lymphoblastic Leukemia, *PEG-asparaginase* Pegylated asparaginase, *CR* complete remission

Patient	M/F	Age at diagnosis (years)	Diagnosis	Maximum documented ammonia concentration ($\mu\text{mol/L}$)	Number of PEG-asparaginase doses before maximum ammonia concentration	Symptoms	Treatment for hyperammonemia	Other toxicities during PEG-asparaginase therapy	Outcome
A (index case)	F	5	Pre B ALL	400	6	Headache, nausea, vomiting, lethargy, admission	Lactulose (1.8 mL/kg), protein restricted diet; Omission of eight doses of PEG-asparaginase	Severe hypertriglyceridemia (1,946 mg/dL), hypercholesterolemia (703 mg/dL), elevated gamma GT (2,163 U/L)	In CR, 33 months after end of therapy
B	F	3	Pre B ALL	258	13	Mild lethargy, anorexia, weakness	None	Moderate hypertriglyceridemia (690 mg/dL)	In CR, 30 months after end of therapy
C	M	3	Common ALL	248	8	Lethargy, nausea	Omission of 1 dose of PEG-asparaginase	Moderate hypertriglyceridemia (717 mg/dL)	In CR, 28 months after end of therapy
D	F	12	Pre B ALL	281	14	Dizziness, lethargy, headache, leading to admission	Lactulose, protein restricted diet; omission of 1 dose of PEG-asparaginase	Mild hypercholesterolemia (286 mg/dL)	In CR, 27 months after end of therapy
E	M	3	Common ALL	366	5	Mild nausea, headache, malaise	None	Severe hypertriglyceridemia (3,645 mg/dL), Hypercholesterolemia (792 mg/dL), thrombosis vena axillaris, elevated gamma GT (1,280 U/L), hypoglycemia (2.4 mmol/L)	In CR, 21 months after end of therapy
F	F	9	Pre B ALL	320	13	Mild lethargy, nausea	None	Severe hypertriglyceridemia (8,725 g/dL), Hypercholesterolemia (448 mg/dL)	In CR, 21 months after end of therapy
G	M	5	Common ALL	123	2	Anorexia	None	Severe hypertriglyceridemia (1,991 mg/dL), Hypercholesterolemia (471 mg/dL)	In CR, 18 months after end of therapy
H	F	4	Common ALL	89	1	None	Not applicable	None	In CR, 17 months after end of therapy

Table 2 Results of metabolic studies in described patients. Reference values: asparagine 34–94 $\mu\text{mol/L}$; aspartate 1–17 $\mu\text{mol/L}$; glutamine 333–809 $\mu\text{mol/L}$; glutamate 14–78 $\mu\text{mol/L}$

Patient	Asparagine and aspartate	Glutamine and glutamate	Urine orotic acid
A (index case)	Asparagine: not available Aspartate: elevated (21 $\mu\text{mol/L}$)	Glutamine: normal (812 $\mu\text{mol/L}$) Glutamate: not available	Normal
B	Not available	Not available	Not available
C	Asparagine: complete depletion (0 $\mu\text{mol/L}$) Aspartate: elevated (30 $\mu\text{mol/L}$)	Glutamine: decreased (325 $\mu\text{mol/L}$) Glutamate: elevated (169 $\mu\text{mol/L}$)	Normal
D	Asparagine: complete depletion (0 $\mu\text{mol/L}$) Aspartate: elevated (30 $\mu\text{mol/L}$)	Glutamine: decreased (1,295 $\mu\text{mol/L}$) Glutamate: elevated (653 $\mu\text{mol/L}$)	Normal
E	Asparagine: complete depletion (0 $\mu\text{mol/L}$) Aspartate: normal (13 $\mu\text{mol/L}$)	Glutamine: decreased (189 $\mu\text{mol/L}$) Glutamate: elevated (441 $\mu\text{mol/L}$)	Normal
F	Asparagine: complete depletion (0 $\mu\text{mol/L}$) Aspartate: elevated (24 $\mu\text{mol/L}$)	Glutamine: decreased (102 $\mu\text{mol/L}$) Glutamate: elevated (733 $\mu\text{mol/L}$)	Not available
G	Asparagine: complete depletion (0 $\mu\text{mol/L}$) Aspartate: elevated (21 $\mu\text{mol/L}$)	Glutamine: decreased (116 $\mu\text{mol/L}$) Glutamate: elevated (463 $\mu\text{mol/L}$)	Normal
H	Asparagine: complete depletion (0 $\mu\text{mol/L}$) Aspartate: normal (8 $\mu\text{mol/L}$)	Glutamine: decreased (54 $\mu\text{mol/L}$) Glutamate: elevated (263 $\mu\text{mol/L}$)	Normal

Fig. 2 Ammonia concentrations in each patient related to the week of treatment. Arrows denote PEG-asparaginase administration. In patient A, 8 gifts were omitted; in patients C and D, the last gift was omitted

Fig. 3 Correlative analysis of ammonia concentrations 1 week after PEG-asparaginase administration and week of treatment

normal in all samples analyzed, excluding other known causes of secondary hyperammonemia.

In conclusion, our data clearly indicate that the clinical and biochemical consequences of asparaginase treatment are likely to be more pronounced when using PEG-asparaginase because of its prolonged half-life. Future studies are indicated to define the exact incidence of symptomatic hyperammonemia in patients receiving PEG-asparaginase, to define the relationship between hyperammonemia and clinical symptoms and to elucidate the link between hyperammonemia and hypertriglyceridemia. Finally, therapeutic drug monitoring by measuring ammonia concentrations, asparagine concentrations, and asparaginase levels might be of clinical use to define if lower doses of or longer intervals between PEG-asparaginase gifts are feasible. In the next DCOG ALL treatment protocol (ALL 11), therapeutic drug monitoring to optimize dosing of PEG-asparaginase will be studied.

Take Home Message

Pegylation of asparaginase, used in the treatment of childhood acute lymphoblastic leukemia, seems to result in a high incidence of symptomatic hyperammonemia due to its prolonged half-life.

Authorship and Disclosures

KH designed the research, collected and analyzed data, and wrote the chapter. BP performed and analyzed laboratory studies and revised the manuscript critically. TK revised the manuscript critically. PH designed the research, analyzed data, and wrote the chapter. MB designed the research, analyzed data, and wrote the chapter. The authors declare no conflict of interest.

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