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ORIGINAL ARTICLE

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Anti-*Erwinia* asparaginase antibodies during treatment of childhood acute lymphoblastic leukemia and their relationship to outcome: a case-control study

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Abstract *Purpose*: A case-control study was performed to determine whether patients who had been treated with *Erwinia* asparaginase as part of their treatment for childhood acute lymphoblastic leukemia (ALL) and who showed relapsed of their disease more often developed anti-asparaginase antibodies than patients who remained in remission. *Methods*: A group of 13 patients who showed relapsed of their disease (median follow-up 35 months) were randomly matched with control patients of the same risk group (two control patients to each case), who had received therapy of the same intensity during the same period (median follow-up 70 months). Anti-*Erwinia* asparaginase antibodies were measured (ELISA method) during maintenance therapy after asparaginase treatment (30,000 IU/m^2 daily for 10 days in all patients plus twice weekly for 2 weeks in intermediate-risk and high-risk ALL patients). *Results*: The overall incidence of anti-*Erwinia* asparaginase antibodies was 8% (3 of 39 patients). There was no statistically significant difference in the incidence of antibody formation between patients who had suffered relapse (1 of 13) and those who had not (2 of 26). In two of the three patients who developed antibodies, the antibodies disappeared after some time, whereas one patient had measurable antibody levels for more than a year after asparaginase therapy. *Conclusions*: In this study, the development of anti-*Erwinia* asparaginase antibodies was rare and was unrelated to the risk of relapse.

Keywords *Erwinia* asparaginase · Antibody · Child · Leukemia · Relapse

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Introduction

Asparaginase is part of the multiagent therapy of acute lymphoblastic leukemia (ALL) [5]. Approximately 30% of patients have been reported to develop antibodies against this foreign protein [11]. In some patients anti-asparaginase antibodies result in hypersensitivity reactions, whereas in others the presence of antibodies does not result in allergic reactions, but is correlated with rapid asparaginase clearance (“silent antibodies”) [1]. Allergic reactions usually necessitate discontinuation of asparaginase therapy or a change of preparation. An association between hypersensitivity reactions, anti-asparaginase antibodies, and altered pharmacokinetics has been found in many studies [1, 2, 6, 9]. The presence of neutralizing antibodies or the withdrawal of subsequent doses after an allergic reaction could result in an inferior cure rate.

Asselin [1] found that high antibody levels are correlated with rapid asparaginase clearance and a significantly lower response rate in relapsed patients,

whereas Woo et al. [11] found no difference in 4-year event free survival (EFS) among patients with newly diagnosed ALL who did and did not develop antibodies, or among patients who did and did not develop allergic reactions.

The present study of the relationship between anti-asparaginase antibodies and relapse was performed as a case-control study, including patients who had received *Erwinia* asparaginase as part of their treatment for ALL.

Patients and methods

Patients were eligible for the study if they: (1) were diagnosed with non-B cell ALL after 1 January 1992 and entered maintenance therapy before 1 January 1997; (2) were treated at one of the four pediatric oncology centers in Denmark; (3) were treated according to the NOPHO ALL-92 program (Nordic Society for Pediatric Haematology and Oncology, protocol initiated in 1992) [5]; and (4) had a blood sample taken during the first 6 months of maintenance therapy available for anti-*Erwinia* asparaginase antibody measurements.

Of 113 patients eligible to enter the study, 13 who had shown relapse of their disease before 31 December 2000 were included in this case-control study (cases). The 13 patients included 7 boys and 6 girls comprising three standard-risk (SR) patients (median age 4.2 years, range 3.3–5.6 years; median white blood cell count (WBC) at diagnosis 4.1×10^9/l, range 2.1–6.0×10^9/l), seven intermediate-risk (IR) patients (median age 6.2 years, range 3.0–9.3 years; median WBC 20.0×10^9/l, range 10.0–45.0×10^9/l), and three high-risk (HR) patients (median age 5.2 years, range 3.8–5.2 years; median WBC 121.0×10^9/l, range 82.0–399.0×10^9/l). All the relapsed patients were diagnosed with B-lineage disease. The risk classification (i.e. intensity of therapy) was based on clinical characteristics at diagnosis (SR were those aged 2.0–9.9 years and WBC $<10\times10^9$/l; IR were those aged 1.0–1.9 years or age 10.0–14.9 years and/or WBC 10–49×10^9/l; and HR were those with WBC $\geq50\times10^9$/l, T cell disease, mediastinal or CNS or testicular or lymphomatous disease, t(4,11), t(9,22), a day-14 bone marrow with more than 25% lymphoblasts, or a day-29 bone marrow with more than 5% lymphoblasts).

The control group of 26 patients (2 per case) was randomly (computer-based) selected from the remaining 100 patients matched for risk group. The group included 13 boys and 13 girls comprising 6 SR patients (median age 3.5 years, range 2.5–5.5 years; median WBC 6.2×10^9/l, range 1.1–9.0×10^9/l), 14 IR patients (median age 5.5 years, range 1.2–15.9 years; median WBC 11.8×10^9/l, range 1.7–41.0×10^9/l), and 6 HR patients (median age 5.3 years, range 3.5–11.5 years; median WBC 100.0×10^9/l, range 80.0–250.0×10^9/l). Two of the control patients had T-lineage immunophenotype and the remaining 24 patients had B-lineage disease. The included patients were diagnosed with ALL from March 1992 until July 1996. One patient relapsed during maintenance therapy. At the end of follow-up (31 December 2000) all patients were off therapy. The median length of follow-up from diagnosis was 35 months (range 17–79 months) for the relapsed patients and 70 months (range 53–105 months) for the control patients.

Therapy

During the induction phase all newly diagnosed ALL patients received *Erwinia* asparaginase 30,000 IU/m^2 i.v. or i.m. daily for 10 days from day 37 to day 46. All IR and HR patients received a second course of *Erwinia* asparaginase treatment during their second induction therapy which consisted of 30,000 IU/m^2 i.v. or i.m. twice a week for 2 weeks (Mondays and Thursdays) from day 169 to day 179 (IR patients) or from day 232 to day 242 (HR patients). The NOPHO ALL-92 protocol has been described in detail by Gustafsson et al. [5].

Anti-*Erwinia* asparaginase antibodies

During maintenance therapy, plasma samples were measured for antibodies. The plasma samples had been taken in connection with the NOPHO ALL-92 study [10], and stored at –70ºC. Plasma samples had been taken at the beginning of maintenance therapy and then at 3-month intervals during maintenance therapy until death, relapse, or end of treatment. The plasma sample taken at the start of maintenance therapy was measured for antibodies. If a blood sample had not been drawn at this time point, the first available blood sample was analyzed. For patients who had measurable antibodies, all samples available during maintenance therapy were analyzed.

Determination of anti-*Erwinia* asparaginase antibodies was performed at the Department of Pharmacology, University of Aarhus. The antibodies (IgG type) were measured by an ELISA method [4, 9]. The detection limit was 1.56 U/ml.

Statistics

Differences in the incidence of antibody formation between the two groups (cases and controls) were compared using Fisher's Exact test. Differences between cases and controls with respect to the number of days between the last asparaginase dose and the first measurement of antibodies, median age and median WBC at diagnosis, were analyzed using the Mann-Whitney Rank Sum test.

Results

Three patients had measurable antibodies and the remaining 36 patients did not (antibody titer below 1.56 U/ml; Table 1). For two of the three patients who had measurable antibodies, later plasma samples were available at 3-month intervals for determination of antibodies. Figure 1 shows that the antibodies disappeared after a few months in one patient (control 8), whereas in another patient (control 12) the antibody titer remained high in all five measurements for more than a year. For the last patient (case 4), only one plasma sample was available.

None of the SR patients, who had received only one course of asparaginase, had measurable levels of antibodies during maintenance therapy. Three IR patients developed antibodies, and none of the HR patients did.

Among the patients who showed relapsed of their disease, one developed antibodies (1/12, exact 95% confidence limits 0.21–38.48), and among the control patients, two developed antibodies (2/26, exact 95% confidence limits 1.03–27.00; $P>0.05$; Table 1). The interval from the last dose of asparaginase to the determination of antibodies was significantly different ($P<0.05$) between the cases (median interval 103 days) and controls (median interval 68 days). Age and WBC at diagnosis did not differ significantly between cases and controls.

Discussion

Two previously reported studies deal with the possible relationship between anti-asparaginase antibody formation and prognosis. Woo et al. [11] performed a study

Table 1. Anti-*Erwinia* asparaginase antibody titer and clinical characteristics of case and control patients

Case/ Control	Gender	Age (years)	Risk group	Days after ASNase	Antibody titer (U/ml)	Relapse (months after cessation of therapy)
Case 1	M	3.3	SR	46	0	2
Case 2	M	5.6	SR	61	0	30
Case 3	M	4.2	SR	82	0	4
Case 4	M	3.0	IR	141	2.1	31
Case 5	F	6.0	IR	74	0	36
Case 6	M	8.7	IR	116	0	56
Case 7	F	9.3	IR	130	0	21
Case 8	F	6.2	IR	70	0	9
Case 9	M	3.2	IR	207	0	6
Case 10	F	8.3	IR	147	0	Relapse during maintenance therapy
Case 11	M	5.2	HR	102	0	11
Case 12	F	5.2	HR	108	0	18
Case 13	F	3.8	HR	103	0	21
Control 1	M	2.5	SR	64	0	–
Control 2	F	2.6	SR	62	0	–
Control 3	F	2.5	SR	46	0	–
Control 4	M	5.5	SR	59	0	–
Control 5	F	4.2	SR	46	0	–
Control 6	M	3.5	SR	68	0	–
Control 7	M	5.5	IR	75	0	–
Control 8	F	14.7	IR	74	12.2	–
Control 9	F	15.9	IR	67	0	–
Control 10	F	1.8	IR	74	0	–
Control 11	F	14.2	IR	74	0	–
Control 12	M	2.5	IR	75	91.3	–
Control 13	F	3.6	IR	46	0	–
Control 14	F	3.6	IR	62	0	–
Control 15	M	5.8	IR	63	0	–
Control 16	F	1.2	IR	53	0	–
Control 17	M	11.3	IR	51	0	–
Control 18	F	4.1	IR	56	0	–
Control 19	M	6.7	IR	60	0	–
Control 20	M	4.7	IR	57	0	–
Control 21	F	8.9	HR	112	0	–
Control 22	M	5.8	HR	113	0	–
Control 23	M	3.5	HR	110	0	–
Control 24	M	5.3	HR	125	0	–
Control 25	M	11.5	HR	112	0	–
Control 26	F	3.6	HR	81	0	–

with the aim of determining the relationship between anti-*E. coli* asparaginase antibodies or hypersensitivity reactions and EFS, and found that EFS did not differ between patients who did and did not have antibodies. Similarly, EFS did not differ between patients who did and did not develop allergic reactions.

Asselin [1] reported that, in a study of patients with relapsed ALL, 23 patients with high IgG antibody titer to asparaginase cleared asparaginase more rapidly and had a significantly poorer response rate (complete and partial remissions) compared to 14 patients with low titers (26% vs 64%). All patients had previously received asparaginase.

In the present study, we found an overall incidence of antibody formation of 8% (3/39) or 10% (3/30) if the SR patients were excluded. In a previous prospective study of *Erwinia* asparaginase, we found an incidence of 21% (4/19) for IR and HR patients [9]. Both incidences are lower than that found by Woo et al. in their study of *E. coli* asparaginase (36%) [11]. The lower incidences in our studies may have been due to (1) the use of *Erwinia* asparaginase in the NOPHO protocol, which is thought to be less immunogenic than the *E. coli* preparations, (2) the larger dose (30,000 IU/m^2 daily for 10 days) compared to other protocols, (3) the simultaneous administration of dexamethasone (10 mg/m^2 per day) during consolidation, and (4) the interval between the last asparaginase dose and the day of measurement (Table 1), as the antibodies may be cleared during this interval in some patients.

In our study, we found that none of the SR patients (cases and controls), who received asparaginase during the induction phase only, developed antibodies. This observation is in accordance with the results of a previous study [9] in which it was found that none of the patients treated by the NOPHO ALL-92 protocol developed antibodies during induction therapy (the first exposure to asparaginase). Three IR patients (one case and two controls) developed antibodies, whereas none of the HR patients had measurable antibodies. Patients

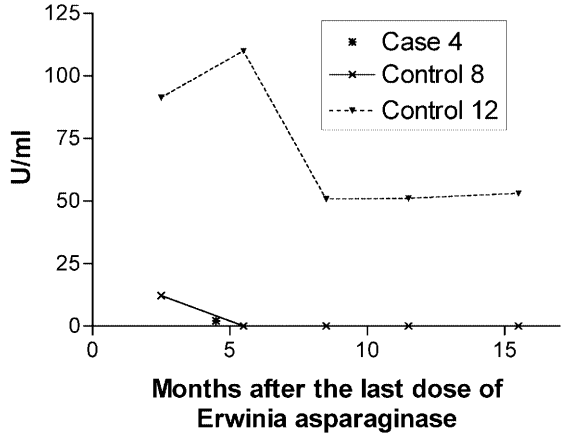

Fig. 1. The concentration of anti-*Erwinia* asparaginase antibodies during maintenance therapy

with a B-lineage immunophenotype have been shown to be more likely to develop antibodies than patients with T-lineage ALL [11], explaining a lower incidence of antibody formation (and in our study its absence) in HR patients. There was no significant difference in the incidence of antibody formation between the relapsed patients and the patients who remained in remission.

For two of the three patients who developed antibodies, blood samples were available at 3-month intervals for determination of the antibody titer one to four times (Fig. 1) after the initial determination. The titer had disappeared after one measurement in one patient (control 8), whereas we observed a continuously high antibody titer in one patient (control 12). Caver et al. [3] have reported that patients during treatment for ALL suffer from a profound abnormality of the B/T lymphocyte ratio (B cell lymphopenia and T cell numbers normal or marginally low and accounting for up to 98% of the lymphocyte populations). Therefore some patients may have lost their antibodies during maintenance therapy.

As a result of previous studies done by our group [7, 8], asparaginase Medac (an *E. coli* preparation) has been introduced into the NOPHO 2000 ALL-protocol. Drug monitoring involving determination of asparaginase activities and anti-asparaginase antibodies is also part of the protocol. The measurement of enzyme activities and antibodies in a large group of patients will make it possible to determine whether the antibodies are of clinical importance, that is whether they result in a more rapid disappearance of the enzyme and thus result in an inferior treatment outcome.

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References

1. Asselin BL (1999) The three asparaginases. Comparative pharmacology and optimal use in childhood leukemia. Adv Exp Med Bull 457:621
2. Capizzi RL, Bertino JR, Skeel RT, Creasey WA, Zanes R, Olayon C, Peterson RG, Handschumacher RE (1971) L-asparaginase, clinical, biochemical, pharmacological, and immunological studies. Ann Intern Med 74:893
3. Caver TE, Slobod KS, Flynn PM, Behm FG, Hudson MM, Turner EV, Webster RG, Boyett JM, Tassie TL, Pui C-H, Hurwitz JL (1998) Profound abnormality of the B/T lymphocyte ratio during chemotherapy for pediatric acute lymphoblastic leukemia. Leukemia 12:619
4. Fabry U, Korholz D, Jurgens H, Gobel U, Wahn V (1985) Anaphylaxis to L-asparaginase during treatment for acute lymphoblastic leukemia in children – evidence of a complement-mediated mechanism. Pediatr Res 19:400
5. Gustafsson G, Schmiegelow K, Forestier E, Clausen N, Glomstein A, Jonmundsson G, Mellander L, Mäkipernaa A, Nygaard R, Saarinen-Pihkala UM (2000) Improving outcome through two decades in childhood ALL in the Nordic countries: the impact of high-dose methotrexate in the reduction of CNS irradiation. Leukemia 14:2267
6. Killander D, Dohlwitz A, Engstedt L, Franzén S, Gahrton G, Gullbring G, Holm G, Holmgren A, Höglund S, Killander A, Lockner D, Mellstedt H, Moe PJ, Palmblad J, Reizenstein P, Skårberg K-O, Swedberg B, Udén A-M, Wadman B, Wide L, Åhström L (1976) Hypersensitive reactions and antibody formation during L-asparaginase treatment of children and adults with acute leukemia. Cancer 37:220
7. Klug Albertsen B, Schrøder H, Jakobsen P, Müller H-J, Carlsen NT, Schmiegelow K (2001) Monitoring of Erwinia asparaginase therapy in childhood ALL in the Nordic countries. Br J Clin Pharmacol 52:433
8. Klug Albertsen B, Schrøder H, Ingerslev J, Jakobsen P, Avramis VI, Müller H-J, Carlsen NT, Schmiegelow K (2001) Comparison of Intramuscular Therapy with Erwinia asparaginase and asparaginase Medac. Pharmacokinetics, pharmacodynamics, formation of antibodies, and influence on the coagulation system. Br J Haematol 115:983
9. Klug Albertsen B, Schrøder H, Jakobsen P, Avramis VI, Müller H-J, Schmiegelow K, Carlsen NT (2002) Antibody formation during intravenous and intramuscular therapy with Erwinia asparaginase. Med Pediatr Oncol 38:310
10. Thomsen JB, Schrøder H, Kristinsson J, Madsen B, Szumlanski C, Weinshilboum R, Anderson JB, Schmiegelow K (1999) Possible carcinogenic effect of 6-mercaptopurine on bone marrow stem cells – relation to thiopurine metabolism. Cancer 86:1080
11. Woo MH, Hak LJ, Storm MC, Sandlund JT, Ribeiro RC, Rivera GK, Rubnitz JE, Harrison PL, Wang B, Evans WE, Pui C-H, Relling MV (2000) Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. J Clin Oncol 18:1525