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INFLUENCE OF TWO DIFFERENT ESCHERICHIA COLI ASPARAGINASE PREPARATIONS ON FIBRINOLYTIC PROTEINS IN CHILDHOOD ALL

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ABSTRACT

Background. **Alterations in hemostasis have frequently been observed in patients with leukemia, and thrombotic events are well documented in patients receiving L-asparaginase (ASP) as a single agent or in combination with vincristine, prednisone (sometimes complemented by an anthracycline). The present study was designed to evaluate prospectively fibrinolytic parameters in leukemic children receiving different *E. coli* ASP preparations (Kyowa ASP, n=20; Bayer ASP, n=20), and to relate changes in the fibrinolytic system to serum ASP activity.**

Materials and Methods. **Blood samples for coagulation studies were obtained together with serum samples for pharmacokinetic monitoring in the same venipuncture (before the first and 6th-7th doses of ASP).**

Results. **Patients receiving Kyowa ASP showed significantly (0.0001) enhanced ASP-activity compared to children treated with the Bayer preparation. Significantly decreased values of fibrinogen ($p<0.001$), plasminogen ($p<0.0002$) and α2-antiplasmin ($p<0.0003$) were found in the Kyowa group, along with significantly enhanced thrombin generation (F1+2; $p<0.001$), t-PA ($p<0.01$) and D-dimer levels ($p<0.05$). In contrast, PAI 1 activity demonstrated no significant difference in the two *E. coli* ASP administered.**

Conclusions. **Changes in fibrinogen, plasminogen, α2-antiplasmin and D-dimer are clearly associated with ASP activity during the course of ASP administration in children with ALL.**

Key words: asparaginase activity, childhood ALL, plasminogen, α2-antiplasmin, t-PA, PAI 1

Alterations in hemostasis have frequently been observed in patients with leukemia, and thrombotic events are well documented in patients receiving L-asparaginase (ASP) as a single agent or in combination with vincristine or prednisone (sometimes complemented by an anthracycline).[1-7]

A wide range of circulating half-lives has been reported when different commercially available asparaginase preparations from *E. coli* and *Erwinia chrysanthemi* were used.[8-10]

The present study was designed to prospectively evaluate fibrinolytic parameters in leukemic children receiving different *E. coli* ASP preparations, and to relate changes in the fibrinolytic system to serum ASP activity.

Materials and Methods

Forty leukemic children diagnosed within an 18-month period and treated according to the ALL-BFM 90 study protocol I (part I: prednisone 60 mg/m^2 days 1-29; *E. coli* asparaginase 10,000 U/m^2 days 12, 15, 18, 21, 24, 27, 30, 33; vincristine 1.5 mg/m^2 and daunorubicin 30 mg/m^2 days 8, 15, 22, 29) received one of the two *E. coli* asparaginase preparations officially approved in Germany: L-asparaginase Crasnitin® (Bayer, Leverkusen, Germany) or Medac® (Medac, Hamburg, Germany; originally purchased from Kyowa Hacco Kyogo, Japan). No patient had an individual or family history of bleeding or thrombophilia. Blood samples for coagulation studies and serum samples for

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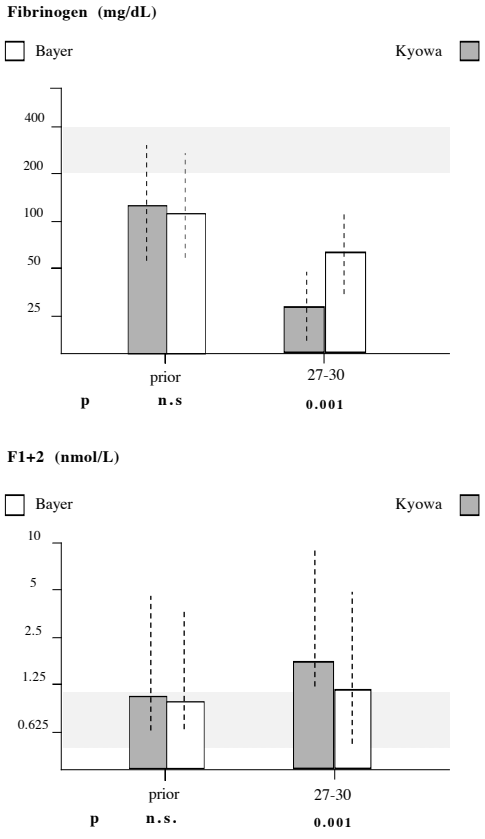


Figure 1. Fibrinogen and thrombin generation (F1+2) before the first and the 7th- 8th ASP administrations (pediatric range: shaded area). Kyowa ASP (dark column) compared to Bayer ASP (light column).

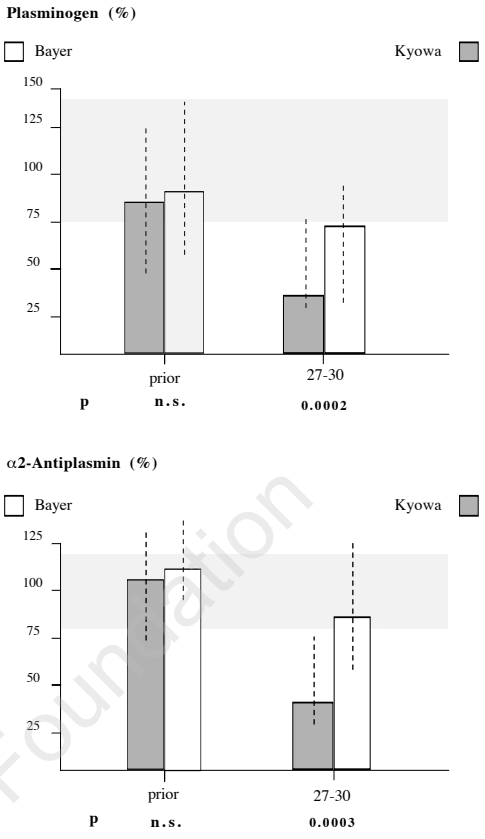


Figure 2. Plasminogen and α2-antiplasmin before the first and the 7th- 8th ASP administrations (pediatric range: shaded area). Kyowa ASP (dark column) compared to Bayer ASP (light column).

pharmacokinetic monitoring were obtained in the same venipuncture before the first and the 6th-7th doses of ASP.

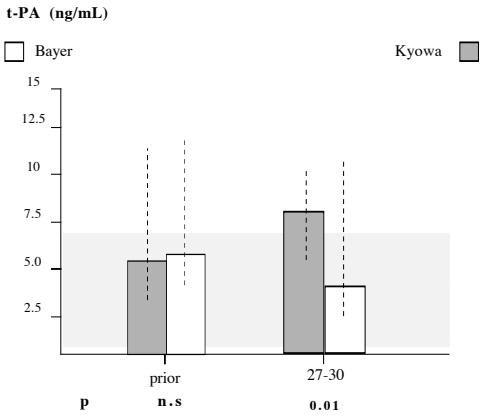
Blood samples were drawn into premarked 3 mL plastic tubes (citrate 3.8%/blood:1:10; Saarstedt® the same venipuncture), immediately placed in iced water and centrifuged at 4°C and 3000 g for 20 minutes. Fibrinogen was measured according to Clauss (Behring Werke, Marburg, Germany). Plasminogen and α2-antiplasmin were assayed by enzymatic procedures using chromogenic substrates S 2765, 2403 (Chromogenix, Mölndal, Sweden). Prothrombin fragment F1+2 (EIA F1+2 micro) and D-dimer (EIA-D-dimer micro) were determined using ELISA kits from Behring Werke, Marburg, Germany. T-PA antigen and PAI 1 activity were quantitated using test kits from Chromogenix, Mölndal, Sweden. Controls included calibration plasma, normal and abnormal control plasma (IL Test™, Instrumentation Laboratory, Italy).

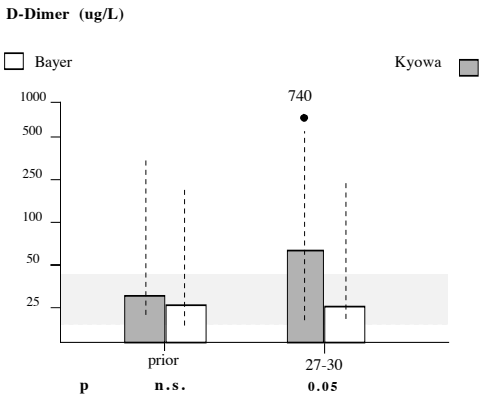
Serum asparaginase activity, defined as ammonia-release per minute after addition of serum to Nessler's solution,[11,12] and asparagine levels[13] were determined simultaneously throughout the testing period.

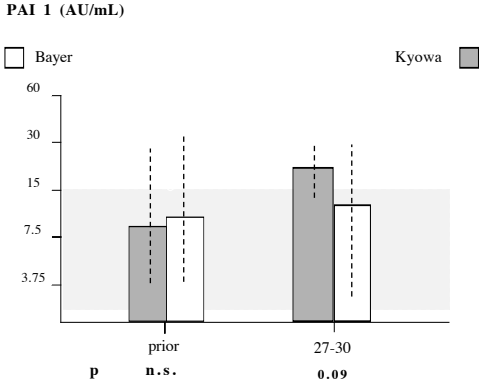
Calculations of medians, ranges and non-parametric statistics (Wilcoxon-Mann-Whitney U-test, Spearman's rank test) were performed with an Apple computer (Macintosh Performa 630) using the Stat View version 4.02 program.

Results

Changes in fibrinolytic parameters were more severe in those patients who received Kyowa ASP. Fibrinogen values were significantly







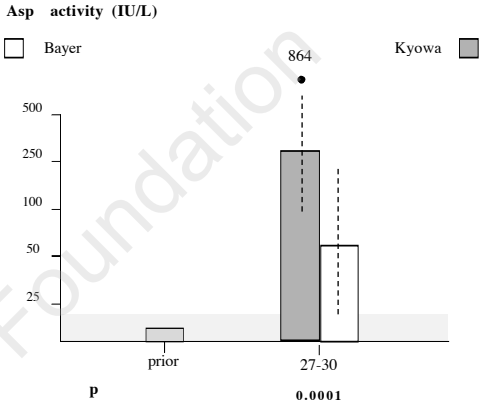


Figure 3. T-PA antigen and PAI 1 activity before the first and the 7th-8th ASP administrations (pediatric range: shaded area): Kyowa ASP (dark column) compared to Bayer ASP (light column).

Figure 4. D-dimer formation and ASP activity before (all patients) and after the 7th-8th ASP administrations (pediatric range: shaded area): Kyowa ASP (dark column) compared to Bayer ASP (light column).

decreased ($p<0.001$) and thrombin generation enhanced (F1+2; $p<0.001$), as shown in Figure 1. Plasminogen (Figure 2: $p<0.0002$), and α2-antiplasmin (Figure 2: $p<0.0003$) levels followed a pattern similar to that of fibrinogen; t-PA values (Figure 3; $p<0.01$) and D-dimer levels (Figure 4; $p<0.05$) were also significantly enhanced. In contrast, PAI 1 activity (Figure 3) showed no significant difference in the two *E. coli* ASP administered.

Furthermore, pharmacokinetic data (Figure 4) from patients receiving Kyowa ASP demonstrated significantly (0.0001) enhanced ASP activity compared to that in children treated with the Bayer type A preparation.

Table 1 reports the correlation between fibrinolytic protein levels and serum asparaginase activity (rho and p values) in blood obtained with the same venipuncture; the highest ASP activity was clearly associated with the lowest values of fibrinogen, plasminogen and α2-

Table 1. Correlation (Spearman's rank test) between fibrinolytic proteinlevels and asparaginase activities (Rho and p value) in leukemic children receiving Bayer type A asparaginase (n=20) or Kyowa asparaginase (n=20).

Parameter	*Rho*	*p value*
Fibrinogen	- 0.495	0.021
Plasminogen	- 0.472	0.025
α2-antiplasmin	- 0.735	0.036
t-PA	0.329	0.188
PAI 1	0.331	0.213
D-dimer	0.581	0.05

antiplasmin, and with enhanced D-dimer.

Both groups of patients showed almost complete asparagine depletion, at a detection limit of 0.1 uM, during the course of ASP treatment.

In the Kyowa group, a 13-year-old boy and a 7-year-old girl developed thrombosis on day 31, and one child experienced intermediate insulin-dependent hyperglycemia. Both patients with vascular occlusion presented increased values of PAI 1, t-PA and D-dimer (upper patient range) along with decreased plasminogen and α2-antiplasmin (lower patient range) prior to the thrombotic event. No antithrombin concentrate[14] or fresh frozen plasma[15] was administered in these patients. No patient treated with the Bayer preparation showed evidence of vascular insults.

Discussion

L-asparaginase is an enzyme that provides specific metabolic therapy for ALL and non-Hodgkin's lymphomas. This enzyme catalyzes the conversion of the amino acid L-asparagine to aspartic acid and ammonia, leading to rapid depletion of the circulating pool of asparagine and glutamine and resulting in decreased protein synthesis.[8]

In agreement with literature data,[1,4,6,7] this study demonstrated a significant decrease in fibrinogen, plasminogen and α2-antiplasmin during the course of *E. coli* asparaginase administration. In addition, F1+2, t-PA and D-dimer levels were significantly enhanced. Furthermore, fibrinogen, plasminogen and α2-antiplasmin were clearly correlated to serum asparaginase activity; thus, the changes in fibrinolytic parameters were more pronounced in those patients receiving Kyowa asparaginase.

In humans, the circulating half-lives of asparaginase enzymes from *E. coli* and *Erwinia chrysanthemi* vary widely.[16,17] Moreover, half-lives differ not only between E. coli strains type A and type B, but also among different commercial E. coli preparations.[8-10,18,19] One of the most obvious distinctions between the two *E. coli* asparaginase preparations administered in this study is the absence of cystine in Kyowa ASP, which also has a lower isoelectric point, a lower clearance and a longer half-life than the Bayer type A ASP. The lower clearance of the Kyowa preparation results in higher through levels of L-asparaginase activity and a prolonged duration of the L-asparaginase depletion. This possibly induces prolonged inhibition of protein synthesis as well as undesired effects on other amino acids and proteins, and in summary may cause a higher rate of side effects even with the same dose. However, our results revealed not only down regulation of fibrinolytic protein activity, but the enhanced D-dimer levels along with decreased values of plasminogen and α2-antiplasmin also signified an activated fibrinolytic system.

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