Check for updates

The second patient, a 60-year-old woman, presented to our hospital in March 2001. In 1990, laboratory studies had shown a positive direct Coombs test, and an idiopathic CHD was diagnosed. Initially, only mild signs of hemolysis with normal hemoglobin level did not require specific therapy. During the year 2000, however, pre-existing Raynaud phenomenon worsened and the hemoglobin level decreased to 10.0 g/dL, reticulocyte percentage was 5.7%, haptoglobin level was below 20 mg/dL, and LDH level was 583 U/L. Physical examination showed neither adenopathy nor liver or spleen enlargement. The direct Coombs test was positive, attributable to complement (C3d, 4+). Serum protein electrophoresis revealed a monoclonal protein (IgM kappa). The BM aspirate and biopsy showed erythroid hyperplasia and lymphoplasmocytic infiltrates (10%), with abnormal B cells by immunophenotyping. Since this patient refused cyclophosphamide chemotherapy, she was treated with rituximab (4×375 mg/m^2). No infusion-related side effects were observed. Five weeks and 11 months after the last rituximab infusion, the hemoglobin level had increased to, respectively, 12.1 g/dL and 12.9 g/dL, reticulocyte percentage had decreased to, respectively, 2.5% and 2.0%, and LDH level had decreased to, respectively, 335 U/L and 253 U/L (Figure 1B).

Our report demonstrates that both CHD patients were successfully treated with rituximab. No side effects occurred, and no additional immunosuppressive or chemotherapeutic agents had to be administered to maintain the response. The effect of the elimination of B cells by this anti-CD20 therapy is novel and has rarely been described. One small trial and only 2 case reports have reported on the use of rituximab in patients with autoimmune hemolytic anemia.[7-9] The largest report comprises 6 children, showing an ongoing complete remission (CR) with rituximab, but with warm reactive Ab (IgG)–associated autoimmune hemolytic anemia, which is vastly better to treat than CHD.[7] The 2 case reports describe a CR to rituximab each in a single patient with refractory CHD, including the diagnosis of an indolent clonal lymphoproliferative disease similar to our second patient.[8,9] Nevertheless, these patients received a combination of rituximab plus cyclophosphamide and corticosteroids,[8] or rituximab plus α-interferon,[9] whereas in our patients previous nonresponsive medication was stopped with the beginning of rituximab in the first patient, and, even more striking and not reported to date, a complete remission was rapidly achieved with rituximab alone in the second patient. Moreover, in these previous reports[8,9] the follow-up was shorter, the disease course milder,[8,9] and recurrence of CHD arose 7 months later, with the patient dying from a stroke 14 months after the treatment initiation.[9] In our 2 patients treatment response is ongoing 9 and 11 months after the start of rituximab, with no CHD recurrence either in the first patient, with no underlying primary lymphoma, or in the second patient, with no further treatment necessity for the lymphoplasmocytic lymphoma.

Of interest is, finally, that pure red cell aplasia (PRCA), as a rare complication in chronic lymphocytic leukemia (CLL), has recently been demonstrated in 2 B-cell CLL patients to also dramatically respond to rituximab,[9] and its action in Waldenström macroglobulinemia, immune thrombocytopenic purpura (ITP), and cryoglobulinemia has clearly been verified.[10-12] The mechanism of action of rituximab seems to be that of an immune modulation, presumably in Waldenström macroglobulinemia due to the elimination of either CD20$^+$ clonotypic precursor B cells or CD20$^+$ plasma cells. The same rationale may well apply to other immunoglobulin-mediated diseases of B lymphocytes, such as cryoglobulinemia, ITP, and CHD. In summary, our report strongly suggests that rituximab can successfully control refractory CHD. With the increasing interest in Ab-based therapies, rituximab appears to be a promising alternative to the conventional medication in CHD. In view of its mild toxicity and the lack of effective alternative treatments, it should be strongly considered in severely affected patients who do not respond to standard therapy.

Monika Engelhardt, Andreas Jakob, Björn Rüter, Martin Trepel, Friedrich Hirsch, and Michael Lübbert

Correspondence: Monika Engelhardt, University of Freiburg Medical Center, Hugstetterstr 55, D-79106 Freiburg, Germany; e-mail: engelhardtm@mm11.ukl.uni-freiburg.de

References

1. Azuma E, Nishihara H, Hanada M, et al. Recurrent cold hemagglutinin disease following allogeneic bone marrow transplantation successfully treated with plasmapheresis, corticosteroid and cyclophosphamide. Bone Marrow Transplant. 1996;18:243-246.
2. Zimmer-Molsberger B, Knauf W, Thiel E. Mycophenolate mofetil for severe autoimmune hemolytic anemia. Lancet. 1997;350:1003-1004.
3. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood. 1994;83:435-445.
4. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapse indolent lymphoma: half of the patients responded to a four-dose treatment program. J Clin Oncol. 1998;16: 2825-2833.
5. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large B-cell lymphoma. N Engl J Med. 2002;346:235-242.
6. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood. 2001;98:952-957.
7. Quartier P, Brethon B, Philippet P, Landman-Parker J, Le Deist F, Fischer A. Treatment of childhood autoimmune hemolytic anemia with rituximab. Lancet. 2001;358:1511-1513.
8. Lee EJ, Kueck B. Rituxan in the treatment of cold agglutinin disease. Blood. 1998;92:3490-3491.
9. Layios N, Van Den Neste E, Jost E, Deneys V, Scheiff JM, Ferrant A. Remission of severe cold agglutinine disease after rituximab therapy. Leukemia. 2001;15:187-188.
10. Ghazal H. Successful treatment of pure red cell aplasia with rituximab in patients with chronic lymphocytic leukemia. Blood. 2002;99:1092-1094.
11. Dimopoulos M, Zervas K, Zomas A, Kiamouris C, Hamilos G. Primary treatment of Waldenstrom's macroglobulinemia with rituximab. Blood. 2001; 98(suppl):367a.
12. Rosenthal E, Pesce A, Karsenti J-M, Allieri-Rosenthal M-A, Cassuto J-P. Polyneuropathy and vasculitis associated with IgG type I cryoglobulinemia can be treated with anti-CD20 monoclonal antibody (Rituximab). Blood. 2001;98(suppl):292b.

To the editor:

Pharmacology of PEG-asparaginase in childhood acute lymphoblastic leukemia (ALL)

Asparaginase (ASNase) has long been considered to be an important element in the management of childhood ALL. Its antileukemic effect is thought to be related to a metabolic deficiency reflected by the blasts' incapability to synthesize asparagine (ASN) from aspartic acid. Treatment with ASNase aims therefore at depleting the blood of ASN in order to exhaust the substrate supply that became selectively essential to the malignant cells. Highly interesting findings on PEG-ASNase, which is a polyethylene

Downloaded from http://ashpublications.net/blood/article-pdf/100/5/1923/1253645/h8170200192 2b.pdf by guest on 02 June 2024

glycol (PEG) conjugate of an ASNase derived from *Escherichia coli*, have recently been reported by Avramis et al.[1] In the context of a randomized trial, the authors evaluated several important pharmacologic parameters after a single intramuscular (IM) administration of 2500 IU/m^2 PEG-ASNase in children with newly diagnosed ALL.

The pharmacokinetic results show that the mean ASNase serum activity peaked on day 5 after the first IM dose at an average of 1000 U/L and was quantifiable within the therapeutic range of above 100 U/L over a period of about 28 days. Elimination from the serum was described by a single exponential function.[1] Remarkably, the presented findings contrast in every aspect with results observed after the intravenous use of the identical dose of PEG-ASNase within the ALL protocols of the Berlin-Frankfurt-Münster (BFM) study group.[2] Using a well-established drug monitoring program at our own institution, we have been able to identify peak ASNase serum activities ranging around 2500 U/L immediately after administration, but the time period with activity values above 100 U/L, which was usually 3 weeks, was significantly shorter than the one reported by Avramis et al.[1] Comparison of these data with intravenous results after 1000 IU/m^2 (Müller et al[3]) have also shown that a substantial increase of the PEG-ASNase dose neither translated into a prolongation of time with activities above 100 U/L nor influenced the rate of patients with early ASNase inactivation. For these reasons and with regard to the predictive use of pharmacokinetic (PK) modeling, we conclude that the elimination of PEG-ASNase from the serum cannot generally be characterized using the linear model as proposed by the authors. Due to the effects of the IM route of administration on the distribution of the ASNase activities, the suggested method might, however, in their study have served as the most appropriate approach to describe PEG-ASNase elimination.

ASN concentrations in the serum and cerebrospinal fluid (CSF) that allow a more accurate estimate of an effect caused by ASNase have also been measured in the study performed by Avramis et al.[1] The authors state that ASN was depleted rapidly from serum and CSF after administration of PEG-ASNase. This is somewhat confusing because, in contrast to the authors' conclusion, ASN is represented in considerable amounts in every figure illustrating the amino acid results. Independent of the applied ASNase or the activity measured at the same time, the lowest mean ASN concentrations are depicted in a range of about 1 μM. In view of in vitro findings demonstrating ongoing leukemic blasts' growth in medium containing comparable amounts of ASN,[4] as well as findings from other working groups showing complete depletion at a level of 0.2 μM after administration of different schedules using native ASNase preparations,[5-7] a clarification of the outlined discrepancy would be desirable for an adequate estimate of the treatment intensity achieved with the schedules presented in the paper. Under methodologic aspects, giving a clear definition of ASN depletion or a description of the preanalytic sample preparation might be helpful since hemolysis of blood after withdrawal is eg known to influence ASN concentrations leading to false high values.

Joao Paulo Vieira Pinheiro, Claudia Lanvers, Gudrun Würthwein, and Joachim Boos

Correspondence: Joachim Boos, University Children's Hospital Muenster, Department of Pediatric Hematology and Oncology, Albert-Schweitzer-Strasse 33, 48129 Münster, Germany; e-mail: onkpharm@uni-muenster.de

References

1. Avramis VI, Sencer S, Periclou AP, et al. A randomized comparison of native *Escherichia coli* asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. Blood. 2002;99:1986-1994.
2. Müller HJ, Beier R, Casimiro da Palma J, et al. PEG-asparaginase (Oncaspar) 2500 IU/m2 BSA in reinduction and relapse treatment in the ALL/NHL-BFM protocols. Cancer Chemother Pharmacol. 2002;49:149-154.
3. Müller HJ, Löning L, Horn A, et al. Pegylated asparaginase (Oncaspar) in children with ALL: drug monitoring in reinduction according to the ALL/NHL-BFM 95 protocols. Br J Haematol. 2000;110:379-384.
4. Cooney D, Handschuhmacher R. L-asparaginase and L-asparagine metabolism. Ann Rev Pharmacol. 1970;10:421-440.
5. Ahlke E, Nowak-Göttl U, Schulze-Westhoff P, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukaemia. Br J Haematol. 1997; 96:675-681.
6. Woo MH, Hak LJ, Storm MC, et al. Cerebrospinal fluid asparagine concentrations after Escherichia coli asparaginase in children with acute lymphoblastic leukemia. J Clin Oncol. 1999;17:1568-1573.
7. Rizzari C, Zucchetti M, Conter V, et al. L-asparagine depletion and L-asparaginase activity in children with acute lymphoblastic leukaemia receiving im or iv Erwinia C or E coli L-asparaginase as first exposure. Ann Oncol. 2000;11:189-193.

Response:

PEG-asparaginase and deamination of serum asparagine in children with standard-risk lymphoblastic leukemia (CCG-1962)

We are aware of Vieira Pinheiro et al's data showing the rapid elimination of PEG-asparaginase at low serum concentrations. We have not seen that late decay with a similar formulation in our pediatric population. Our findings are consistent with those reported by Asselin et al[1] using the same PEG-ASNase formulation as that used in the Children's Cancer Group studies. The Europeans use a different form of pegylated native *Escherichia coli* asparaginase produced in Japan and marketed by Medac than that used in the United States. Dr Boos's group reported that the native asparaginase marketed by Medac has different pharmacokinetic properties than other native *E coli* asparaginase preparations.[2] The native *E coli* asparaginase used in the United States is produced by Merck and is pegylated by a different chemistry according to the manufacturer (Enzon, Bridgewater, NJ). This may have lead to a pegylated product with different characteristics in drug disposition and elimination. The rapid fall in asparaginase activity at late time points suggests that their enzyme preparation may contain a mixture of variously pegylated products with different half-lives.

The route of administration in our pediatric trial was intramuscular, while the Boos group gives their drug intravenously. Our population analyses estimated a long half-life of absorption from the injection site of this drug. This could have lead to a depot effect that masked any late decline in enzyme activity. Nevertheless, we also found a first-order disappearance of PEG-asparaginase in 24 adult patients treated intravenously.[3] It should be added that neither the native *E coli* asparaginase nor the PEG-asparaginase marketed by Medac are licensed in the United States. We would like to conduct a randomized study between the 2 PEG-asparaginase formulations if the Medac product becomes licensed for use in the United States.

We have consistently found low residual asparagine concentrations in some patient samples with high levels of enzyme activity. We rapidly chilled the samples to prevent continued ex vivo deamination of asparagine, and we found that very few samples had visual signs of hemolysis. Asselin et al have reported that

Downloaded from http://ashpublications.net/blood/article-pdf/100/5/1923/1253645/h8170200192 2b.pdf by guest on 02 June 2024

asparagine concentrations return toward normal much faster when care is taken to inhibit ex vivo enzyme activity.[4] We sent a blinded set of our samples to Dr Asselin, and she confirmed the asparagine values we reported. Liver perfusion studies and modeling of asparaginase treatment suggest that there is a high rate of asparagine input into the circulation from diet and the tissues.[5,6] An equilibrium between the rate of asparagine input and the asparaginase activity can result in a low (nonzero) steady-state asparagine concentration.

Vassilios I. Avramis and John S. Holcenberg

Correspondence: Vassilios I. Avramis, USC Keck School of Medicine, Children's Hospital Los Angeles, Division of Hematology/Oncology, 4650 Sunset Blvd, Los Angeles, CA 90027

References

1. Asselin BL, Whitin JC, Coppola DL, et al. Comparative pharmacokinetic studies of three asparaginase preparations. J Clin Oncol. 1993;11:1780-1786.
2. Boos J, Werber G, Ahlke E, et al. Monitoring of asparaginase activity and asparagine levels in children on different asparaginase preparations. Eur J Cancer. 1996;32A:1544-1550.
3. Douer D, Cohen LJ, Periclou AP, et al. PEG-Asparaginase (PEG-ASP) in a remission induction regimen for newly diagnosed acute lymphoblastic leukemia (ALL) adults. Blood. 1998;92(suppl):400a.
4. Asselin BL, Lorenson MY, Whitin JC, et al. Measurement of serum L-asparagine in the presence of L-asparaginase requires the presence of an L-asparaginase inhibitor. Cancer Res. 1991;51:6568-6573.
5. Woods JA, Handschumacher RE. Hepatic regulation of plasma L-asparagine. Am J Physiol. 1973;224:740-745.
6. Holcenberg JS, Roberts J. Enzymes as drugs. Ann Rev Pharmacol Toxicol. 1977;17:97-116.

To the editor:

Idiopathic thrombocytopenic purpura, *Helicobacter pylori* infection, and HLA class II alleles

Recently, Emilia et al[1] reported a high prevalence of *Helicobacter pylori* infection in patients with idiopathic thrombocytopenic purpura (ITP) and a significant increase in platelet count after bacterium eradication. In this study, we analyzed the correlation between *H pylori* infection and HLA class II alleles in 39 ITP patients (median age, 48.9 years; range, 21-82 years; 17 males, 22 females; M/F ratio, 0.8) observed at our department between December 1998 and April 2002. We compared the frequency of the HLA-DR/-DQ antigens in these patients with that of 150 healthy bone marrow donors, matched for sex and age (Table 1). The frequency of HLA-DRB1*11 and -DQB1*03 alleles were significantly lower in ITP patients than in healthy controls. None of the other alleles (HLA-DRB1*1, *15, *16, *03, *04, *12, *13, *14, *07, *08, *0910, *1001; and -DQB1*02, *04, *05, *06) was differently expressed in ITP patients and healthy controls. The 39 patients were then compared for the presence of *H pylori* infection: 24 patients were *H pylori*–positive and 15 patients were *H pylori*–negative (Table 1). These 2 groups differed for median age (56.5 years, range, 21-82 years, for *H pylori*–positive patients; 41 years, range, 22-70 years, for *H pylori*–negative patients; $P = .03$), for sex (9 males and 15 females, M/F ratio, 0.6, for *H pylori*–positive patients; 8 males and 7 females, M/F ratio, 1.1, for *H pylori*–negative patients), and for HLA class II alleles distribution. *H pylori*–negative patients showed an HLA-DRB1*03 frequency significantly higher and a -DRB1*11, *14 and -DQB1*03 frequencies significantly lower than in *H pylori*–positive patients. The lower frequencies of HLA-DRB1*11 and of -DQB1*03 alleles observed in our ITP patients seems to be a typical feature of *H pylori*–negative cases. No significant differences in any of the class II alleles was observed in *H pylori*–positive patients as compared with controls.

So far, there is little evidence of an association between major histocompatibility complex class II and ITP.[2-5] A higher prevalence of

Table 1. Comparison of HLA-DRB1/-DQB1 frequencies of ITP patients and controls and relationship with *Helicobacter pylori* infection

HLA class II alleles	ITP patients (%), n = 39	Controls (%), n = 150	P*	ITP patients (%), n = 39		
				H pylori$^+$, n = 24	*H pylori*$^-$, n = 15	P†
DRB1						
*01	8 (20.5)	32 (21.3)	NS	5 (20.8)	3 (20.0)	NS
*15	7 (17.9)	30 (20.0)	NS	3 (12.5)	4 (26.7)	NS
*16	5 (12.8)	12 (10.2)	NS	2 (8.3)	3 (20.0)	NS
*03	10 (25.6)	28 (18.6)	NS	3 (12.5)	7 (46.7)	.01
*04	6 (15.4)	21 (14.0)	NS	4 (16.7)	2 (13.3)	NS
*11	12 (30.8)	68 (45.3)	.04	10 (41.7)	2 (13.3)	.03
*12	0	4 (2.6)	NS	0	0	—
*13	6 (15.4)	28 (18.6)	NS	4 (16.7)	2 (13.3)	NS
*14	6 (15.4)	15 (10.0)	NS	6 (25.0)	0	.02
*07	9 (23.1)	26 (17.3)	NS	6 (25.0)	3 (20.0)	NS
*08	3 (7.7)	6 (4.0)	NS	2 (8.3)	1 (6.7)	NS
*0910	0	2 (1.3)	NS	0	0	—
*1001	0	6 (4.0)	NS	0	0	—
DQB1						
*02	17 (43.6)	48 (32.0)	NS	9 (37.5)	8 (33.3)	NS
*03	17 (43.6)	94 (62.6)	.03	14 (58.3)	3 (20.0)	.02
*04	3 (7.7)	7 (4.6)	NS	2 (8.3)	1 (6.7)	NS
*05	17 (43.6)	66 (44.0)	NS	11 (45.8)	6 (40.0)	NS
*06	10 (25.6)	50 (33.3)	NS	5 (20.8)	5 (33.3)	NS

The χ^2 method with Yates correction and Fisher exact test were used for data analysis.
NS indicates not significant.
*ITP patients versus healthy controls.
†*H pylori*–positive patients versus *H pylori*–negative patients.

Downloaded from http://ashpublications.net/blood/article-pdf/100/5/1923/1253645/h81702001922b.pdf by guest on 02 June 2024