given 90 grams of IVIgG. Eight days after immunoadsorption, danazol was replaced by azathioprine and a third course of vincristine was given. Six days later, the platelet count began to rise. Pharmacologic therapy was tapered down until withdrawal and the patient is in complete remission so far.

Snyder *et al.* reported in 1992 a durable response in 36% of refractory ITP patients treated with Prosorba columns. The clinical response was associated with a significant decrease in specific serum platelet autoantibodies, PAIgG and circulating immune-complexes (CIC).[2] In these only two cases we have treated, we observed the immediate disappearance of anti-platelet GPIIb-IIIa and anti HLA class I antibodies from serum while the clinical response was time-delayed. However, the significance of removing circulating platelet autoantibodies in chronic ITP is questionable, since fewer than 50% of ITP patients have detectable antibodies in the serum and many of them are non-pathogenic. The immunomodulatory effect of the immunoadsorption is a better explanation of the late response observed in our patients. However, further studies are required to explain and validate the use of immunoadsorption treatment in refractory ITP.

Fabrizio Fabris, Guido Luzzatto, Roberto Ramon, Maria Luigia Randi, Giustina De Silvestro, Antonio Girolami*

*Department of Medical and Surgical Sciences, University of Padua, Medical School; *Transfusion Center of Padua City Hospital, Padua, Italy*

Key words

Protein-A column, immune thrombocytopenia, platelet autoantibodies.

Correspondence

Fabrizio Fabris MD, Istituto di Semeiotica Medica, via Ospedale 105, 35100 Padua, Italy. Phone: international +39.049.-8212668 – Fax: international +39.049.65739 E-mail: ffabris@tin.it

References

1. Guthrie TH Jr, Oral A. Immune thrombocytopenia purpura: A pilot study of staphylococcal protein A immunomodulation in refractory patients. Semin Hematol 1989; 26(Suppl 1):3-9.
2. Snyder HW Jr, Cochran SK, Balint JP Jr, et al. Experience with Protein A-immunoadsorption in treatment-resistant adult immune thrombocytopenic purpura. Blood 1992; 79:2237-45.
3. George JN, Woolf SH, Raskop GE, et al. Idiopathic thrombocitopenic purpura: A practice guideline developed by explicit methods for the American Society of Hematology. Blood 1996; 88:3-40.
4. Nilsson IM, Berntorp E, Freiburghaus C. Treatment of patients with factor VIII and IX inhibitors. Thromb Haemost 1993; 70:56-9.
5. Fujisawa K, O'Toole TE, Tani P, et al. Autoantibodies to the presumptive cytoplasmic domain of platelet glycoprotein IIIa in patients with chronic immune thrombocytopenic purpura. Blood 1991; 77:2207-13.
6. Bercthold P, Dale GL, Tani P, McMillan R. Inhibition of autoantibody binding to platelet glycoprotein IIb/IIIa by anti-idiotypic antibodies in intravenous gamma globulin. Blood 1989; 74:2414-7.

Programmed versus non-programmed freezing of umbilical cord blood

Programmed freezing is an expensive procedure that requires the use of sophisticated equipment, not available in many centers. We designed a prospective study to compare programmed and non-programmed freezing for cord blood. Our results suggest the feasibility of non-programmed freezing for umbilical cord blood, simplifying the method and decreasing costs in a cord blood bank.

Sir,

Many authors have established the optimal conditions for cryopreservation of umbilical cord blood to be a controlled cooling rate of 1°C/min.[1-3] However, programmed freezing is an expensive procedure that requires the use of sophisticated equipment, not available in many centers.

We designed a prospective study to compare programmed and non-programmed freezing for cord blood. For this purpose, 39 cord blood units were collected, volume reduced and cryopreserved in two 25 mL aliquots with 10% DMSO final concentration, following Rubinstein's method.[4] One of the aliquots was cryopreserved in a controlled rate freezer (Planer Biomed, Kryo 10) with a cooling-rate of 1°C/min, and the other one was placed directly into a –80°C mechanical freezer (Koxka). After 24 hours, the –80°C frozen cord blood was stored in a liquid nitrogen tank in the vapor phase. After 7 days, the UCB was thawed by submerging the bag in a 37°C water bath and washing the cells with thawing solution containing dextran and human

Table 1. Recovery of nucleated total cells, CD34+ cells and colony-forming units after thawing.

	N	*Mean*	*Median*	*SD*	*Min*	*Max*	*p*
TNC x10^8							
-80°C	40	3.76	3.6	1.63	0.67	7.8	
-120°C	38	3.57	3.32	1.6	0.69	8.3	0.541
CD34 x10^6							
-80°C	40	1.79	1.4	1.48	0.18	8.1	
-120°C	38	1.55	1.3	1.02	0.17	4.7	0.498
CFUs x10^4							
-80°C	39	43.85	34.91	34.15	2.1	127	
-120°C	33	40.21	27.05	34.55	2.1	131.3	0.278
TNC Rec (%)							
-80°C	40	88	89	34.15	61.1	150	
-120°C	38	85.6	84.24	11.22	59.09	110	0.109
CD34 Rec (%)							
-80°C	33	98.8	85	41.83	25	175	
-120°C	31	92.57	90	33.05	32.14	166.67	0.421
CFUs Rec (%)							
-80°C	28	70	69.69	39.55	11.49	151.43	
-120°C	22	53.36	45.55	36.94	5.96	140	0.199
Viability (%)							
-80°C	32	71	74	11.03	46	87	
-120°C	30	69.13	69.5	13.72	38	94	0.461

CNT: total nucleated cells. CFUs: colony-forming units. Rec: recovery expressed as percentage.

©Ferrata Storti Foundation

albumin 2.5% final concentration. Before cryopreservation and after thawing, samples for cell counts, CD34 analysis, viability, clonogenic assays and microbiology were drawn directly from the bag. Cells were counted with a Sysmex K800 autoanalyzer (Toa Medical Electronics, Kobe, Japan). $CD34^+$ cells were quantified by flow cytometry (Procount, Becton Dickinson). Cell viability was assessed by ethidium bromide and acridine orange. Clonogenic assays were performed using a commercially prepared complete methylcellulose medium (Methocult GF H4434), supporting growth of CFU-GM, BFU-E and CFU-GEMM. Sterility controls were performed using an automated blood culture system (BacT/Alert: Organon Teknica) at 35°C for 14 days. The Statistical Package for Social Science (SPSS) v. 8.0 was used to perform the statistical analysis. All studied variables showed a normal distribution. Means were compared using the t-test. A *p* value of <0.05 was considered statistically significant.

The mean collection volume without anticoagulant was 78 mL (range 40-137). At collection, mean values of total nucleated cells, $CD34^+$ cells and colony-forming units per bag were 4.28×10^8 (range 0.65-9.5), 1.94×10^6 (range 0.3-5.5) and 68.6×10^4 (range 14-197), respectively. Mean cell viability was 89.5% (range 56-95%). Results of thawing for non-programmed and programmed freezing are shown in the Table. There were no significant differences in mean recovery of $CD34^+$ cells, total nucleated cells or CFUs. Only one microbiological culture was positive for *S. viridans* in a thawed sample of a non-programmed cryopreserved unit.

These conclusions are similar to those obtained for peripheral progenitor cells[5,6] and suggest that non-programmed freezing of cord blood units is feasible, simplifying the method and decreasing costs in a cord blood bank. In spite of this, programmed freezing is probably recommended because it allows a temperature registry and is easier to standarize. Non-programmed freezing could be reserved for those banks that do not have adequate devices or in emergency situations. Besides, more studies are needed to determine whether there are clinical differences in grafting between cord blood units cryopreserved in these two different ways.

Pilar Solves, Luis Larrea, Mª Angeles Soler, Vicente Mirabet, Francisco Carbonell, Elena María Franco

Umbilical Cord Blood Bank, Cryopreservation Unit, Valencia Transfusion Center, Valencia, Spain

Key words

Umbilical cord blood, cryopreservation, non-programmed freezing.

Correspondence

Pilar Solves, M.D., Centro de Transfusión de la Comunidad Valenciana, Avda del Cid, 65-A, 46014 Valencia, Spain. Fax: international +34-96-963502469 – E-mail: rodenas_ter@gva.es

References

1. Campos L, Roubi N, Guyotat D. Definition of optimal conditions for collection and cryopreservation of umbilical cord hematopoietic cells. Cryobiology 1995; 32:511-5.
2. Donaldson C, Armitage WJ, Denning-Kendall PA, Nicol AJ, Bradley BA, Hows JM. Optimal cryopreservation of human umbilical cord blood. Bone Marrow Transplant 1996; 18:725-31.
3. Shlebak AA, Marley SB, Roberts IAG, Davidson RJ, Goldman JM, Gordon MY. Optimal timing for processing and cryopreservation of umbilical cord haematopoietic stem cells for clinical transplantation. Bone Marrow Transplant 1999; 23:131-6.
4. Rubinstein P, Dobrila L, Rosenfield RE, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA 1995; 92:10119-22.
5. Galmés A, Besalduch J, Bargay J, et al. Cryopreservation of hematopoietic progenitor cells with 5-percent dimethyl sulfoxide at –80°C without rate-controlled freezing. Transfusion 1996; 36:794-7.
6. Perez-Oteyza J, Bornstein R, Corral M, et al. Controlled-rate versus uncontrolled-rate cryopreservation of peripheral blood progenitor cells: a prospective multicenter study. Group for cryobiology and biology of bone marrow transplantation (CBTMO), Spain. Haematologica 1998; 83:1001-5.

Identification of one novel and three other point mutations in the protein C gene of five unrelated Brazilian patients with hereditary protein C deficiency

Hereditary protein C (PC) deficiency is an autosomally inherited disorder associated with a high risk of thrombosis.[1-5] We report three previously described point mutations[6] and one novel mutation (family E) in five Brazilian families with PC deficiency.

Sir,

According to the last PC gene database,[6] 160 mutations have so far been described. We determined the genetic basis of PC deficiency in five Brazilian patients with deep venous thrombosis, and their available relatives. The patients' mean age was 23 years (range 11 to 36).

Plasma PC levels (activity and antigen) were analyzed by coagulation (Bioclot PC Biopool) and *rocket* immunoelectrophoresis, respectively. Venous blood samples in EDTA were obtained for DNA analysis by polymerase chain reaction (PCR). The amplified fragments were submitted to SSCP and CSGE analysis for mutation screening. PCR fragments, which revealed an altered pattern were sequenced to determine molecular alterations. Results of the PC activity assays for the five families are shown in Table 1. In three patients the PC antigen test could not be performed. The other two patients had reduced levels of PC antigen, confirming type I deficiency.

Our results suggest a dominant autosomal pattern in relation to plasma PC deficiency and a recessive autosomal pattern regarding to clinical expression of thrombosis (Figure 1).

We identified four different substitutions (Table 1). The novel mutation is a 3190G to T transversion, converting Gly83 in Cys, in exon 5 affecting the first EGF domain. Most missense mutations in this domain are associated with a type I deficiency and

© Ferrata Storti Foundation