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Original Research Report

No Differences in Colony Formation of Peripheral Blood Stem Cells Frozen with 5% or 10% Dimethyl Sulfoxide

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ABSTRACT

High-dose chemotherapy with autologous stem cell rescue usually requires cryopreservation of the cells. For several years, 10% dimethyl sulfoxide (DMSO) has been used as the standard cryoprotectant. Because DMSO infusion can lead to toxic clinical complications in a dose-related manner, we wanted to evaluate if reduction to 5% DMSO would be possible. We have compared colony formation in the myeloid, erythropoietic, and megakaryocyte linages in peripheral blood progenitor cell (PBPC) samples cryopreserved in parallel with 5% and 10% DMSO. Twenty-seven PBPC samples from patients with malignant diseases were investigated after 3 months of cryopreservation in liquid N_2, and samples from 14 of these patients were investigated after 1 year. A significantly higher colony formation was demonstrated for colony-forming units-erythrocyte (CFU-E) and CFU-granulocyte, erythrocyte, macrophage, megakaryocyte (GEMM) both at 3 months and at 1 year in the 5% samples. For CFU-granulocyte-macrophage (GM) and CFU-megakaryocyte (Mk) no significant difference was demonstrated neither at 3 months nor at 1 year in samples frozen with 5% and 10% DMSO. Also, there was a statistically significant correlation between the CFU-total and CFU-Mk-total, indicating that the CFU-total might be used as an evaluation of megakaryocyte progenitors. Viability testing with the Trypan Blue exclusion test showed that cells cryopreserved in 5% DMSO had significantly higher viability than the cells cryopreserved in 10% DMSO. We conclude that 5% DMSO is at least as good for cryopreservation of small-volume PBPC samples as the conventional 10% DMSO, and our results suggest that the possibility of using 5% DMSO for cryopreservation of autologous PBPC grafts should be further investigated in clinical studies.

INTRODUCTION

HIGH-DOSE CHEMOTHERAPY followed by autologous peripheral blood progenitor cell (PBPC) transplantation is now used in the treatment of chemosensitive malignancies (1–3). PBPC are harvested and usually stored in liquid N_2 until reinfusion at a temperature low enough to block all enzymatic pathways and metabolism in the cells (4). Controlled-rate freezing with cryopreservation of PBPC in 10% dimethyl sulfoxide (DMSO) has been the standard procedure at most institutions (5,6). DMSO is relatively freely permeable across the cell membrane (7,8) and prevents intracellular formation of ice crystals and disruption of cell membranes (9). DMSO has also been tried in combination with hydroxyethyl starch (HES) (4,10–13), which coats the outside of cells and may thereby prevent post-thawing clumping or gel formation (4,13). However, Donaldson et al. (13) have con-

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cluded that HES did not improve the recovery of $CD34^+$ stem cells after cryopreservation in 5% DMSO.

Infusion of DMSO-preserved PBPC is often associated with toxic reactions, and nausea, vomiting, cardiac dysfunction, anaphylaxia, and acute renal failure have been reported (14). These side effects are usually proportional to the amount of infused DMSO (15). Health personnel are also experiencing physical symptoms such as headaches and gastrointestinal reactions both from the DMSO odor and from its metabolite dimethyl sulfide (DMS). About 45% of injected DMSO is secreted through the urine, whereas DMS is secreted through the skin, breath, feces, and urine from the patients for up to 2 days after infusion. Thus, DMSO toxicity can be a problem for several days after stem cell infusion (16).

Autologous stem cell transplantation is usually a safe procedure, with low treatment-related mortality. This safety has been achieved through the use of peripheral blood-mobilized cells, careful estimation of collected $CD34^+$ cells, and improved supportive care (17). The improved safety also allows autotransplantation to be tried in new patient groups, e.g., elderly patients. The present results are based on stem cell preservation with 10% DMSO, and the possible use of 5% DMSO will require a careful evaluation before an alteration of the present procedures can be further considered. Furthermore, the effects of cryopreservation may differ between small sample volumes and the large cryobags used for preservation of PBPC grafts. The initial laboratory studies therefore have to be followed by additional clinical studies. These studies should include both in vitro evaluation of PBPC grafts frozen in cryobags with 5% and 10% DMSO, as well as a detailed characterization of hematopoietic and immunological reconstitution.

Long-lasting thrombocytopenia has previously been a major problem after stem cell transplantation. An alteration of present cryopreservation procedures thus has to be evaluated with regard to preservation of clonogenic progenitors, especially megakaryocyte progenitors and the risk of posttransplant thrombocytopenia. To our knowledge, no previous study has assessed whether colony formation differs in PBPC frozen with either 5% or 10% DMSO as the sole cryoprotectant and kept frozen below −140°C. The aim of our study was to investigate whether the number of clonogenic progenitors, and especially the megakaryocyte precursors, is maintained when using 5% instead of 10% DMSO for cryopreservation.

MATERIALS AND METHODS

Patients

A total number of 27 autologous PBPC products from 27 consecutive patients were studied. The patients suffered from multiple myeloma ($n = 15$), non-Hodgkin's lymphoma ($n = 8$), Hodgkin's lymphoma, osteogenic sarcoma, Ewing sarcoma, and testicular cancer.

Harvesting and cryopreservation techniques

PBPC were harvested after mobilization with granulocyte colony-stimulating factor (G-CSF) and chemotherapy and collected on a Cobe Spectra (Cobe Laboratories, Gloucester, UK) separator into citrated autologous plasma. The yield of $CD34^+$ cells in the PBPC concentrates was $1.2–13 \times 10^6$/kg body weight.

The cell concentrations were adjusted with autologous plasma to $100–200 \times 10^6$ cells/ml before addition of DMSO (Cryoserv, Edvards Lifesciences, Irvine, CA). A sample of a few milliliters was removed from the PBPC, and DMSO was added to a final concentration of 5%. The sample was distributed in 0.5-ml aliquots into cryotubes (Nunc, Copenhagen, Denmark) and placed on ice. Meanwhile, DMSO was added to the PBPC concentrate (on ice) to a final concentration of 10% and a few-milliliter sample from this PBPC concentrate was distributed into cryotubes as above. The 5% and 10% samples together with the PBPC concentrate were thereafter frozen to −160°C in a controlled programmed freezer: from 4°C to −5°C with −2°C/min, thereafter with −1°C/min to −40°C, and last with −5°C/min to −160°C (Planer Cryo 10, Planer Concentrate Ltd, England). The samples and the PBPC autograft were then transferred to liquid N_2. This was the procedure for the first 17 patients (all samples cryopreserved up to 1 year were from this group). Later we changed the procedure to obtain an even better similarity between the 5% and 10% DMSO samples: Two small samples from the PBPC were removed and DMSO was added to final concentrations of 5% and 10%, respectively, and placed on ice while DMSO was added to the PBPC to a final concentration of 10%, then frozen together in the programmed freezer.

The quality of our routines for preparation and storage of autologous stem cell grafts has been evaluated repeatedly during the same time period of the present study. The mean recovery of $CD34^+$ cells compared in 19 samples before freezing and after thawing was 78%.

Progenitor cell thawing and colony-forming cell assay

Samples with 5% and 10% DMSO were always handled in parallel. After 3 months (samples from 27 patients) and 1 year (samples from 14 of the 27 patients), one vial of each sample was thawed in a water bath at 37°C until the ice crystals just disappeared. Ten microliters of cell suspension from each vial was immediately diluted in 990 μl of Hanks' buffer w/o (Ca^{2+} and Mg^{2+}), and 330 μl from each dilution was thereafter suspended

into 3 ml of methylcellulose medium with erythropoietin and phytohemagglutinin-leukocyte conditioned medium (Methocult™ H4433, Stemcell, Vancouver) to a final concentration of 1.0–2.2 $\times$ 10^5 cells/ml.

The cells were then distributed into a 24-well culture plates (Costar 3524). From each of the two media containing cells, 0.5 ml of cell suspension/well $\times$ 4 were filled into eight wells (the empty wells filled with 0.9% saline). The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 14 days colony-forming unit-erythroid (CFU-E; 40 or more cells/colony), colony-forming unit-granulocyte, macrophage (CFU-GM), and colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM) colonies were then enumerated under an inverted light microscope (all wells blinded). The results are presented as the number of CFU/μl original (before DMSO addition) seeded cells (mean of four parallel cultures). The CFU total is defined as the sum of CFU-E, CFU-GM, and CFU-GEMM.

All reagents used in these assays have been standardized by the producer. The practical procedures in our laboratory have also been standardized, and all assays were performed by the same experienced technician. The reproducibility of our methods has been documented by independent examination by many other laboratories in parallel assays and by evaluation in international workshops (originized by StemCell Technologies).

Megakaryocytic progenitor assay

Megakaryocyte progenitors were always analyzed in parallel with other CFUs (see above), and 200 or 250 μl of thawed cells were then suspended in 8.5 ml of Hank's buffer before centrifugation at 200 $\times$ g for 12 min to get rid of platelets, plasma, and DMSO. The cells were thereafter washed once in Hank's buffer (515 $\times$ g, 9 min) and resuspended in Iscove's modified Dulbecco's medium (IMDM) with 0.1 mg/ml gentamicin. From this suspension, 0.1 ml containing 2–10 $\times$ 10^5 cells was added to 2 ml of Megacult™-C medium with cytokines (human megakaryocytic progenitor assay, Stemcell, Vancouver). This CFU-Mk assay has been optimized for accurate quantitation, standardized for Mk progenitors, and performed by an experienced scientist. The cells were incubated in the collagen-based system for 14 days in a humidified atmosphere of 5% CO_2 at 37°C before they were fixed and stained.

The Mk cells were stained with a primary antibody to the Mk-specific CD41 antigen (GPIIb/IIIa) linked to a secondary biotinylated antibody–alkaline phosphatase avidin conjugated detection system to amplify the primary signal. The stained slides were kept in the dark at 4°C until Mk colonies were counted in an inverted light microscope. CFU-Mk 20–49 cells/colony, CFU-MK >50 cells/colony, and CFU-Mk/non-Mk colonies (CFU-Mk-Mix, containing other linages in addition to Mk) were counted. CFU-Mk-total is defined as the sum of these colonies. Platelets also express GPIIb/IIIa and are stained pink as the megakaryocytes and will be seen on the slides. Because the platelets are much smaller and without nuclei, they are easily differentiated from Mk colonies. Samples were blinded, and CFU-Mk colonies were counted in an inverted light microscope by the same person throughout the experimental period.

Viability

The 5% and 10% samples were checked for viability with Trypan Blue dye exclusion (Sigma) simultaneously with dilution of the samples into methylcellulose medium.

Statistical analysis

The Wilcoxon's test for paired samples (two-tailed test) was used for statistical comparisons, and Kendall's τ-test was used for correlation analysis (SPSS software package; SPSS, Chicago, IL). Differences were regarded as statistically significant when $p < 0.05$. SPSS software has also been used to determine the means and SDs shown in Table 1.

RESULTS

CFU-GM, CFU-E, and CFU-GEMM after cryopreservation in 5% and 10% DMSO

Although the frequencies of clonogenic cells showed a wide variation between the patients, the differences in individual products were relatively small when comparing samples frozen with 5% and 10% DMSO. Figure 1 illustrates the individual values of CFU-GM and CFU-E after 3 months. Out of 27 cases, 2 increased more than 50% compared to 10% samples, 5 increased >25%, while 1 decreased >25% of the CFU-GM values. For CFU-E, 6 cases increased more than 50% and 4 >25%, whereas only one decreased more than 25%.

The values from the 10% vials and the vials from the cryobags appeared uniform. The data from 27 samples in parallel were therefore pooled for each type of colony. The median values of CFU-GM, CFU-E, and CFU-GEMM were higher for 5% samples than for 10% samples, and for CFU-E and CFU-GEMM the comparison was significant ($p < 0.040$ and 0.004 respectively; Table 1). The numbers of CFU-GM, CFU-E, and CFU-GEMM showed significant correlations between 5% and 10% DMSO samples (Kendall's test, $p < 0.01$ for all three). The ratio between the median values for 5% and 10% samples were all above 1.00. Samples from 14 of these patients were also examined after 1 year of cryopreser-

TABLE 1. COLONY FORMATION AFTER CRYOPRESERVATION OF PBPC IN 5% AND 10% DMSO

Time interval	*Patient number*	*Type of colonies*	*Colony formation*[a]				*Ratio (colonies in 5% versus 10% DMSO)*[b]		*Kendall τ-test correlation*	*Wilcoxon comparison*
			5% DMSO		*10% DMSO*					
			Median (range)	*Mean ± SD*	*Median (range)*	*Mean ± SD*	*Median (range)*	*Mean ± SD*		
3 months	27	CFU-GM	57 (9–225)	64 ± 52	52 (8–214)	60 ± 46	1.04 (0.43–1.88)	1.07 ± 0.30	0.01	NS[c]
		CFU-3	193 (34–809)	237 ± 176	166 (38–510)	199 ± 141	1.01 (0.69–4.43)	1.36 ± 0.76	0.01	0.040
		CFU-GEMM	13 (0–127)	22 ± 28	9 (0–80)	15 ± 18	1.07 (0.00–4.50)	1.44 ± 0.89	0.01	0.004
		CFU-Mk>50	10 (1–73)	14 ± 16	10 (0–38)	13 ± 12	1.22 (0.38–2.53)	1.23 ± 0.55	0.01	NS
		CFU-Mk-total	39 (2–155)	51 ± 43	40 (1–183)	50 ± 45	1.17 (0.52–1.94)	1.14 ± 0.42	0.01	NS
1 year	14	CFU-GM	66 (21–316)	87 ± 80	60 (16–302)	87 ± 79	1.05 (0.50–2.25)	1.12 ± 0.46	0.01	NS
		CFU-E	190 (45–366)	210 ± 99	159 (32–380)	173 ± 93	1.30 (0.83–2.01)	1.29 ± 0.34	0.01	0.035
		CFU-GEMM	20 (4–184)	38 ± 46	15 (2–108)	26 ± 30	1.40 (0.67–4.00)	1.72 ± 1.02	0.01	0.003
		CFU-Mk>50	16 (1–27)	13 ± 10	12 (0–29)	12 ± 10	1.25 (0.46–5.75)	1.64 ± 1.35	0.01	NS
		CFU-Mk-total	53 (2–112)	48 ± 36	39 (1–106)	41 ± 33	1.22 (0.49–2.70)	1.27 ± 0.49	0.01	NS

[a]The results are presented as number of colonies per microliter of seeded cells (before DMSO addition), and the table shows the median numbers and range.
[b]The table shows the median ratio and range.
[c]NS, Not significantly different.

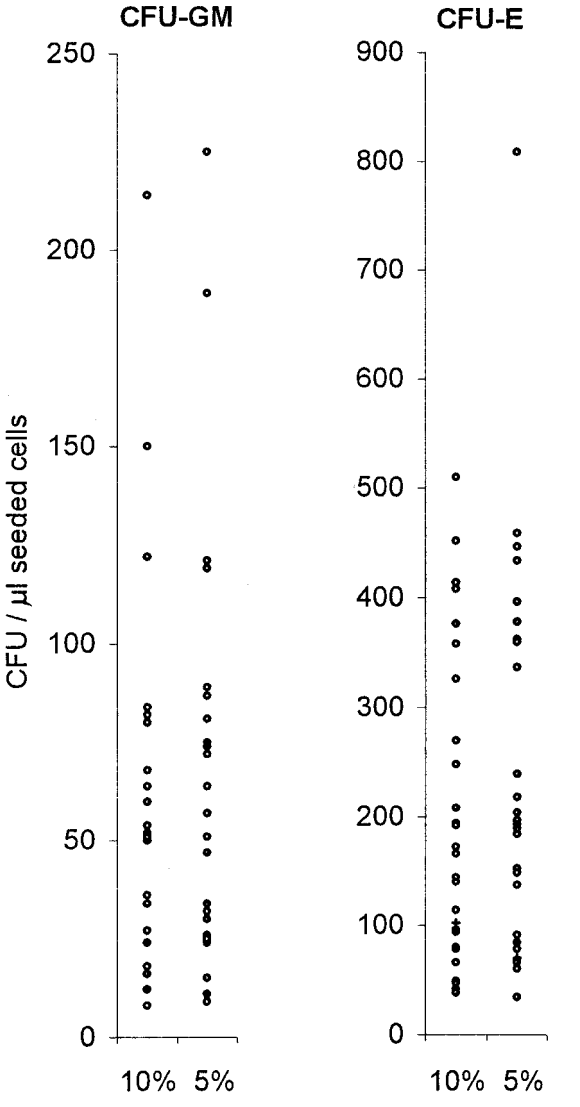

FIG. 1. CFU-GM and CFU-E colony formation in samples from 27 different patients. Parallel samples with 5% and 10% DMSO were stored frozen in liquid N_2 for 3 months.

vation (Table 1). Increased numbers of CFU-GEMM and CFU-E were detected for samples with 5% DMSO as compared to 10% ($p < 0.003$ and 0.035, respectively). The means and SDs are also given in the Table 1, again showing higher values for 5% samples.

CFU-Mk after cryopreservation in 5% and 10% DMSO

CFU-Mk was also examined for samples cryopreserved in 5% and 10% DMSO. Figure 2 illustrates the individual values of CFU-Mk >50 after 3 months and after 1 year. Out of 27 cases, 6 increased more than 50% and 6 increased >25%. Three cases decreased more than 50% and 4 cases >25%. Out of 14 cases after 1 year of cryopreservation, 5 cases increased more than 50%, 2 cases >25%, and 3 cases decreased >25%, all compared to 10% samples.

The number of CFU-Mk >50 and CFU-Mk-total did not differ significantly for the 5% and 10% samples tested at 3 months ($n = 27$) and 1 year ($n = 14$) of storage (Wilcoxon's test for paired samples). The values of CFU-Mks showed a significant correlation for samples with 5% and 10% DMSO with Kendall τ-test ($p < 0.01$) both at 3 months and 1 year. Table 1 shows that there is a

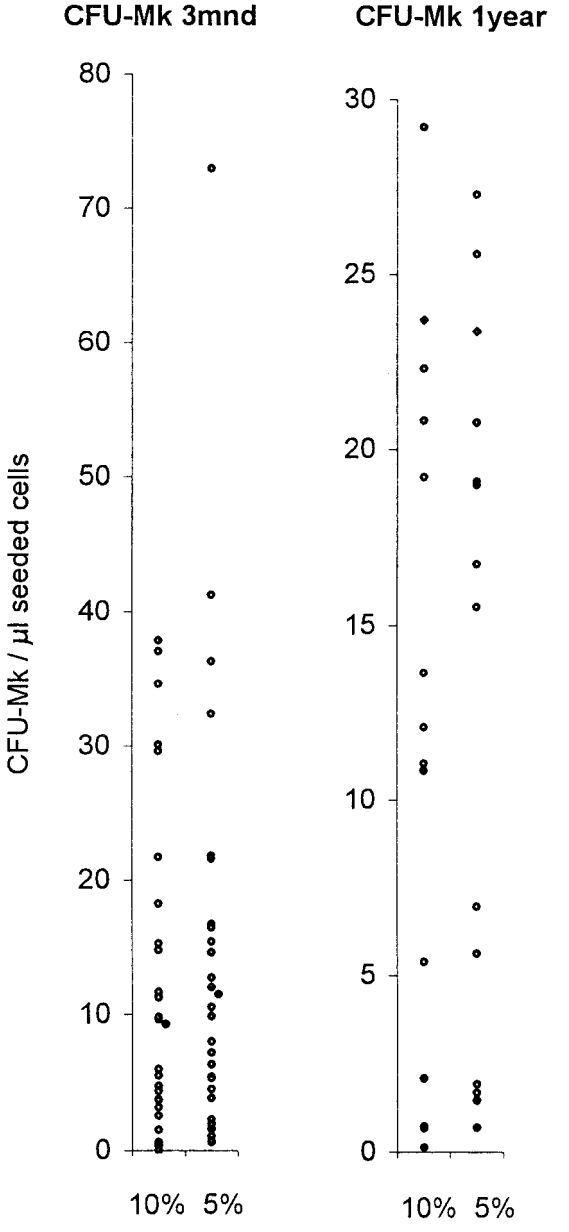

FIG. 2. CFU-Mk >50 colony formation in parallel samples from 27 different patients. Parallel samples were stored frozen in liquid N_2 for 3 months; and CFU-Mk >50 colony formation from 14 of these patients were also investigated after 1 year.

higher median value for 5% samples than for 10% samples after 1 year when testing for both CFU-Mk >50 and CFU-Mk-total, while at 3 months the values are similar for both these CFU colonies. The median 5% versus 10% ratio was above 1.15 for CFU-Mk >50 and CFU-Mk-total both at 3 and 12 months.

All patients showed satisfactory hematopoietic reconstitution with platelet counts exceeding 50×10^9/L within 20 days (range 11–20) and granulocyte counts $>0.5 \times 10^9$/L within 20 days (range 9–20) after autotransplantation.

Viability of nucleated cells and recovery of CD34$^+$ cells in the PBPC autografts

The viability test with Trypan Blue dye exclusion showed that the cells cryopreserved in 5% DMSO (median number of viable cells 85%, range 72–94%) had a significant higher viability than the cells preserved in 10% DMSO (median 78%, range 55–92%; $p < 0.001$). The recovery of CD34$^+$ cells (numbers before and after freezing) was compared for 5% and 10% samples for 7 patients, and no statistically significant difference was observed (mean recovery 88% for 5% DMSO and 78% for 10%).

DISCUSSION

High-dose chemotherapy with autologous stem cell rescue usually requires cryopreservation of the stem cells until conditioning therapy and later reinfusion of the stored PBPC (5,6). The stem cells are usually preserved in 10% DMSO. Our present results suggest that cryopreservation of PBPC in 5% rather than 10% DMSO is at least equally good when samples are stored for up to 1 year in liquid N_2. This decreased DMSO concentration would result in a lower amount of DMSO reinfused to the patient, and therefore decreased toxic effects after reinfusion would be expected.

A previous study demonstrated that the freezing procedure has a major influence on the preservation of clonogenic cells (18). Therefore, all of our samples were handled according to generally accepted guidelines for autologous PBPC grafts to ensure that our results are representative for the clinical use of PBPC autolog grafts. The quality of these routines was documented through regular evaluation of CD34$^+$ cell recovery during the study period. Samples with 5% and 10% DMSO from the same patient were always handled in parallel. Because 90% of PBPC harvested patients are autotransplanted within 3 months, we did not examine samples that were stored for more than 12 months.

A comparison of CD34$^+$ cell recovery (i.e., number of cells before and after freezing) in paired samples with 5% and 10% DMSO was only available for 7 patients, and no difference was observed. However, a high recovery of CD34$^+$ cells preserved in 10% DMSO was documented in several samples throughout the study period. Taken together with our observation that the numbers of clonogenic progenitors in 5% samples were equal to or higher than in the 10% samples, it can be concluded that both 5% and 10% results in a high recovery of progenitor cells compared with the prefreezing levels.

Our results demonstrated that cryopreservation of clonogenic cells in 5% DMSO was at least as good as routine preservation in 10% DMSO solution, and this was true both for CFU-E, CFU-GM, CFU-GEMM, and CFU-Mk stored for up to 1 year in liquid N_2. The numbers of CFU in 5% and 10% DMSO samples also showed a significant correlation. In contrast, Galmes et al. (19) have previously described a decreased recovery of certain CFU subsets (CFU-GM, CFU-E) when hematopoietic progenitors was cryopreserved in 5% and stored for 1 year at -80°C. Taken together with our present results, these observations suggest that storage at -140°C or below in liquid N_2 may be essential when hematopoietic stem cells are preserved in 5% DMSO. We did not examine DMSO concentrations below 5% because previous studies have demonstrated that stem cell recovery is dramatically reduced when lower concentrations are used for cryopreservation (13). Successful engraftment at these lower DMSO concentrations seems to depend on combination with HES (12).

The analysis of CFU-Mk is time consuming and not suitable for routine evaluation of PBPC grafts. However, CFU-Mk showed a significant correlation with CFU-total in the routine erythropoietin/conditioned medium assay, and this observation suggests that this simplified assay simultaneously indicate the content of megakaryoblasts.

The clinical toxicity after reinfusion of cryopreserved PBPC grafts depends both on the amount of DMSO and the amount of reinfused damaged and dead cells (10,15,20–22). The majority of nucleated cells in our grafts were differentiated normal leukocytes, and cryopreservation in 5%, as compared to 10% DMSO, was associated with increased viability of these cells. Thus, decreased toxicity with 5% DMSO would be expected both because of the decreased amount of DMSO and because of decreased release of intracellular components from dead cells in the autografts.

Douay et al. (18) have pointed out that freezing geometry is so important that conclusions from small sample in cryotubes cannot be drawn by simple extrapolation on cryobags. In clinical practice, comparison between PBPC

cells cryopreserved in cryobags with 5% and 10% DMSO can only be achieved in the majority of patients by performing additional apheresis procedures to get enough $CD34^+$ cells to fill an additional cryobag with 5% DMSO. In the present study, a well-established programmed freezing technique has been used. Because the cells in the samples cryopreserved with 5% and 10% DMSO have been handled strictly in parallel, we believe that a comparison between the cells in the cryotubes are reasonable. However, the optimal next step might be, in patients with a high $CD34^+$ cell count in the apheresis product, to compare cells frozen in cryobags with 5 and 10% DMSO before it is envisaged to introduce a new freezing protocol with 5% DMSO into clinical routine.

In conclusion, the results from the present study suggest that colony formation and cell viability are not hampered when PBPC cells are frozen only with 5% DMSO. These results alone do not justify for alteration in the present clinical routines using 10% DMSO for cryopreservation of PBSC grafts, but our results suggest that the possibility of using 5% DMSO should be investigated further in clinical studies.

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REFERENCES

1. Stiff PJ, AR Koester, MK Weidner, K Dvorak and RI Fisher. (1987). Autologous bone marrow transplantation using unfractionated cells cryopreserved in dimethylsulfoxide and hydroxyethylstarch without controlled-rate freezing. Blood 70:974–978.
2. Bensinger W, J Singer, F Appelbaum, K Lilleby, K Longin, S Rowley, E Clarke, R Clift, J Hansen and T Shields. (1993). Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. Blood 81:3158–3163.
3. Beaujean F, J-H Bourhis, Ch Bayle, H Jouau, M Divine, C Rieux, M Janvier, Ch Le Forestier and JL Pico. (1998). Successful cryopreservation of purified autologous $CD34^+$ cells: influence of freezing parameters on cell recovery and engraftment. Bone Marrow Transplant 22:1091–1096.
4. Makino S, M Harada, K Akashi, S Taniguchi, T Shibuya, S Inaba and Y Niho. (1991). A simplified method for cryopreservation of peripheral blood stem cells at −80°C without rate-controlled freezing. Bone Marrow Transplant 8:239–244.
5. Haas R, AD Ho, U Bredthauer, S Cayeux, G Egerer, W Knauf and W Hunstein. (1990). Successful autologous transplantation of blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor. Exp Hematol 18:94–98.
6. Rowley SD, WI Bensinger, TA Gooley and CD Buchner. (1994). Effect of cell concentration on bone marrow and peripheral blood stem cell cryopreservation. Blood 83: 2731–2736.
7. Szmant HH. (1975). Physical properties of dimethyl sulfoxide and its function in biological systems. Ann NY Acad Sci 243:20–23.
8. Rowley SD and GL Anderson. (1993). Effect of DMSO exposure without cryopreservation on hematopoietic progenitor cells. Bone Marrow Transplant 11:389–393.
9. Branch DR, S Calderwood, MA Cecutti, R Herst and H Solh. (1994). Hematopoietic progenitor cells are resistant to dimethyl sulfoxide toxicity. Transfusion 84:887–890.
10. Gorlin J. (1996). Stem cell cryopreservation. J Infusional Chemother 6:23–27.
11. Sputtek A, S Jetter, K Hummel and P Kühnl. (1997). Cryopreservation of peripheral blood progenitor cells: Characteristics of suitable techniques. Transfusionsmedizin 34:79–83.
12. Halle P, O Tournilhac, W Knopinska-Posluszny, J Kanold, P Gembara, N Boiret, C Rapatel, M Berger, P Travade, S Angielski, J Bonhomme and F Deméocq. (2001). Uncontrolled-rate freezing and storage at −80°C, with only 3.5-percent DMSO in cryoprotective solution for 109 autologous peripheral blood progenitor cell transplantations. Transfusion 41:667–673.
13. Donaldson C, WJ Armitage, PA Denning-Kendall, AJ Nicol, BA Bradley and JM Hows. (1996). Optimal cryopreservation of human umbilical cord blood. Bone Marrow Transplant 18:725–731.
14. Ayello J, C Hesdorffer and RF Reiss. (1995). A semiautomated technique for volume reduction of stem cell suspensions for autotransplantation. J Hematother 4: 545–549.
15. Zambelli A, G Poggi, GA Da Prada, P Pedrazzoli, A Cuomo, D Miotti, C Perotti, P Preti and G Robustelli Della Cuna. (1998). Clinical toxicity of cryopreserved circulating progenitor cells infusion. Anticancer Res 18:4705–4708.
16. Prior D, A Mitchell, M Nebauer and M Smith. (2000). Oncology nurses' experience of dimethyl sulfoxide odor. Cancer Nursing 23:134–140.
17. Bruserud O, G Tjonnfjord, BT Gjertsen, B Foss and P Ernst. (2000). New strategies in the treatment of acute myelogenous leukemia: Mobilization and transplantation of autologous peripheral blood stem cells in adult patients. Stem Cells 18:343–351.
18. Douay L, M Lopez and NC Gorin. (1986). A technical bias: differences in cooling rates prevent ampoules from being reliable index of stem cell cryopreservation in large volumes. Cryobiology 23:296–301.
19. Galmès A, J Besalduch, J Bargay, A Novo, M Morey, JM Guerra and MA Duran. (1999). Long-term storage at −80°C of hematopoietic progenitor cells with 5-percent dimethyl sulfoxide as the sole cryoprotectant. Transfusion 39:70–73.
20. Davis JM, SD Rowley, HG Braine, S Piantadosi and GW

Santos. (1990). Clinical toxicity of cryopreserved bone marrow graft infusion. Blood 75:781–786.
21. Valeri CR and LE Pivack. (1996). Effects of temperature, duration of storage, and the freezing container on in vitro measurements in human peripheral blood mononuclear cells. Transfusion 36:303–308.
22. Abrahamsen JF, AM Bakken, O Bruserud and BT Gjertsen. (2002). Flow cytometric measurement of apoptosis and necrosis in crypreserved PBPC concentrates from patients with malignant diseases. Bone Marrow Transplant 29:165–171.

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