

Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres

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Summary:

The cryoprotectant dimethyl sulfoxide (DMSO) is known to have toxic side effects, yet guidelines for its use in stem cell transplantation do not exist. To assess current practice in the use of DMSO and the incidence of DMSO-related complications, a single page questionnaire was mailed to 444 EBMT centres involved in autologous transplantation. The responses from 97 centres showed a wide variation in practice between transplant units regarding the concentration of DMSO used, daily DMSO dose restriction and the use of cell washing. The overall incidence of DMSO toxicity was approximately one in 70 transplants and most cases were cardiovascular and respiratory in nature. There was a trend to reduced complication rates in centres using lower concentrations of DMSO or washing cells prior to return. A large-scale prospective study of the strategies for reduction in exposure to DMSO and reduction in toxic effects is required before guidelines in the use of DMSO in stem cell cryopreservation can be promulgated.

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Autologous stem cell transplantation using high-dose chemotherapy requires the storage of frozen stem cells in liquid nitrogen for their subsequent reinfusion. Prior to storage, dimethyl sulfoxide (DMSO) is added to the cells as a cryoprotectant. Cryoprotectants act by penetrating the cell where they bind to water molecules in solution. This in turn blocks the efflux of water from the cytoplasm during freezing, preventing cellular dehydration or shrinkage and maintaining stable intracellular salt concentrations and pH levels. By slowing the rate of freezing they also prevent the formation of harmful ice crystals within the cell.

DMSO is known to have toxic effects. Animal studies using a swine rete testis model have shown that DMSO exhibits a dose-dependent vasoconstrictor effect.^{1,2} Cases of DMSO toxicity have also been reported in humans. This toxicity has been implicated due to the development of acute side effects during the actual reinfusion of cryopreserved cells. General side effects such as nausea, vomiting and abdominal cramps are seen in up to 50% of patients³ and are believed to arise due to a vagal response caused by the intravenous infusion of a cold liquid. Cardiovascular and respiratory problems such as hypotension and bradycardia⁴ or more rarely hypertension and tachycardia, fatal arrhythmias,⁵ respiratory arrest^{6,7} and diffuse alveolar haemorrhage⁸ have been reported. A number of centres have described cases of neurological toxicity such as reversible leukoencephalopathy,⁹ epileptic seizures^{10,11} and stroke.^{12,13} Elevation of lactate dehydrogenase levels¹⁴ and haemoglobinaemia¹⁵ have also been reported.

About 10% DMSO has been used as a standard cryoprotectant in stem cell transplantation. It has been suggested that lowering the dose of DMSO may be advisable. Lower concentrations, for example 5% DMSO^{16,17} or 3.5% DMSO¹⁸ are an effective cryoprotectant. Stem cell washing to remove DMSO appears to have no adverse effect on haematological recovery and may reduce toxicity¹⁹ and fractionation of stem cell administration may similarly reduce side effects.²⁰ In contrast, it has been hypothesised that DMSO may have beneficial antitumour effects *in vivo* and that reduction of DMSO dose may be detrimental.²¹

It is not known if a reduction in the amount of DMSO infused leads to a reduction in side effects, and there are no current guidelines for its use and most transplant centres are thought to have developed their own protocol for DMSO use and administration. Thus, the aim of this study was to assess current practice in the use of DMSO in EBMT transplant centres and to obtain outline data on its toxicity.

Methods

A single page questionnaire was designed, piloted and mailed to 444 EBMT transplant centres in April 2003. The study was completed in December 2003. A return of >20%

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would be viewed as a satisfactory response typical for this sort of survey.

In view of the nature of the survey and the fact that toxic events were not specified in detail, the data have not been subject to extensive statistical analysis.

Results

Replies were received from 97 centres, a response rate of 22%. In all, 95 centres returned completed questionnaires and two centres had not carried out any autologous transplantation. All 95 responders used DMSO as their sole cryoprotectant.

DMSO concentration

Seventy-eight centres used a final concentration of 10% DMSO in the cryopreserved cells and nine centres used 5% DMSO. A concentration of 7.5% DMSO was used by two centres; 20, 8, 7, 6, 5.5 and 2.2% were used by one centre each.

Cell concentration

Sixty one centres limited the frozen cell concentration in the cryopreserved bags. Of these, 23 centres used a limit of 1×10^8 cells/ml and 26 used 2×10^8 cells/ml. Twelve centres used a total of seven other concentrations, 26 centres did not limit cell concentration and there were eight nonresponders.

Forty-one centres used a filter in the infusion line, 29 did not and 20 infused directly using a syringe. Of these, four centres used either a line including a filter or a syringe.

DMSO administration

There was an upper limit in the amount of DMSO given per day used by 57 centres as follows: 80 g ($n=5$), 60 g ($n=9$), 40 g ($n=12$), 20 g ($n=2$) and others ($n=29$). In the 'others' group there was a total of 14 different limits used, the most frequently cited being 1 g/kg in 13 centres. Of the remainder, 33 centres had no upper limit of DMSO given per day and five centres did not respond.

Further variation was seen in the return of stem cells; 33 preferred to return cells in split doses while 30 did so in single doses and 32 did not respond. Five centres exclusively washed cells before return while 12 did so 'sometimes.' One centre tested for sulphur sensitivity and another specifically questioned recipients regarding a previous history of sulphur allergy.

DMSO toxicity incidence

Of the 95 responding centres, 57 had seen DMSO toxicity (60%). There were at least 470 cases of toxicity reported, although the exact number was difficult to estimate since 11 centres that had experienced DMSO toxicity failed to report the number of cases seen. In total, approximately 34 000 transplants had been carried out by the 95 centres giving a minimum overall incidence (ie the total number of adverse events divided by the total number of transplants

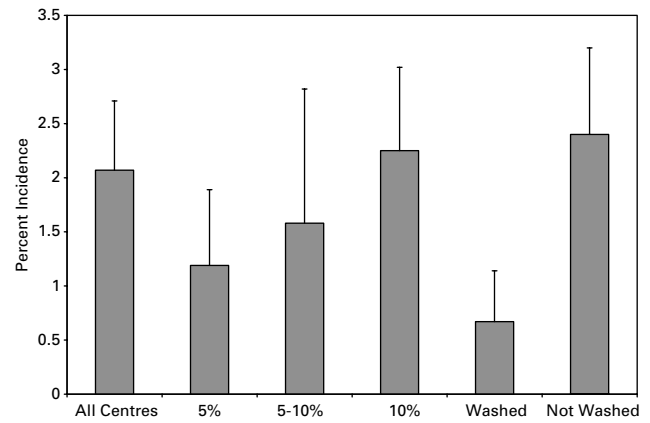


Figure 1 Mean centre incidence of DMSO toxicity by DMSO reduction strategy. Error bars show standard errors.

Table 1 Toxicity according to organ system reported by transplant centres

Toxicity	Probable (no. of centres)	Possible (no. of centres)
Cardiovascular	26	14
Respiratory	14	15
CNS	4	8
Renal	4	8
Others	9	4
Total	57	49

performed by all centres) of 1.4%; approximately one in every 70 transplants carried out. The mean incidence per centre of DMSO toxicity was 2.1%.

Effect of DMSO reduction strategies

Use of 10% DMSO (78 centres) was associated with an overall incidence of toxicity of 1.5%, while use of DMSO reduction strategies (concentration <10% or washing of cells before return – 22 centres) was associated with an overall toxicity incidence of 0.3%. The effect of different reduction strategies is shown on mean centre incidence of toxicity in Figure 1.

Nature of DMSO toxicity

A total of 38 centres had seen probable or possible cases of cardiovascular toxicity related to DMSO, while 27 had seen respiratory problems, 12 CNS effects, 11 renal disturbance and 13 others including hepatic dysfunction and anaphylaxis, as detailed in Table 1. Some patients may have had more than one serious toxicity – this was not specified in the questionnaire.

Discussion

The addition of DMSO to stem cell concentrates is a crucial step in undertaking stem cell transplantation for the vast majority of patients giving time to allow recovery from stem cell mobilisation procedures and possibly further

chemotherapy prior to the administration of conditioning therapy. Despite this central role, no guidelines exist for its usage and it is clear from the data obtained from this survey that there is wide variation in practice among transplant centres.

Inspection of the data does suggest that strategies to reduce the amount of DMSO given to patients as part of their stem cell transplant may help to reduce serious toxicity in addition to alleviating 'minor' toxicities of nausea, vomiting, etc. Despite publication of a hypothesis that DMSO might have a role in curing the underlying malignancy in autologous transplantation,²¹ there has been no support for this concept. In contrast, the lowest incidence of toxicity was seen in centres that washed cells before transplantation or used lower concentration of DMSO. DMSO toxicity appears to be common and larger transplant centres might expect to see at least one case of severe toxicity every year. The majority of cases are cardiovascular and respiratory in nature. Other effects such as CNS, renal, hepatic and anaphylaxis are less common. As the overall transplant related mortality from autologous procedures falls, the morbidity caused by DMSO takes on increasing importance. From this brief questionnaire we were unable to extract data regarding the severity or mortality related to DMSO but case reports of fatalities do exist.⁵

Although the need to protect the viability of the cryopreserved stem cells is paramount it does seem reasonable that strategies to reduce DMSO should be investigated in a prospective manner. We would suggest that the time is right for a more detailed prospective analysis of DMSO toxicity to be undertaken by transplant centres with a view to production of guidelines, which should lead to a fall in the incidence of morbidity and mortality on DMSO administration. Such guidelines should also then set a standard for the introduction of any alternative cryoprotectants, which might be considered for clinical use.

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