Cryopreservation of Mesenchymal Stem Cells- Can and should we avoid DMSO? [Powerpoint Presentation]

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Cryopreservation of Mesenchymal Stem Cells - Can and should we avoid DMSO?

Dr. Alexandra Stolzing,
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Loughborough University & Leipzig University
Cryopreservation using DMSO

Advantages

• proven efficacy as a cryoprotectant
• widely used, accepted and approved
• cheap and easy

Disadvantages... side effects
Cryopreservation using DMSO

Acute life-threatening cardiovascular toxicity with umbilical cord blood infusion: The role of dextran 60 in the apheresis process

Robert W. Ma, John M. Poon, David D. Ma, and Keith C. Fay

LETTER TO THE EDITOR

Ischemic stroke associated with the infusion of DMSO-cryopreserved auto-PBSCs

Nadia M. Romanos - Silvia Lechante

LETTER TO THE EDITOR

Temporary vision loss because of dimethyl sulfoxide in autologous stem cell transplantation

LETTER TO THE EDITOR

Neurotoxicity upon infusion of dimethylsulfoxide- cryopreserved peripheral blood stem cells in patients with pre-existing cerebral disease

Lutz P. Mueller*, Sebastian Theurich*, Maximilian Christendt, Wilfried Grothe, Anke W

Centre for Biological Engineering
Loughborough University
Cryopreservation using DMSO – side effects

A faster reconstitution of hematopoiesis after autologous transplantation of hematopoietic cells cryopreserved in 7.5% dimethyl sulfoxide if compared to 10% dimethyl sulfoxide containing medium

Iwona Mitrus, Andrzej Smagur, Sebastian Giebel, Joanna Gliwinska, Magdalena Prokop, Magdalena Glowala-Kosinska, Agata Chwieduk, Maria Sadus-Wojciechowska, Andrzej Tukiendorf, Jerzy Holowiecki

| Hematopoietic recovery after autoHSCT, need for transfusions and infusion-related complications according to Me₂SO concentration used for cryopreservation. |
|---|---|---|---|
| | 10% Me₂SO | 7.5% Me₂SO | p |
| N | 52 | 56 |  |
| Me₂SO volume transfused (ml) | 30 (10–160) | 22.5 (7.5–160) | 0.02 |
| WBC > 1.0 × 10⁹/L recovery (median day, range) | 11 (10–13) | 11 (9–12) | 0.03 |
| ANC > 0.5 × 10⁹/L recovery (median day, range) | 11 (10–13) | 11 (9–13) | 0.04 |
| PLT > 50 × 10⁹/L recovery (median day, range) | 12.5 (0–19) | 12 (0–21) | 0.36 |
| RBC transfusions (median no., range) | 0 (0–2) | 0 (0–4) | 0.27 |
| PLT transfusions (median no., range) | 1 (0–6) | 1 (0–5) | 0.2 |
| PBPCs infusion-related complications (grade 1 or 2) |  |
| Nausea | 15 (29%) | 17 (30%) | 1.0 |
| Vomiting | 4 (8%) | 6 (11%) | 0.74 |
| Dizziness | 1 (2%) | - | 0.48 |
| Weakness | - | 1 (2%) | 1.0 |
| Any complication | 20 (38%) | 24 (43%) | 0.7 |

56 patients with 10%

52 patients with 7.5%
Cryopreservation using DMSO – side effects

Recovery of neutrophils

Recovery of leukocytes

![Graphs showing recovery of neutrophils and leukocytes with different DMSO concentrations.](image)

- DMSO 7.5% vs. DMSO 10% for neutrophils
- DMSO 7.5% vs. DMSO 10% for leukocytes
Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres

P Windrum¹, TCM Morris¹, MB Drake¹, D Niederwieser² and T Ruutu³, on behalf of the EBMT Chronic Leukaemia Working Party Complications Subcommittee

Study: use of DMSO in 97 European Blood and Marrow Transplant Group centers undertaking autologous transplantation in 34 000 patients.

1 out 70 patients had severe side effects.
60% of centre reported side effects.
Cryopreservation using DMSO – side effects

Five centres exclusively washed cells before return while 12 did so ‘sometimes.’
Cryopreservation using DMSO – side effects

TRANSPLANTATION AND CELLULAR ENGINEERING

Should the standard dimethyl sulfoxide concentration be reduced? Results of a European Group for Blood and Marrow Transplantation prospective noninterventional study on usage and side effects of dimethyl sulfoxide

Curly Morris,1 Liesbeth de Wreede,2 Marijke Scholten,2 Ronald Brand,2 Anja van Biezen,2 Anna Sureda3 Ebbe Dickmeiss4 Marek Trnovy5 Jana Amerlev6 Patrizia Chiusolo7

**Study:** use of DMSO in 64 European Blood and Marrow Transplant Group centers undertaking autologous transplantation in 1,651 patients.
Cryopreservation using DMSO – side effects

- direct DMSO toxicity
- apoptotic cells and cell debris
- red blood lysis
- low temperature of infused product
- electrolyte imbalance

Side effects “probably related to DMSO” found in 862 patients (52%)
1 out of 7 patients had severe side effects

HOWEVER: 80% of centers still use 10% DMSO. Only 3 of these wash
DMSO – handling/processing

Washing?
• washing DMSO could be a good prophylaxis
• risk of loosing cells

Dilution?
• in what solution to dilute and in what volume

How soon to infuse?
• stability of thawed product and accepted time before infusion
• are there differences to how cells are infused (devices used)

How to thaw the cells?
• thawing in the lab or at bed side
• how to handle multiple bags
• training level of the operator
Few translated into clinical practice

Some cell therapy companies (Athersys & Regenesys) are testing DMSO-free solutions
MSC cell cryopreservation - a barrier to translation of stem cell products into the clinic?
Cryopreservation of MSC

- First isolated by Friedenstein in 1974
- Fibroblastoid cells - spindle-shaped
- Adherent to tissue culture glass or plastic
- High growth potential
- Immune modulation properties
- Anti-inflammatory
- Capable to be induced to differentiate into: osteoblasts, chondrocytes, adipocytes
Cryopreservation of MSC

- Majority of MSC product regulatory submissions (80%) want to use cryopreservation (Mendicino et al., 2014)
- A smaller proportion (35%) describe the use of cell banking systems
- Viability need to be >70% for intravenously administered MSCs (FDA)
Cryopreserving MSC I – reducing DMSO

Isolation → Passage 1-3 → Freezing 1°C/min → Day 0 counting with tryptan blue → Day 3 counting with MTT → Differentiation into osteoblasts for 14 days
Cryopreserving MSC I – reducing DMSO

Viability on day 3

Concentration of DMSO [%]

Concentration of HES450 [%]

Fresh cells

10% DMSO

4% DMSO, 6% HES 450

10% HES 450

Viability [%]

0 20 40 60 80 100 120 140

*
Cryopreserving MSC I – osteogenic diff.

MSC Viability [%]

Concentration of DMSO [%]

Concentration of HES [%]

*
Cryopreserving MSC I – reducing DMSO

<table>
<thead>
<tr>
<th></th>
<th>Fresh cells</th>
<th>10% DMSO</th>
<th>10% HES450</th>
<th>5%HES450 + 5%DMSO</th>
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Cryopreserving MSC I – reducing DMSO

Viability on day 3

Viability [%]

MW of HES [kDa]

Fresh cells

109, 209, 309, 409, 509, 609, 10% DMSO

Loughborough University Centre for Biological Engineering
Cryopreserving MSC I – titrating DMSO

• DMSO concentrations above 4% - good survival
• MSC differentiation is not negatively affected by low DMSO concentrations (with the exception of chondrogenesis)
• Additional cryopreservation compounds are necessary to improve survival in samples without DMSO
• HES with a very high MW is better for cryopreservation
• HES with a low substitution rate is better for cryopreservation
Cryopreserving MSC II – substituting DMSO

Viable cells [%]

- Fresh cells
- 10% DMSO 90% FCS
- 5% DMSO 95% FCS
- 10% DMSO
- 5% DMSO
- 5% HES200 0.3M sorbitol 10% dextran5
- 5% HES200 0.3M sorbitol 5% dextran5
- 5% HES200 0.3M sorbitol 10% dextran450
- 5% HES200 0.3M sorbitol 5% dextran450

- Ringer acetate
- Gelafusal

Significance levels:
- *: p < 0.05
- **: p < 0.01
- ***: p < 0.001
Cryopreserving MSC II – substituting DMSO

10% DMSO + Ring Acetate
5% DMSO + Ring Acetate
Dextran+ Sorbitol+ Hes

Osteoblast
Adipocyte
Chondrocyte
Cryopreserving MSC II – deleting DMSO

- DMSO-free cryopreservation solutions can be a viable alternative
- Compounds are available as medical grade
- Cells can be left at ambient temperature without toxicity effects of the cryoprotectant
- No washing required

Effect on MSC:
- No phenotypical changes
- Osteogenic and adipogenic diff. are not impaired
- But chondrogenic differentiation is impaired
Summary

• Cryopreservation of cell therapies requires attention to side effects, clinical and laboratory logistics, and the unique effect on particular cell types & behaviours

• Some DMSO-free cryopreservation solutions for expanded MSC are equally effective compared to 10% DMSO solutions in terms of cell survival, but seem to impair specifically chondrogenic differentiation

• Fish- and plant derived anti-freeze proteins are effective in reducing or substituting DMSO in MSC cryopreservation
Team members
- James Bui
- Jerome Lay
- Dr. Yahaira Naaldijk
- Dr. Victoriya Fedorova