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?

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Senior Lecturer, Regenerative Medicine,
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
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 a,*, Andrzej Smagur a, Sebastian Giebel a, Joanna Gliwinska b, Magdalena Prokop b, Magdalena Glowala-Kosinska a, Agata Chwieduk a, Maria Sadus-Wojciechowska a, Andrzej Tukiendorf c, Jerzy Holowiecki a

<table>
<thead>
<tr>
<th>Hematopoietic recovery after autoHSCT, need for transfusions and infusion-related complications according to Me₂SO concentration used for cryopreservation.</th>
<th>10% Me₂SO</th>
<th>7.5% Me₂SO</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>52</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Me₂SO volume transfused (ml) a</td>
<td>30 (10–160)</td>
<td>22.5 (7.5–45)</td>
<td>0.02</td>
</tr>
<tr>
<td>WBC &gt;1.0 x 10⁹/L recovery (median day, range)</td>
<td>11 (10–13)</td>
<td>11 (9–12)</td>
<td>0.03</td>
</tr>
<tr>
<td>ANC &gt;0.5 x 10⁹/L recovery (median day, range)</td>
<td>11 (10–13)</td>
<td>11 (9–13)</td>
<td>0.04</td>
</tr>
<tr>
<td>PLT &gt;50 x 10⁹/L recovery (median day, range)</td>
<td>12.5 (0–19)</td>
<td>12 (0–21)</td>
<td>0.36</td>
</tr>
<tr>
<td>RBC transfusions (median no., range)</td>
<td>0 (0–2)</td>
<td>0 (0–4)</td>
<td>0.27</td>
</tr>
<tr>
<td>PLT transfusions (median no., range)</td>
<td>1 (0–6)</td>
<td>1 (0–5)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

PBPCs infusion-related complications (grade 1 or 2) b

<table>
<thead>
<tr>
<th></th>
<th>10% Me₂SO</th>
<th>7.5% Me₂SO</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>15 (29%)</td>
<td>17 (30%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (8%)</td>
<td>6 (11%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1 (2%)</td>
<td>-</td>
<td>0.48</td>
</tr>
<tr>
<td>Weakness</td>
<td>-</td>
<td>1 (2%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Any complication</td>
<td>20 (38%)</td>
<td>24 (43%)</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Cryopreservation using DMSO – side effects

Recovery of neutrophils

Recovery of leukocytes

![Graphs showing the recovery of neutrophils and leukocytes with DMSO concentrations of 7.5% and 10%. The graphs indicate statistical significance with p-values of 0.04 and 0.03 respectively.](image-url)
Cryopreservation using DMSO – side effects

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.’
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
Alternatives to DMSO?

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

[Diagram showing the process steps with images of cultured cells, freezing equipment, and MTT staining results.]
Cryopreserving MSC I – reducing DMSO

Viability on day 3

Fresh cells

Concentration of DMSO [%]

0 1 2 3 4 5 6 7 8 9 10

Concentration of HES450 [%]

0 1 2 3 4 5 6 7 8 9 10

Viability [%]

0 20 40 60 80 100 120 140

*
Cryopreserving MSC I – osteogenic diff.

MSC Viability [%]

Concentration of DMSO [%]

Concentration of HES [%]

<table>
<thead>
<tr>
<th>fresh cells</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tbody>
<tr>
<td>0</td>
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<td>*</td>
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<td></td>
<td></td>
<td>80</td>
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<tr>
<td>1</td>
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<td>60</td>
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<td>0</td>
</tr>
</tbody>
</table>

* indicates a significant difference.
# 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>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone</strong></td>
<td><img src="image1.png" alt="Bone Image" /></td>
<td><img src="image2.png" alt="Bone Image" /></td>
<td><img src="image3.png" alt="Bone Image" /></td>
<td><img src="image4.png" alt="Bone Image" /></td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td><img src="image5.png" alt="Fat Image" /></td>
<td><img src="image6.png" alt="Fat Image" /></td>
<td><img src="image7.png" alt="Fat Image" /></td>
<td><img src="image8.png" alt="Fat Image" /></td>
</tr>
<tr>
<td><strong>Chondrosarcoma</strong></td>
<td><img src="image9.png" alt="Chondrosarcoma Image" /></td>
<td><img src="image10.png" alt="Chondrosarcoma Image" /></td>
<td><img src="image11.png" alt="Chondrosarcoma Image" /></td>
<td><img src="image12.png" alt="Chondrosarcoma Image" /></td>
</tr>
</tbody>
</table>

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Cryopreserving MSC I – reducing DMSO.
Cryopreserving MSC I – reducing DMSO

Viability on day 3

Viability [%] vs. MW of HES [kDa]

- Fresh cells
- 109
- 209
- 309
- 409
- 509
- 609
- 10% DMSO

* indicates a significant difference.
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 in [%]

- Fresh cells
- 5%HES200 0.3Msorbitol 10% dextran450
- 5%HES200 0.3Msorbitol 10% dextran450
- 5%HES200 0.3Msorbitol 5% dextran450
- 5%HES200 0.3Msorbitol 5% dextran5

* Ringer acetate
& Gelafusal
§§
***

0 20 40 60 80 100 120 140

Loughborough University Centre for Biological Engineering
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