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
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 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

56 patients with 10%

52 patients with 7.5%

<table>
<thead>
<tr>
<th></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 × 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 × 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 × 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>
<tr>
<td>PBPCs infusion-related complications (grade 1 or 2)b</td>
<td>15 (29%)</td>
<td>17 (30%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Nausea</td>
<td>4 (8%)</td>
<td>6 (11%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (2%)</td>
<td>-</td>
<td>0.48</td>
</tr>
<tr>
<td>Dizziness</td>
<td>-</td>
<td>1 (2%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Weakness</td>
<td>-</td>
<td>1 (2%)</td>
<td></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 probability of recovery over time for neutrophils and leukocytes using different DMSO concentrations. The graphs indicate statistically significant differences in recovery times between the groups.
Cryopreservation using DMSO – side effects

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

TRANSPANTATION 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 Suredo 3 Ebbe Dickmeiss 4 Marek Tronov 5 Jane Annerlev 6 Patrizia Chiusolo 7

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
Cryopreserving MSC I – reducing DMSO

Viability on day 3

Concentration of DMSO [%]

Concentration of HES450 [%]

Fresh cells

Viability [%]
Cryopreserving MSC I – osteogenic diff.

MSC Viability [%]

Concentration of DMSO [%]

Concentration of HES [%]

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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>![Bone image]</td>
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</tr>
</tbody>
</table>
Cryopreserving MSC I – reducing DMSO

Viability on day 3

<table>
<thead>
<tr>
<th>MW of HES [kDa]</th>
<th>Viability [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh cells</td>
<td>100</td>
</tr>
<tr>
<td>109</td>
<td>10%</td>
</tr>
<tr>
<td>209</td>
<td></td>
</tr>
<tr>
<td>309</td>
<td></td>
</tr>
<tr>
<td>409</td>
<td></td>
</tr>
<tr>
<td>509</td>
<td></td>
</tr>
<tr>
<td>609</td>
<td></td>
</tr>
<tr>
<td>10% DMSO</td>
<td>120</td>
</tr>
</tbody>
</table>

* Significant difference compared to fresh cells, p < 0.05.
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

Fresh cells 10% DMSO 90% FCS 5% DMSO 95% FCS 10% DMSO 5% DMSO 5% HES200 0.3M sorbitol 10% dextran 5 5% HES200 0.3M sorbitol 5% dextran 5 5% HES200 0.3M sorbitol 10% dextran 450 5% HES200 0.3M sorbitol 5% dextran 450

Viable cells [%]

Ringer acetate
Gelafusal

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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 differentiation 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