Hydroxyethyl Starch (HES) – An Alternative for the Freezing of Human Red Blood Cells

Andreas Sputtek

Universitätsklinikum Hamburg-Eppendorf
Inst. f. Transfusionsmedizin,
Hamburg, Germany

www.sputtek.de
1950    Smith A. U.
Prevention of haemolysis during freezing and thawing of red blood-cells
Lancet 259, 910-911 (1950)

("After freezing quickly to, and storage at, -79 °C for ... 3 months, and subsequent thawing at +40 °C, most of the red blood corpuscles survived and were unaltered in shape.")
Discovery of the Cryoprotectant Glycerol

- 1950  Medawar P. (University College, London)

"...observed in microscopic sections of skin which had been frozen and thawed in glycerol solutions that there were intact red blood cells in the blood vessels and in the skin."

Sloviter H. H.
First transfusions of red blood cells previously frozen and thawed
Vox Sang. 48, 254-256 (1985)
Discovery of the Cryoprotectant Glycerol

1951  Sloviter H. A.
Recovery of human red blood-cells after freezing
Lancet 261, 823-824 (1951)

("The slow removal of glycerol from red blood-cells has been successfully accomplished by dialysis against saline solutions containing progressively decreasing concentrations of glycerol.")
First Clinical Application of Frozen/Thawed Red Blood Cells

April 16, 1951
Hammersmith Hospital
London

Mollison P. L., Sloviter H. A.
Successful transfusion of previously frozen human red cells
Lancet 261, 862-864 (1951)

("... one patient severely ill with chronic leukaemia, who had already received many transfusions, was transfused with approximately 100 ml. of a suspension of previously frozen red cells. No unfavourable effects were observed and differential agglutination tests showed that the circulation contained approximately the expected number of red cells.")
Red Cell Preservation Processes

High-glycerol slow-cooling techniques

1960  **Tullis Process**

- final glycerol concentration 40% to 50%
- slow cooling (about 1 K/min) to -80 °C
- storage temperature -80 °C
- continuous-flow centrifugal equipment required to add the glycerol prior to freezing and to remove it after thawing

Red Cell Preservation Processes

High-glycerol slow-cooling techniques

1963 Huggins Process

- original cryoprotectant dimethyl sulfoxide, later on abandoned in favor of glycerol
- reversible agglomeration of red cells in a nonionic medium used for the removal of the cryoprotectant
- clinical experience in combat casualties

Huggins H. E.
Preservation of blood for transfusion with dimethyl sulfoxide and a novel washing technique, Surgery 54, 191 (1963)
<table>
<thead>
<tr>
<th>Year</th>
<th>Process</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963-1968</td>
<td>Pert/Krijnen/Rowe Process</td>
<td>- Red cell recovery depends on both additive concentration and heat transfer during cooling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Final glycerol concentration 14% to 18%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- For rapid cooling/storage liquid nitrogen required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- No specialized mechanical devices for removal of the cryoprotectant required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Dominant method in Europe</td>
</tr>
</tbody>
</table>

Pert, J. H., Schork, P. K., Moore, R.
A new method of blood preservation using liquid nitrogen and a glycerol-sucrose additive, Clin. Res. 11, 197 (1963)

Krijnen, H. W., Kuivenhoven, A. C. J., De Wit, J. J. F. M.
The preservation of blood cells in the frozen state. Experiences and current methods in the Netherlands, Cryobiology 5, 136-143 (1968)

Rowe, A. W., Eyster, E., Kellner, A.
Liquid nitrogen preservation of red blood cells for transfusion: a low glycerol-rapid freeze procedure, Cryobiology 5, 119-128 (1968)
Advantages of Cryopreserved Red Cells

- Reducing the infectious risks of homologous blood (Quarantine)
- Extension of preoperative collection period for autologous blood prior to elective surgery
- Date of surgery being not restricted by outdating of autologous units
- Unlimited preservation without subsequent loss in quality (autologous deposits; inappropriate relief planning; temporary shortages)
- Reducing problems regarding compatible blood (rare blood groups; multiple antibodies)
<table>
<thead>
<tr>
<th>Cryoprotectant</th>
<th>Glycerol</th>
<th>HES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>Purification of RBC prior to freezing</td>
<td>not required</td>
<td>required</td>
</tr>
<tr>
<td>Addition of cryoprotectant</td>
<td>critical</td>
<td>not critical</td>
</tr>
<tr>
<td>Cooling rate</td>
<td>not critical</td>
<td>critical</td>
</tr>
<tr>
<td>Storage below</td>
<td>-80 °C</td>
<td>-130 °C</td>
</tr>
<tr>
<td>Warming rate</td>
<td>less critical</td>
<td>critical</td>
</tr>
<tr>
<td>Removal of cryoprotectant</td>
<td>required</td>
<td>(not) required</td>
</tr>
<tr>
<td>Inadequate removal</td>
<td>critical</td>
<td>not critical</td>
</tr>
<tr>
<td>Storage at 4 °C after thawing</td>
<td>≤ 24 h</td>
<td>not critical</td>
</tr>
<tr>
<td>Leukocyte depletion</td>
<td>incomplete</td>
<td>incomplete</td>
</tr>
<tr>
<td>Viral transmission</td>
<td>possible</td>
<td>possible</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>critical</td>
<td>?</td>
</tr>
</tbody>
</table>
Advantages of HES for the Cryopreservation of Red Cells Compared to Glycerol

- HES is a well-established colloidal plasma substitute
- HES protected red blood cell concentrates can be thawed ready for use within 75 sec
- HES can be transfused together with the red blood cell concentrate
- HES does not penetrate the membrane of red cells. This is the reason why it can be removed by a simple washing step within 20 min prior to transfusion.
$T \ [\text{°C}]$

\begin{align*}
\text{H} &= 200 \ \text{K/min}
\end{align*}
Cryopreservation of RBC with Hydroxyethyl Starch (HES)

- In vitro investigations
- Animal experiments (n > 25)
- Experiments in healthy volunteers (n = 7)
- First patients
- Clinical study (n = 36)*
- Clinical application

* = in accordance with the ethical standards of the Committee on Human Experimentation of the Medical Society of Hamburg
Removal of 2 units of whole blood after induction of anesthesia, then:

**Group 1**

*Conventionally stored unit*

(RBC in PAGGS-M, buffy coat removed, stored for 39 +/- 12 d in 200 ml PAGGS-M)
230 ml RBC concentrate (HCT 80%)
500 ml HES 10%, 200.000/0,5
500 ml NaCl 0,9%

180 ml RBC
50 g HES
V = 1230 ml

**Group 2**

*Cryopreserved unit*

(washed with 290 ml isotonic saline)
230 ml RBC concentrate (HCT 80%)
500 ml HES 10%, 200.000/0,5
500 ml NaCl 0,9%

180 ml RBC
50 g HES
V = 1230 ml

**Group 3**

*Cryopreserved unit*

(no post-thaw washing)
450 ml RBC concentrate (HCT 40%)
in 11.5 Gew.-% HES 200.000 / 0,5
780 ml NaCl 0,9%

180 ml RBC
50 g HES
V = 1230 ml
Tissue Oxygenation (M. quadriceps)

\[ tpO_2 \text{ (mmHg)} \]

- Pre Donation
- Post Donation
- 10 min after Transfusion
- 20 min after Transfusion

- Control
- Cryo (washed)
- Cryo (unwashed)
Conclusions

- The transfusion of one unit of HES-cryopreserved autologous red blood cell concentrates is safe.

- The improvement of muscular tissue oxygenation by transfusion of the cryopreserved red cell concentrates (washed or not washed after thawing) is at least equivalent to the transfusion of one conventionally stored unit.

- A post-thaw washing step for the reduction of the "free" hemoglobin and the HES can be performed rapidly.

- Further investigations are deemed necessary to evaluate the effects of larger volumes of HES-cryopreserved red cells.

- The effects of homologous transfusions have to be studied.
Modular Storage System