Influence of gamma irradiation on red cell quality
Are changes to current standards and practices required?

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Blood Matters - Halifax
Disclosures

• I am an employee of Canadian Blood Services
• I am a member of the Scientific / Educational Advisory Board of: Biomet Biologics Inc., Biolife Solutions LLC.
• I have received consulting fees from HemoCue AB and Biomet Biologics Inc.

No products from these companies will be discussed in this presentation
Gamma and X-ray Irradiation

Irradiation results in single and double-stranded DNA breaks
Indications for Gamma Irradiation

• reduce risk of transfusion-associated graft-versus-host disease in immunocompromised recipients
  – rare, usually fatal complication following transfusion of viable T lymphocytes with HLA disparity
• all transfusions from 1st or 2nd degree relatives, all intrauterine transfusions, allogeneic stem cell recipients

Effect of γ Irradiation of Red Blood Cell Units on T-Cell Inactivation as Assessed by Limiting Dilution Analysis: Implications for Preventing Transfusion-Associated Graft-Versus-Host Disease

By Mary M. Pelszynski, Gary Moroff, Naomi L.C. Luban, Barbara J. Taylor, and Ralph R. Quinones

Guidelines for RCC Irradiation

• AABB / CSA Guidelines
  – Irradiation can be performed at any time during RCC storage (up to d 42) and stored for up to 28 d (or the original expiry)

• Council of Europe (17th Edition)
  – RCCs may be irradiated up to 28 d after collection but must be transfused as soon as possible, but no later than 14 d after irradiation and no later than 28 d after collection

• British Committee for Standards in Haematology
  – RCCs may be irradiated at any time up to 14 d after collection and may be stored for a further 14 d
International Guidelines

Post-Collection Storage (d)

0  14  28  35 / 42

Irradiation at d 21 not allowed
Current Practice in Canada

- gamma irradiation performed by Canadian Blood Services or at regional hospitals
- irradiation of blood bags and syringes is common
- target 25 Gy (15 – 50 Gy) gamma irradiation
- X-ray irradiators starting to be adopted
Effect of Irradiation on RBCs

- biconcave discoid
- lipid peroxidation
- decreased deformability
- potassium leakage
- swelling
- membrane remodeling
- Hb-bound extracellular vesicles
- hemolysis
The effect of timing of gamma-irradiation on hemolysis and potassium release in leukoreduced red cell concentrates stored in SAGM


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• 896 SAGM, LR units irradiated (25 Gy)
• 8-40 d after collection and stored for 1 – 28 d
Hemolysis of Irradiated RCCs

- Hemolysis increases with storage time before and after irradiation.
- Storage time after irradiation is the most predictive explanatory variable for hemolysis.

Donor sex (p=0.015) and age (p<0.001) predicted hemolysis.

**Predicted Hemolysis**

![Graph showing predicted hemolysis over days before irradiation.](image-url)
Potassium Levels in Irradiated RCCs

- within 72 h after irradiation, RCCs have potassium levels that exceed those seen in d 42 units
BEST Study #74

Irradiation Of RCCs

Changes in Guidelines (Europe)

• European Guidelines
  – **Previous**: Red cell components may be irradiated up to 14 days after collection and thereafter stored until the 28th day after collection. Exceptions are defined in specific monographs.
  – **17th Edition**: Red cell components may be irradiated up to 28 days after collection. Irradiated cells must be transfused as soon as possible but no later than 14 days after irradiation and in any case no later than 28 days after collection. More stringent requirements are included in specific component monographs.

• few studies on irradiation at various intervals of red cell shelf life
BEST 74 Study Design

- initiated in February 2014 at 7 international blood centres
- per center 4x7 paired units (pool-and-split);
  - 2 M / 2 F pools
  - every week one unit per series irradiated
- stored for 43 days, with weekly sampling
- after irradiation: 24 h and 72 h sample extra
- 256 sample points per participating blood centre
- measured: hemolysis, potassium, hematocrit, total Hb, ATP, microparticles, deformability, morphology, metHb, lipid peroxidation, mechanical fragility, PS exposure
RCC Unit Characteristics

pre-irradiation

<table>
<thead>
<tr>
<th>Centre</th>
<th>Units</th>
<th>Hematocrit</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CPD / SAGM</td>
<td>60 ± 3 L/L</td>
<td>49.8 ± 4.6 g/unit</td>
</tr>
<tr>
<td>ARCBS</td>
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<tr>
<td>B</td>
<td>CPD / SAGM</td>
<td>60 ± 3 L/L</td>
<td>52.8 ± 5.6 g/unit</td>
</tr>
<tr>
<td>Canadian Blood Services</td>
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<tr>
<td>C</td>
<td>CPD / SAGM</td>
<td>65 ± 2 L/L</td>
<td>65.5 ± 5.2 g/unit</td>
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<tr>
<td>IBTS</td>
<td></td>
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</tr>
<tr>
<td>D</td>
<td>CP2D / AS-3</td>
<td>58 ± 2 L/L</td>
<td>54.7 ± 4.4 g/unit</td>
</tr>
<tr>
<td>Hema-Quebec</td>
<td></td>
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</tr>
<tr>
<td>E</td>
<td>CPD / SAGM</td>
<td>57 ± 1 L/L</td>
<td>52.8 ± 4.5 g/unit</td>
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<tr>
<td>Sanquin</td>
<td></td>
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<tr>
<td>F</td>
<td>CPD / SAGM</td>
<td>57 ± 2 L/L</td>
<td>52.9 ± 5.5 g/unit</td>
</tr>
<tr>
<td>SNTBS</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G</td>
<td>CPD / PAGGSM</td>
<td>62 ± 2 L/L</td>
<td>56.3 ± 4.3 g/unit</td>
</tr>
<tr>
<td>GRCBS</td>
<td></td>
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</tbody>
</table>
Effect of Timing of Irradiation

- all parameters significantly different from day to day (p<0.001) due to storage lesion except for total hemoglobin (p=0.1077)

- after controlling for day of irradiation:
  - hematocrit (p=0.0087)*
  - free hemoglobin (p<0.0001)
  - hemolysis (p<0.0001)
  - potassium (p<0.0001)
  - PS-exposure (p=0.0296)
  - ATP (p<0.0001)

* hematocrit only Day 1 and Day 8 irradiation significantly higher than control and other test points
• timing of irradiation influences hemolysis
  • higher hemolysis in units irradiated early and then stored
  • rate of change of hemolysis increases if RCCs stored longer before irradiation
• gender effect: female < male
• hemolysis levels affected by type of additive solution (AS-3 / PAGGSM < SAGM)
Potassium (mM) – All Centres

- **timing of irradiation influences potassium release**
  - rate of change of K⁺ increases if RCCs stored longer before irradiation
  - **levels exceed 43 d control within 7 d of irradiation**
- gender effect: female < male
- potassium levels affected by type of additive solution (AS-3 < PAGGSM / SAGM)
Influence of Donor Sex

- Irradiated female pools had absolute:
  - Lower hematocrit ($p<0.01$)
  - Lower hemolysis ($p=0.045$)
  - Lower potassium ($p=0.0032$)
  - Equivalent ATP ($p=0.0623$)

- Rate of change of variables following irradiation were not affected by gender.

- Female RCCs less susceptible to irradiation injury?
General Conclusions from BEST Study

• If hemolysis is guidance:
  – new regulatory CoE rule is basically right: irradiation within first 28 days and used within these 28 days to avoid higher free hemoglobin load compared to non-irradiated units stored for 43 days

• If potassium is guidance:
  – use within 7 days after irradiation during first 10-14 days to avoid higher potassium load compared to non-irradiated units stored for 43 days or immediately after irradiation if stored > 14 days

• in general not advised to have stock of irradiated units due to rapid ageing/damage
Irradiation of Washed RCCs

• Canadian Blood Services implemented ACP-215 in October 2013
  – provides 7 d extended outdate on washed RCCs
• concerns over K⁺ levels in washed then irradiated RCCs
Effect of Timing of Irradiation on Washed RCCs
48 h Expiry on Irradiated, Washed RCCs

<table>
<thead>
<tr>
<th></th>
<th>BC-produced (n = 20)</th>
<th>WB-produced (n = 20)</th>
<th>Mean ± SD (n = 40)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>289 ± 18</td>
<td>336 ± 16</td>
<td>312 ± 29‡</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hb content (g/unit)</td>
<td>47 ± 6</td>
<td>60 ± 5</td>
<td>54 ± 8‡</td>
<td>&lt;0.01</td>
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<tr>
<td>Hct</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spun (%)</td>
<td>51 ± 3</td>
<td>57 ± 2</td>
<td>54 ± 4‡</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Advia (L/L)</td>
<td>0.53 ± 0.03</td>
<td>0.59 ± 0.02</td>
<td>0.56 ± 0.04‡</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hemolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drabkin's (%)</td>
<td>0.35 ± 0.06</td>
<td>0.33 ± 0.09</td>
<td>0.34 ± 0.07‡</td>
<td>0.65</td>
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<tr>
<td>HemoCue (%)</td>
<td>0.30 ± 0.05</td>
<td>0.32 ± 0.09</td>
<td>0.31 ± 0.07‡</td>
<td>0.42</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>96 ± 2</td>
<td>98 ± 2</td>
<td>97 ± 2‡</td>
<td>0.02</td>
</tr>
<tr>
<td>Extracellular K⁺ (mmol/L)</td>
<td>24.4 ± 2.7</td>
<td>27.8 ± 2.5</td>
<td>26.1 ± 3.1‡</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>102 ± 6</td>
<td>101 ± 5</td>
<td>102 ± 5‡</td>
<td>0.81</td>
</tr>
<tr>
<td>Sterility (% with no growth)</td>
<td>100% (20/20 no growth)</td>
<td>100% (20/20 no growth)</td>
<td>100% (40/40 no growth)‡</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Irradiation of Cryopreserved RCCs

• cryopreservation of rare RCCs from familial donors for directed transfusions presents risk for TA-GVHD
  – BCSH does not recommend irradiating cryopreserved RCCs
  – however, immunocompetent lymphocytes have been shown to be present in cryopreserved LR, RCCs
• we examined the effect of pre- and post-cryopreservation irradiation on RCC quality
# Effect of Timing of Irradiation on Cryopreserved RCCs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Pre-Cryo Irradiation</th>
<th>Post-Cryo Irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Volume (mL)</td>
<td>290 ± 14</td>
<td>283 ± 4</td>
<td>284 ± 4</td>
</tr>
<tr>
<td>Hemoglobin (g/unit)</td>
<td>48.3 ± 1.1</td>
<td>48.6 ± 1.1</td>
<td>48.3 ± 0.4</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>56.3 ± 2.1</td>
<td>58.0 ± 1.0</td>
<td>57.3 ± 0.6</td>
</tr>
<tr>
<td>Hemolysis (%)</td>
<td>1.13 ± 0.03</td>
<td>1.01 ± 0.1</td>
<td>0.97 ± 0.10</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.2 ± 0.7</td>
<td>10.1 ± 0.4*</td>
<td>13.9 ± 0.3*</td>
</tr>
<tr>
<td>Deformability (Eimax)</td>
<td>0.511 ± 0.007</td>
<td>0.510 ± 0.001</td>
<td>0.520 ± 0.003*</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>94.2 ± 2.5</td>
<td>94.9 ± 3.0</td>
<td>93.9 ± 1.0</td>
</tr>
</tbody>
</table>

Mean ± SD (n=3); 24-h post-cryopreservation assessment
* Comparison to control (p<0.05); ‡ Comparison to Pre-Cryo (p<0.05)

- post-cryopreservation irradiation may enhance damage to RBCs
  - current 24 h expiry of cryopreserved RCCs mitigates extent of damage
Conclusions

What does current evidence tell us?

• timing of gamma irradiation has significant impact on RCC quality
• irradiation of washed or cryopreserved units require special considerations
• irradiation following current CSA / AABB guidelines can result in:
  – RCCs with levels of hemolysis and potassium that would exceed those in 42 d stored products
  – levels may be harmful for specific recipients
Actions and Recommendations

• NAC is considering recommendation to align with European Standards
  – restricting irradiation of RCCs to < 14 d old units and applying an expiry of 28 d post-collection
  – assessment of impact of supply and inventory to be performed

• hospitals should evaluate current practices and consider evidence on the effects of irradiation on RCC and platelet quality

• a review of current CSA / CSTM and AABB standards is warranted
Canadian Blood Services
Société canadienne du sang

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