Effect of X-irradiation on the quality of red cell concentrates

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Introduction  The irradiation of blood components is used to inactivate T-lymphocytes to prevent transfusion-associated graft-versus-host (GVH) disease in susceptible recipients. X-irradiation can be used as an alternative to gamma irradiation and does not involve the use of a radioactive source. This study investigated the effect of X-irradiation on the quality of various red cell concentrates (RCC).

Methods  Whole blood units were processed into RCC in additive solution (SAG-M) or RCC in plasma suitable for intra-uterine (IUT) or neonatal exchange transfusion. RCC-SAG-M were irradiated at day 14 and sampled before and 0, 7 and 14 days following irradiation. RCC-IUT and RCC-Exchange were irradiated on day 4 and sampled before and 0 and 24 h following irradiation. Extracellular potassium levels and free plasma haemoglobin (haemolysis) were compared in X or gamma-irradiated units during storage.

Results  X-irradiation of RCC in SAG-M, or RCC for IUT or Exchange resulted in haemolysis comparable with gamma irradiation and levels were below the current UK/CE limit of 0.8% at the end of shelf life. X-irradiation of RCC-SAG-M or RCC-IUT with haematocrit of 75% and 85% resulted in potassium leakage comparable with gamma-irradiation. However, X-irradiation of RCC for neonatal exchange transfusion showed statistically significantly higher supernatant potassium levels after 24 h than gamma irradiation, although this difference is considered to be clinically insignificant.

Conclusion  X-irradiation of RCC in SAG-M or IUT or RCC in plasma suitable for neonatal exchange transfusion resulted in acceptable levels of haemolysis and potassium leakage compared with the current process of gamma irradiation.

Key words: gamma irradiation, red cell concentrates, SAG-M, X-irradiation.
leakage from red cells, particularly if the red cell components are stored for a prolonged period following irradiation. There are conflicting reports on the effect of gamma-irradiation on 24 h post-transfusion recovery [10, 11].

There are very few publications on the \textit{in vitro} effects of X-irradiation on RBCs and comparison with gamma-irradiation [12], at a time when many blood centres are considering X-irradiation as an alternative to gamma irradiation and it is important to know whether the damage caused to RCC components by X-irradiation is comparable with that caused by gamma-irradiation. A limitation of a recent study [12], comparing X-ray with gamma irradiation was that the citrate phosphate dextrose-1 (CPD-1) red cell units were not leucocyte depleted and, since many blood centres are leucodepleting units, it is important to know the effect of X-irradiation on such red cell components.

The aim of this study was to assess whether X-irradiation of RCC components met UK/CE specifications and to compare data with existing reference data from gamma-irradiated components, some of which have previously been published [13]. However, during the study it became apparent that reference data were not available for RCC-IUT for direct comparison and therefore additional gamma-irradiated components for IUT were produced and analysed. We assessed red cells stored in saline adenine glucose-mannitol (SAG-M) additive solution, red cells stored in plasma suitable for neonatal exchange transfusion (haematocrit of 55%), and red cells stored in plasma at two high haematocrit (Hct) levels, both suitable for intra-uterine transfusion (Hct 70–80% and 80–90%). Two markers of erythrocyte quality were assessed: haemolysis and potassium leakage.

**Materials and methods**

The effects of X or gamma-irradiation on three types of red cell components (RCC) were studied – RCC in SAGM, RCC for Exchange Transfusion and RCC for Intra-uterine Transfusion. The RCC units were irradiated on the last permitted day according to UK guidelines to test the worst-case scenario. The study design is shown in Fig. 1.

**Whole blood collection**

Whole blood (target 470 ml, range 405–495 ml) donations from healthy volunteers were collected into 63 ml citrate-phosphate-dextrose anticoagulant using standard top–top (FQE 6280LB; MacoPharma, Middlesex, UK) and bottom–top (RCB 436CU3; Pall Corp., Portsmouth, UK) blood collection bags according to standard NHSBT procedure as described below.

**RCC in SAGM**

Ten RCC-SAGM units were made on the day of collection (day 0), using the Top and Top process and Bottom and Top process for X-irradiation in this study. Twenty RCC-SAGM had previously been made in the same way for gamma-irradiation in another study [13] and were used as reference samples.

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Fig. 1 Schematic flow chart of the study design. Red cell concentrate (RCC) in SAGM (10 X-irradiated and 20 gamma-irradiated), RCC for exchange (10 X-irradiated and 20 gamma-irradiated) and two sets of RCC for IUT: one with Hct between 70% and 80% (10 X-irradiated and 10 gamma-irradiated) and another with Hct 80–90% (10 X-irradiated and 10 gamma-irradiated).

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data in this study. Briefly, for Top and Top processing, whole blood was leucocyte depleted (R3 filter), centrifuged (3399 g for 15 min at 4°C, Sorval RC3BP; Kendro Laboratory Products, Bishops Stortford, UK) and plasma removed using an automated expressor (Optipress II, Fenwal, Inc., Lake Zurich, IL). SAGM (100 ml) was added to the red cell component. For Bottom and Top processing, the whole blood was centrifuged (2800 g for 11.5 min at 22°C), an automated expressor (Optipress II) used to remove the plasma and the red cell component leaving the buffy coat component in the original pack. SAGM (100 ml) was added to the red cell component which was then leucocyte depleted (RCM3 filter). All RCC SAGM units were stored at 2–6°C depleted (RCM3 filter). All RCC SAGM units were stored at 2–6°C (achieved a central dose of 35 GY, Raycell X-ray irradiator, MDS Nordion). RCC-SAGM units were irradiated at day 14 of storage. Samples (12–15 ml) were taken using sterile connection (TSCD; Terumo, Leuven, Belgium) of a sample pouch (VSE 000A, MacoPharma, Middlesex, UK) before irradiation (day 14 of storage), immediately following irradiation and at days 14, 21 and 28 of storage (0, 7 and 14 days following irradiation, Fig. 1). RCC-IUT and RCC-Exchange units were X-irradiated on day 4 of storage. Samples were taken before irradiation (day 4), immediately following irradiation on day 4 and on day 5 of storage (0 and 24 h following irradiation). All units of RCC were stored at 2–6°C between sampling.

Sample analysis
Due to the high haematocrit of RCC-IUT components, samples were diluted (1:2) using saline before full blood count (FBC) analysis on the Advia 2120 haematology analyser (Siemens, Camberley, Surrey, UK) and results corrected for the dilution. All samples were double-centrifuged (2000 g for 10 min) and the RCC supernatant removed for potassium and haemolysis testing. Extracellular potassium concentrations were measured using a biochemistry analyser (Vitros DT60II, Ortho-Clinical Diagnostics, High Wycombe, UK) and extracellular haemoglobin levels were estimated using a direct spectrophotometric technique [16].

Statistical analysis
Statistical analysis was performed using Prism 5 (GraphPad Software, San Diego, CA, USA). The data were tested for normality using the D’Agostino and Pearson omnibus normality test. If the data were normally distributed, the groups were compared using one- or two-way ANOVA and Tukey’s Multiple Comparison post-test. If the data were not normally distributed, data were compared using the Kruskal–Wallis test and Dunn’s Multiple Comparison Test.

Results
The volume and haematocrit of all gamma and X-irradiated RCC in SAGM, RCC for exchange and RCC for IUT met current UK specifications [14] (Tables 1a–1c). There were no statistically significant differences ($P > 0.05$) in the volume and haematocrit of the X-irradiated and gamma-irradiated components.

RCC in SAGM
There was no difference in supernatant potassium concentration and haemolysis before and immediately following X-irradiation in RCC-SAGM units that had been stored for
X-irradiation on the quality of red cell concentrates

Table 1a  Volume and haematocrit of X-irradiated RCC-SAGM, RCC for exchange and RCC for IUT units

<table>
<thead>
<tr>
<th>X-irradiated units</th>
<th>RCC-SAGM (n = 10)</th>
<th>RCC-Exchange (n = 10)</th>
<th>RCC-IUT (Hct 70–80%) (n = 10)</th>
<th>RCC-IUT (Hct 80–90%) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>267 (219–297)</td>
<td>312 (265–348)</td>
<td>218 (210–247)</td>
<td>190 (174–211)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>60.7 (56.7–64.6)</td>
<td>55.6 (53.8–57.0)</td>
<td>77.4 (72.4–80.6)</td>
<td>87.9 (83.8–91.4)</td>
</tr>
</tbody>
</table>

Data are median (min–max) of the units.

Table 1b  Volume and haematocrit of gamma-irradiated RCC-SAGM, RCC for exchange and RCC for IUT units

<table>
<thead>
<tr>
<th>Gamma-irradiated units</th>
<th>RCC-SAGM (n = 20)</th>
<th>RCC-Exchange (n = 20)</th>
<th>RCC-IUT (Hct 70–80%) (n = 10)</th>
<th>RCC-IUT (Hct 80–90%) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>276 (254–303)</td>
<td>297 (241–381)</td>
<td>224 (197–249)</td>
<td>191 (168–204)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>62.0 (58.1–65.7)</td>
<td>56.8 (52.1–59.0)</td>
<td>77.0 (66.6–78.4)</td>
<td>88.6 (86.6–93.4)</td>
</tr>
</tbody>
</table>

Data are median (min–max) of the units.

Table 1c  Component specifications

<table>
<thead>
<tr>
<th>RCC-SAGM</th>
<th>RCC-Exchange</th>
<th>RCC-IUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>220–340&lt;sup&gt;a&lt;/sup&gt;</td>
<td>220–395&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>50–70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50–55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haemoglobin (g/unit)</td>
<td>&gt;40&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>&gt;40&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>UK specifications [14].
<sup>b</sup>NHSBT specifications.

14 days prior to irradiation. During storage for 14 days after X-irradiation the supernatant potassium and haemolysis increased from 28.8 to 63.9 mmol/l and 0.19% to 0.36%, respectively (Fig. 2). The supernatant potassium in X- and gamma-irradiated RCC-SAGM units equate to 6.86 and 6.45 mmol/unit, respectively, at the end of shelf life (day 28, 14 days post-irradiation). There was no significant difference in supernatant potassium (either in terms of mmol/l supernatant or mmol per unit) or haemolysis between X- and gamma-irradiated RCC in SAGM at the end of shelf life. Haemolysis in X-irradiated RCC-SAGM units complied with guidelines from the UK (<0.8% in >75% of units) [14], Council of Europe (<0.8%) [15] and AABB (<1%) [17].

RCC for exchange transfusion (stored in plasma at Hct of 50–60%)

There was no difference in supernatant potassium concentration and haemolysis immediately following X-irradiation in the RCC for exchange transfusion units that were stored for 4 days prior to irradiation. Twenty-four hours after X-irradiation the median supernatant potassium of RCC for exchange transfusion units had increased from 9.7 to 22.7 mmol/l (Fig. 3). Supernatant potassium concentration in X-irradiated units was significantly higher (P < 0.001), approximately 22%, than gamma-irradiated RCC 24 h following irradiation. The supernatant potassium in X- and gamma-irradiated RCC for exchange transfusion units equate to 3.07 mmol/unit (10 μmol/ml of RCC) and 2.20 mmol/unit (8 μmol/ml of RCC), respectively at the end of shelf life (24 h following irradiation). There was no increase in the level of haemolysis 24 h following X-irradiation and no difference between X- and gamma-irradiated RCC for exchange transfusion. At the end of shelf life, levels of haemolysis complied with guidelines from the UK (<0.8% in >75% of units) [14], Council of Europe (<0.8%) [15] and AABB (<1%) [17].

RCC for IUT (stored in plasma at Hct >70%)

Supernatant potassium levels in RCC-IUT (median Hct 77%) on day 4 were 21.7 mmol/l. This was unchanged immediately after X-irradiation, but increased to 42.1 mmol/l after 24 h storage (Fig. 4). The concentration of supernatant potassium in RCC-IUT at a higher Hct (median 88%) on day 4 was 37.7 mmol/l increasing to 84.0 mmol/l after X-irradiation and 24 h storage. However, when expressed as total amount of supernatant potassium per unit (mmol) there was no difference between IUT units prepared at 77% or 88%. The total supernatant potassium in X- and gamma-irradiated RCC-IUT units (median Hct 77%) was 2.4 mmol/unit (11 μmol/ml of RCC) and 2.5 mmol/unit (11 μmol/ml of RCC), respectively and at a higher Hct (88%) was 1.87 mmol/unit (9 μmol/ml of RCC) and 2.28 mmol/unit (9 μmol/ml of RCC), respectively, at the end of shelf life (24 h following irradiation). There was no significant difference in the supernatant potassium either in terms of supernatant concentration (mmol/l) or total

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amount per unit (mmol) between gamma and X-irradiated RCC-IUT units 24 h post-irradiation (Fig. 4).

Haemolysis levels in RCC-IUT (median Hct 77%) and RCC-IUT (median Hct 88%) units on day 4 were 0.07% and 0.12%, respectively. There was no significant increase in haemolysis after X-irradiation and storage for 24 h of either group of RCC-IUT units. There was no significant difference in levels of haemolysis between X- and gamma-irradiated RCC for IUT at the end of shelf life, and all RCC-IUT complied with current UK specifications (<0.8% in >75% of units), with only 1 of 10 units in the gamma irradiated packs exceeding the 0.8% limit. However, there was a trend towards higher haemolysis in gamma irradiated units with 88% haematocrit.

Discussion
The irradiation of cellular blood components is used to inactivate residual lymphocytes to prevent TA-GvHD.
X-irradiation has been shown to affect lymphocyte function equal to the effect of gamma-irradiation when equivalent doses (25 Gy) are used [12]. A consequence of irradiation is that erythrocytes are also damaged leading to increased haemolysis and potassium leakage into the extracellular fluid. Red cell components stored after irradiation have significantly increased supernatant potassium concentration compared to RCC components that are not irradiated [10, 18, 19]. Therefore, irradiated components have a shorter shelf life to reduce recipient exposure to high potassium concentrations and free haemoglobin.

In this study we X-irradiated RCC in SAGM and RCC for exchange and compared the data with that from gamma irradiated RCC which had been generated in the same way as in this study. All RCC units met the current UK specifications and were suitable for clinical use. Due to a lack of reference data from RCC-IUT treated with gamma irradiation, additional RCC components for IUT were produced at two Hct levels, gamma-irradiated and compared with X-irradiated RCC-IUT units.

Supernatant potassium levels and haemolysis increased as expected following X-irradiation. The supernatant potassium levels in RCC SAG-M doubled 7 days after X-irradiation, but no significant difference was seen between X-ray and gamma irradiation, on the last day that the component can be used for transfusion (day 5, 24 h post-irradiation). Horizontal bars represent median values. ns = not significant (P > 0.05), X-ray vs. gamma.

Fig. 4 Potassium and haemolysis levels in red cell units for IUT, gamma (n = 10) or X (n = 10) irradiated on day 4 of storage with 88% (a and b) and 77% (c and d) Hct. K+ expressed as mmol/l of supernatant and haemolysis expressed as % of total Hb in unit of IUT. Statistical comparison between X-ray and gamma irradiation, on the last day that the component can be used for transfusion (day 5, 24 h post-irradiation). Horizontal bars represent median values. ns = not significant (P > 0.05), X-ray vs. gamma.
explained by the lower haematocrit of units (56–64%) used in our study.

In RCC for Exchange and IUT, the supernatant potassium concentration increased two- to three-fold within 24 h of X-irradiation, similar to increases reported in other studies using gamma irradiation [20, 22]. The X-irradiated RCC for Exchange transfusion had significantly higher supernatant potassium (22.7 mmol/l) than gamma irradiated units (17.7 mmol/l) after 24 h storage. A further pool and split study would be warranted to confirm or refute whether this difference is due to the source of radiation. However, these levels are lower than previously reported in non-leucodepleted X-irradiated RCC in CPDA-1 with haematocrit of 70–80% [12] and are lower than the 80–100 mmol/l reported to be the maximum tolerable infusion through a peripheral vein [21].

There was no significant difference in the potassium concentration 24 h following gamma or X-irradiation of RCC-IUT at 77% or 88% Hct. Potassium levels in X-irradiated RCC-IUT units (24 h post-irradiation) with 88% Hct and RCC-IUT with 77% Hct in our study were up to 103 and 55 mmol/l (1.80 and 2.36 mmol/unit), respectively. However, the potassium concentration and percent haemolysis are both influenced by the haematocrit of the component. For instance at higher haematocrit there are more cells to leak into a smaller volume of supernatant and the calculated concentrations are therefore higher. However, in IUT it is the absolute amount of potassium in a given volume of RCC that is important. Win et al. [23] showed that infusion of 0.95 mmol total potassium in a mean volume of 84 ml red cells with Hct 66% (11 mmol/ml RCC) caused no significant increase in potassium levels in foetal plasma immediately after intra-uterine transfusion. Potassium levels in X-irradiated RCC-IUT in our study were similar (approximately 10 mmol/ml RCC) and therefore are likely to have no clinically significant effect.

The data from this study show no significant increase in haemolysis 24 h after X-irradiation of Exchange and IUT units. Although RCC in SAG-M showed an increase in haemolysis 14 days after irradiation, this did not exceed the current UK and CE limits of 0.8% haemolysis or the AABB limit of 1%. Furthermore, when X-irradiation was compared with gamma-irradiation, no significant differences were seen in haemolysis in RCC in SAG-M, RCC-Exchange and RCC-IUT at the end of their shelf life. However, one unit 24 h post-gamma-irradiation (RCC for IUT with 88% Hct) exceeded the 0.8% limit for haemolysis and also had supernatant potassium concentration of 130 mmol/l, suggesting a donor specific effect. A link between undefined specific whole blood donor characteristics and haemolysis has been observed in stored RCC units [24]. Since the primary effect of gamma- and X-irradiation at the same doses (25 Gy) on lymphocytes is equivalent [12] and the damage to erythrocytes is similar, there are considerable benefits of using X-irradiation over gamma-irradiation: X-ray irradiators are non-radioactive; have fewer regulatory requirements; training, protection and monitoring of staff is less onerous; and the potential for misuse/bioterrorism is reduced.

In summary X-irradiation of RCC in SAG-M or RCC for IUTs showed an effect on potassium leakage similar to gamma irradiation, but resulted in higher levels of supernatant potassium in exchange units. However, this was not considered a clinically significant increase and the components are considered safe for clinical use. Levels of haemolysis in RCC in SAG-M, and those suitable for neonatal exchange or IUT transfusion appear to be comparable after X or gamma irradiation but a further study with increased power (large sample size and/or pool and split design) is warranted to confirm non-inferiority. The data presented here, together with published data on leucocyte inactivation, has been considered by the committee responsible for the UK Guidelines for Blood Transfusion Services, and X-irradiation of red cells has been accepted as a suitable alternative to gamma irradiation. X-irradiation of cellular blood components is now in routine use in England.

Acknowledgements

The authors would like to thank Dr Helen New (Consultant Haematologist, NHSBT and Department of Paediatrics, St Mary’s Hospital, UK) and Dr Sheila MacLennan (Consultant Haematologist and Clinical Director-Products, NHSBT) for their critical appraisal of the manuscript.

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