

Quality control of blood irradiation with a teletherapy unit: damage to stored red blood cells after cobalt-60 gamma irradiation

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BACKGROUND: Previous publications have documented the damage caused to red blood cells (RBCs) irradiated with X-rays produced by a linear accelerator and with gamma rays derived from a ^{137}Cs source. The biologic effects on RBCs of gamma rays from a ^{60}Co source, however, have not been characterized.

STUDY DESIGN AND METHODS: This study investigated the effect of 3000 and 4000 cGy on the in vitro properties of RBCs preserved with preservative solution and irradiated with a cobalt teletherapy unit. A thermal device equipped with a data acquisition system was used to maintain and monitor the blood temperature during irradiation. The device was rotated at 2 r.p.m. in the irradiation beam by means of an automated system. The spatial distribution of the absorbed dose over the irradiated volume was obtained with phantom and thermoluminescent dosimeters (TLDs). Levels of Hb, K^+ , and Cl^- were assessed by spectrophotometric techniques over a period of 45 days. The change in the topology of the RBC membrane was investigated by flow cytometry.

RESULTS: Irradiation caused significant changes in the extracellular levels of K^+ and Hb and in the organizational structure of the phospholipid bilayer of the RBC membrane. Blood temperature ranged from 2 to 4°C during irradiation. Rotation at 2 r.p.m. distributed the dose homogeneously (92%-104%) and did not damage the RBCs.

CONCLUSIONS: The method used to store the blood bags during irradiation guaranteed that all damage caused to the cells was exclusively due to the action of radiation at the doses applied. It was demonstrated that prolonged storage of ^{60}Co -irradiated RBCs results in loss of membrane phospholipids asymmetry, exposing phosphatidylserine (PS) on the cells' surface with a time and dose dependence, which can reduce the in vivo recovery of these cells. A time- and dose-dependence effect on the extracellular K^+ and plasma-free Hb levels was also observed. The magnitude of all these effects, however, seems not to be clinically important and can support the storage of irradiated RBC units for at least 28 days.

Immunocompetent T cells present in transfused whole blood and blood components may become engrafted and may proliferate and induce an immunologic response in patients whose immune system is unable to reject them.¹⁻³ This rare, but fatal, reaction is known as transfusion-associated graft-versus-host disease (TA-GVHD) and may occur in severely immunocompromised patients, including patients with congenital immunodeficiencies and marrow transplant recipients, as well as in cancer patients treated with chemotherapy or radiotherapy.⁴⁻⁸ TA-GVHD has also been reported in immunocompetent patients who receive blood from donors homozygous for shared human leukocyte antigen (HLA) haplotypes.⁹

Irradiation of blood and blood components before transfusion has been the only proven method for preventing this reaction.¹⁰ Gamma and X-rays, both representing ionizing radiation, break the DNA molecules of T lymphocytes and prevent the latter from inducing an immunologic response in the recipient. Blood irradiation can be

ABBREVIATIONS: PS = phosphatidylserine; TA-GVHD = transfusion-associated graft-versus-host disease; TLD(s) = thermoluminescent dosimeter(s).

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performed with commercial irradiators specifically designed for this purpose, usually located in blood banks. These irradiators incorporate one to four gamma-ray sources, usually ^{137}Cs . In the absence of a blood irradiator, however, other alternatives such as X-rays produced by a linear accelerator or gamma rays derived from the ^{60}Co source of a cobalt teletherapy machine can be used for this purpose.¹¹⁻¹⁵ Ultraviolet light (ultraviolet B) also inactivates T lymphocytes. This wavelength (280-320 nm), however, cannot penetrate currently licensed polyvinylchloride, polyolefin, and other plastic blood bags and therefore cannot be used to prevent TA-GVHD. Current filtration technology cannot consistently produce the levels of lymphocytes removal required, and at least one case of TA-GVHD has been observed after transfusion of filtered blood products.¹⁰

Studies on the radiosensitivity of T cells to X-rays (generated by a linear accelerator) and to gamma rays (generated by ^{137}Cs sources of a specific irradiator and by the ^{60}Co source of a teletherapy unit) have shown that a minimum dose of 2500 cGy is necessary to prevent TA-GVHD.¹⁶⁻¹⁸ These studies were conducted on T cells isolated from irradiated red blood cell (RBC) units^{16,18} and from platelet (PLT) concentrates obtained by apheresis.¹⁷ In addition to damaging T lymphocytes, irradiation also damages other blood cells such as RBCs, PLTs, and granulocytes.^{10-12,19-28} It is still unclear, however, what dose eliminates the risk of TA-GVHD while preserving the quality of the transfused product. The US Food and Drug Administration (FDA),²⁹ the AABB,³⁰ and the UK Red Book³¹ require a minimum dose of 2500 cGy at the middle plane. The Council of Europe guidelines state that irradiation must be at least 2500 cGy but no more than 5000 cGy.³²

Cobalt teletherapy units are less requested than linear accelerators in a radiotherapy center, permitting their use to irradiate blood. Considering the lack of information about the biologic damage to cellular blood products provoked by gamma rays emitted by a ^{60}Co source, and the dose range recommended,²⁹⁻³² we decided to investigate the influence of 3000 and 4000 cGy of gamma rays emitted by a cobalt teletherapy machine on RBCs stored in plastic bags (PL-146, Baxter Healthcare Corp., Deerfield, IL) containing preservative solution (ADSOL, Baxter Healthcare Corp.).

To guarantee temperatures below 6°C, the RBC units were stored in the thermal device of an automated mechanical system used to store blood bags during irradiation with a teletherapy unit.¹⁵ To minimize the heterogeneity of the dose in the irradiated volume, the thermal device was rotated at 2 r.p.m. in front of the irradiation beam. The distribution of the doses in the volume was determined with thermoluminescent dosimeters (TLDs) and a homogeneous phantom consisting of clear polystyrene plastic.¹⁵ The influence of this rotation rate on the

physical integrity of the RBC membrane was determined on the basis of the elevation of the extracellular levels of potassium (K^+), chloride (Cl^-), plasma-free hemoglobin (Hb), and alteration of the asymmetry of the phospholipid bilayer of the membrane. The results showed that the method used to store the blood bags during irradiation maintained the blood temperature below 6°C and homogenized the distribution of the dose in the volume without injuring the RBC membrane. This guaranteed that the damage produced to the cells of interest was due only to the action of radiation at the doses applied.

MATERIALS AND METHODS

Irradiation system and dosimetry

In this study, all materials were irradiated with a cobalt teletherapy unit (Gammatron S-80, Siemens, Munich, Germany). The dose rate was determined with a calibrated dosimetric system consisting of an electrometer (DI4, PTW, Freiburg, Germany) and an ionization chamber (M23332, PTW). The dosimetry of the ^{60}Co beam was performed according to an international dosimetry protocol.³³ Dosimetry in the volume of irradiated blood was simulated with a cylindrical clear polystyrene phantom with shape and size (20 cm height and 20 cm in diameter) matching those of the thermal device used to store the blood bags during irradiation.¹⁵ Cavities inside the phantom were filled with TLDs (LiF-100, Harshaw Chemical Co., Solon, OH) and the phantom was rotated at 2 r.p.m. in front of the irradiation beam with an automated mechanical system.¹⁵ The phantom was irradiated with a $30 \times 30\text{-cm}^2$ field at the surface of the phantom and an 80-cm source-to-phantom surface distance. The irradiation time was selected to obtain a dose of 1200 cGy at the phantom volume center. A total of 85 TLDs were individually calibrated and annealed according to manufacturer recommendations (1 hr at 400°C followed by 2 hr at 100°C). The TLD response was analyzed with a reader (Model 2000-B/2000-C, Harshaw). The dose in each cavity was measured with three TLD readings that were averaged and normalized to the dose value obtained at the phantom center. The dose distribution on the central plane of the phantom was represented by isodose curves according to the method described in the literature.¹⁵

Blood collection and irradiation

The RBC units were prepared by centrifugation from blood collected from 10 healthy adult volunteers. The RBC unit obtained (approx. 250 mL) from a single donor, nonleukoreduced, was stored in a PL-146 plastic bag containing ADSOL preservative solution (Baxter Healthcare Corp.). To avoid biologic variability, each RBC unit collected from a single donor was equally divided into three

bags; one was used as control (nonirradiated) and the other two were used to test the effects of doses of 3000 and 4000 cGy on the RBCs. Six hours after collection, the blood bags were individually protected with plastic wrap, stored in the thermal device (which contained water at the temperature of 2°C), and irradiated. The thermal device was rotated at 2 r.p.m. in front of the irradiation beam with the automated mechanical system described above. A 30 × 30-cm² field on the surface of the device and an 80-cm source-to-device surface distance were used based on the dose distribution results obtained previously for the phantom. The dose released into the blood bags was confirmed by TLDs calibrated and annealed as described above. After irradiation, the blood bags were stored in a refrigerator at 2 to 4°C for 45 days. The effects of 3000 and 4000 cGy on the RBCs were determined 15 hours after irradiation and on Days 10, 20, 35, and 45. On each of these days, a sample of RBC unit, approximately 5 mL, was collected from each blood bag with a sterile connecting system (Baxter) and the content of Hb, K⁺, and Cl⁻ was determined in the plasma separated from the RBC samples.

Free Hb, potassium, and chloride levels

One volume of plasma (approx. 100 µL) was separated from each RBC sample by centrifugation at 20°C for 20 minutes at 1500 × g with a refrigerated centrifuge (Fanen, Guarulhos, SP, Brazil). Plasma-free Hb levels were determined by the technique of first-derivative recording with a spectrophotometer (Spectronic, Milton Roy, Ivyland, PA).³⁴ The same plasma volume was separated from the RBC samples by centrifugation according to the specifications described above and K⁺ levels were determined by flame photometry.³⁵ Each plasma volume, approximately 100 µL, was diluted 1:100 with diluting solutions (Celm, Barueri, SP, Brazil), and the quantities of K⁺ were determined by flame photometry with a flame photometer (FC 280, Celm). These measurements were checked against a standard solution (Celm) containing 140 mmol per L K⁺. The quantity of Cl⁻ in each plasma volume, approximately 100 µL, was determined with a chloride meter (920 M, Corning, New York, NY), and the measurements were checked against a standard solution (Corning) containing 100 mmol per L Cl⁻.

Phospholipid bilayer

The physical integrity of the asymmetry of the phospholipid bilayer of the RBC membrane was evaluated 15 hours after irradiation and on Days 35 and 45 by measuring the amount of cells exposing phosphatidylserine (PS) in the outer leaflet. The procedures used in this experiment were based on the method used to investigate changes in the asymmetry of the phospholipid bilayer of pathological

cells with flow cytometry.³⁶ One RBC sample, approximately 2 mL, was collected from each blood bag with the sterile connecting system described above. The RBC samples were washed three times with 0.1 mol per L phosphate buffer, pH 7.0, and centrifuged at 4°C for 5 minutes at 1500 × g. The cells were then diluted 1:50 with 0.1 mol per L phosphate buffer and 485 µL calcium buffer at 4°C, and 5 µL of fluorescein isothiocyanate–annexin V (Nexins Research, Kattendijk, the Netherlands) was added to 10 µL of this solution. The samples were incubated on ice in the dark for 10 minutes and analyzed with a flow cytometer (FACSort, Becton Dickinson, San Jose, CA) equipped with a 488-nm argon laser. A total of 10⁴ events per sample were analyzed with the software (CellQuest, Becton Dickinson). The RBC population was defined by size in forward and side scatter plots, and the percentage of annexin V-positive cells was determined on the basis of an unlabeled control prepared according to the method cited above.

Detection of microorganisms

To guarantee that the contents of the blood bags were not contaminated with aerobic or anaerobic microorganisms, the RBC units were monitored with an automated microbial detection system (BacT/ALERT, Organon Teknika, St Laurent, Québec, Canada). Monitoring was performed at the end of Day 45 of storage by analyzing RBC samples, approximately 2 mL, collected from the blood bags with the closed collection system described above. The samples were inoculated individually under a laminar flow into flasks containing culture medium and a colorimetric sensor (Model FAN, Organon Teknika). The contents of the flasks were incubated at 35 to 37°C and continuously monitored for 7 days with the BacT/ALERT system (Organon Teknika).

Effect of 2 r.p.m.

The effect of rotation on the *in vitro* properties of the RBCs was studied with RBC units separated from whole blood collected from 10 donors with the same method and materials as described above. To avoid biologic variability, each RBC unit collected from a single donor, approximately 250 mL, was equally divided into two bags. The RBC content of one bag was used as control while the content of the other was used to test the effects of a 2 r.p.m. rotation rate on the integrity of RBC membranes. The blood bags were protected with plastic wrap and stored in the thermal device which contained water at the temperature of 2°C. The thermal device was then rotated at 2 r.p.m. for 4 hours with the help of the automated system described above. The blood bags were then stored in a refrigerator at 2 to 4°C for 45 days. The extracellular levels of K⁺, Cl⁻, plasma-free Hb, and the change in the asymmetry of the phospholipid bilayer of the RBC mem-

brane were determined with the same method as employed to measure these variables along the storage time in the irradiated RBC units.

Statistical analyses

Statistical analyses were performed with repeated analysis of variance considering a significance level of $\alpha = 0.05$ and with the Bonferroni method to the analysis of multiple comparisons. The data were reported as mean \pm 1 SD ($n = 10$).

RESULTS

Dosimetry

The dose rate measured was 183 cGy per minute. Dose distributions in the target volume, simulated with the use of the phantom, ranged from 92 to 104 percent and were plotted on isodose curves (Fig. 1). The isodose curves were obtained with a total of 25 cavities distributed along the central plane of the phantom. Dosimetry measurements were carried out with an uncertainty of less than 3 percent.

Levels of free Hb, potassium, and chloride

The plasma-free Hb levels in the irradiated and control RBC units increased along the 45 days of storage (Fig. 2A). At 15 hours after irradiation, no significant difference ($p = 0.160$) was observed in free Hb levels between RBC units irradiated with 3000 cGy and control. These levels,

however, were significantly higher ($p = 0.002$) in the RBC units irradiated with 4000 cGy compared to control. On Day 10, the levels of free Hb were significantly higher ($p < 0.001$) in the RBC units irradiated with 3000 cGy compared to control, although no significant difference ($p = 0.578$) in free Hb levels was observed between RBC units irradiated with 3000 and 4000 cGy. Starting on Day 20, the levels of free Hb were significantly higher ($p < 0.001$) in the units irradiated with 4000 cGy than in the units irradiated with 3000 cGy. The levels of free Hb observed along the 45 days of storage of the irradiated and control RBC units are summarized in Fig. 2A.

At 6 hours before irradiation, extracellular K^+ level in the RBC units was 2.4 ± 0.6 mmol per L. Extracellular K^+ levels, however, were duplicated in the RBC units irradiated with 3000 cGy compared to control within only 15 hours after irradiation, while these levels were triple in the RBC units irradiated with 4000 cGy. These differences persisted up to Day 10, but decreased from 10 up to Day 45. Extracellular K^+ levels, however, were significantly higher in the RBC units irradiated with 4000 cGy compared to control ($p < 0.001$) or to RBC units irradiated with 3000 cGy ($p = 0.001$) at the end of Day 45. The extracellular K^+ levels observed along the 45 days of storage of the irradiated and control RBC units are summarized in Fig. 2B.

Extracellular Cl^- levels decreased in both the irradiated and the nonirradiated RBC units (Fig. 2C). No significant difference ($p > 0.05$) in Cl^- levels, however, was observed along the 45 days between irradiated and nonirradiated RBC units. Thus, as shown by the data in Fig. 2C, the doses of 3000 and 4000 cGy did not change the levels of extracellular Cl^- in the irradiated RBC units.

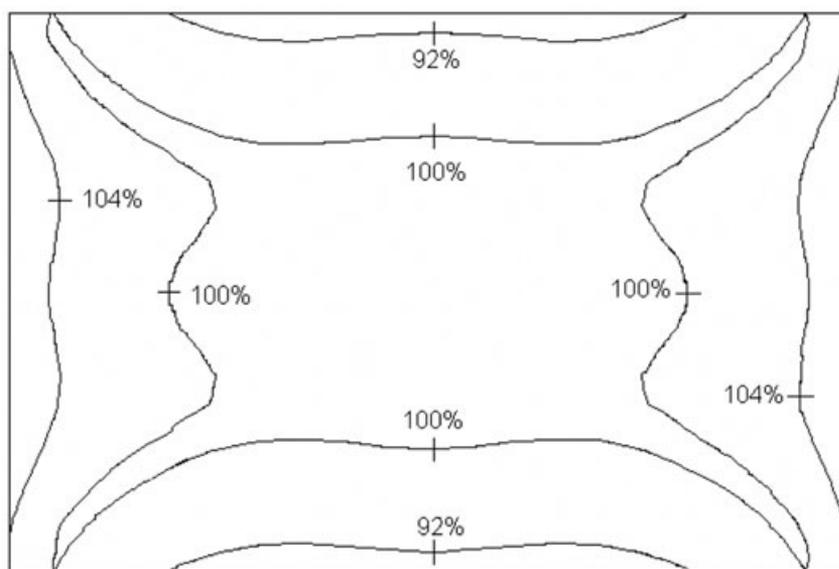


Fig. 1. Isodose curves measured at the central plane when the phantom was rotated at 2 r.p.m. and irradiated with a cobalt teletherapy unit.

Changes in the asymmetry of the membrane phospholipid bilayer

At 15 hours after irradiation, no significant change ($p > 0.05$) was observed in the membrane phospholipid asymmetry of irradiated RBCs compared to nonirradiated cells. Although the number of cells with altered membrane had increased in irradiated RBC units, this difference remained unchanged up to Day 35. On Day 45, the number of cells with a deformed membrane was significantly higher in the units irradiated with 4000 cGy compared to those irradiated with 3000 cGy ($p = 0.029$) or with the control ($p < 0.001$). The data regarding the loss of phospholipid asymmetry in RBCs caused by irradiation as a function of time of storage are summarized in Fig. 3.

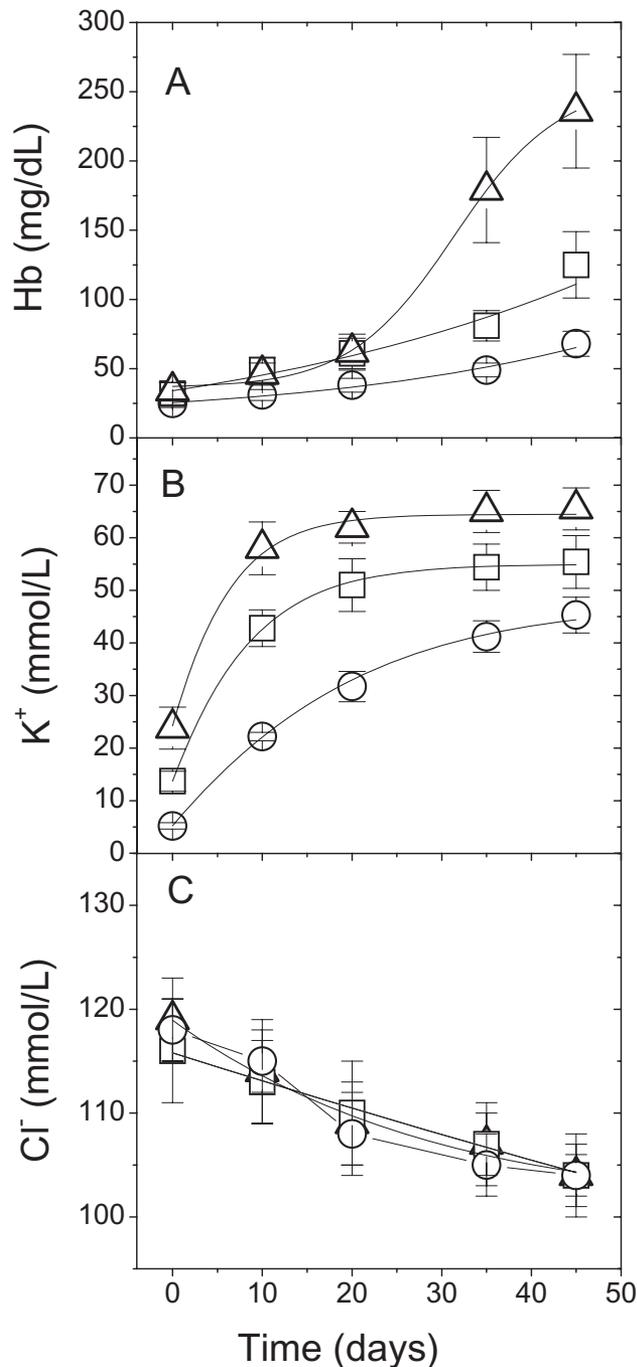


Fig. 2. Effects of ^{60}Co gamma rays on Hb (A), K^+ levels (B), and Cl^- levels (C) at the doses of 0 cGy (O), 3000 cGy (□), and 4000 cGy (Δ), measured (mean \pm 1 SD; $n = 10$) on Days 0, 10, 20, 35, and 45 of storage. Fifteen hours after the beginning of irradiation was considered to be Day 0.

Injuries caused to the RBC membrane by rotation at 2 r.p.m.

Rotation of the RBC units at 2 r.p.m. for 4 hours caused no significant change compared to control ($p > 0.05$) in plasma-free Hb levels or in extracellular levels of K^+ and

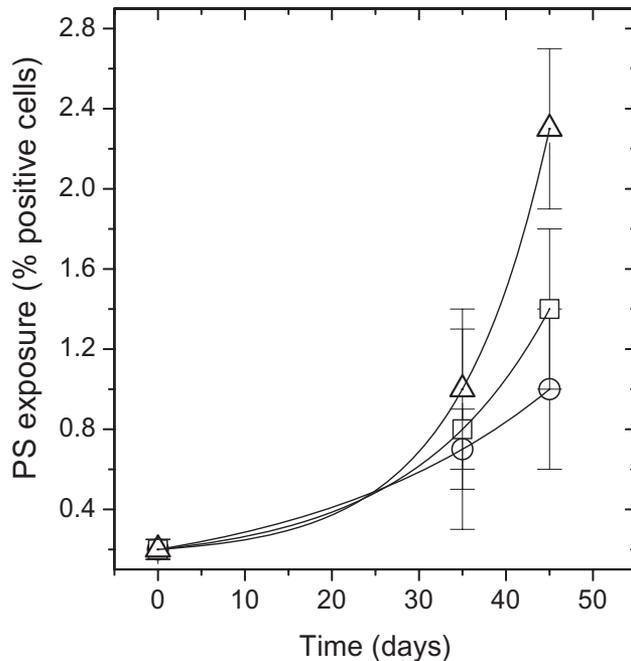


Fig. 3. Percentage of cells exposing PS (mean \pm 1 SD; $n = 10$) as a result of the effect of ^{60}Co gamma-ray interaction with the RBC phospholipid bilayer at the doses of 0 cGy (O), 3000 cGy (□), and 4000 cGy (Δ), measured on Days 0, 35, and 45 of storage. Fifteen hours after the beginning of irradiation was considered to be Day 0.

Cl^- , along 45 days of storage after the rotation. Plasma-free Hb levels in the RBC units rotated at 2 r.p.m. varied from 20 ± 2 mg per dL, 15 hours after the rotation, to 81 ± 10 mg per dL, at the end of 45 days of storage, whereas these levels in the control units varied from 20 ± 4 to 83 ± 10 mg per dL, for the same time period. The extracellular K^+ levels in the RBC units rotated at 2 r.p.m. varied from 5.0 ± 1.0 mmol per L, 15 hours after the rotation, to 48.0 ± 3.0 mmol per L, at the end of 45 days of storage, whereas these levels in the control units varied from 5.0 ± 1.0 to 47.0 ± 4.0 mmol per L, for the same time period. The extracellular Cl^- levels in the RBC units rotated at 2 r.p.m. varied from 116.0 ± 4.0 mmol per L, 15 hours after the rotation, to 102.0 ± 3.0 mmol per L, at the end of 45 days of storage, whereas these levels in the control units varied from 117.0 ± 3.0 to 103.0 ± 4.0 mmol per L, for the same time period. Rotation of the RBC units at 2 r.p.m. for 4 hours also caused no significant change ($p > 0.05$) in the asymmetry of the phospholipid bilayer of the RBC membrane. The percentage of cells exposing PS in the RBC units rotated at 2 r.p.m. varied from 0.26 ± 0.01 percent, 15 hours after the rotation, to 0.67 ± 0.3 percent, at the end of 45 days of storage, whereas this percentage in the control units varied from 0.27 ± 0.02 to 0.69 ± 0.3 percent, for the same time period. These values are given as means \pm 1 SD ($n = 10$).

DISCUSSION

In normal human RBCs, the transmembrane distribution of phospholipids is asymmetric, with phosphatidylcholine and sphingomyelin predominantly located in the outer leaflet, whereas most of the phosphatidylethanolamine and practically all of the PS are in the inner leaflet. Amino phospholipid asymmetry is maintained by an Mg-ATPase enzyme (aminophospholipid translocase or flippase), which continuously pumps PS and phosphatidylethanolamine from the outer to the inner leaflet of the membrane.³⁷ In this study, we have demonstrated that prolonged storage results in loss of phospholipid asymmetry in the membrane of irradiated RBCs, exposing PS on the cells' surface with a time and dose dependence according to one exponential function (Fig. 3). This finding is important because the effects of gamma-ray interaction with the RBCs' phospholipid bilayer have not been characterized. In this study, we also demonstrated a time- and dose-dependence effect on the extracellular K⁺ and plasma-free Hb levels. We have observed, for both doses tested, that K⁺ and Hb leakage through the RBC membrane increased with storage time according to a sigmoid function (Figs. 2A and 2B), although the curves associated with plasma-free Hb levels (Fig. 2A) and extracellular K⁺ levels (Fig. 2B) have shown a different behavior at the beginning and at the end of the storage time. As Fig. 2B shows, up to Day 10, the slope of the curves for 3000 and 4000 cGy was higher compared to the slope of the curve associate for the control. This difference in slope (i.e., difference in leakage rate across the RBC membrane), however, diminished slowly after the 10th day of storage. The mechanism of potassium leakage from the RBCs during storage has not been elucidated, and this finding appears to support the proposition that irradiation damage to the RBC membrane progresses for a limited number of days, after which membrane-associated repair may occur.²² In our study, we also observed significant changes in extracellular Cl⁻ levels in the control RBC units as a function of storage time, but we were unable to detect a change in these levels when irradiated RBC units were compared to control (Fig. 2C).

In this study, irradiated blood units showed a significantly higher relative percentage of RBCs expressing PS in the outer leaflet than control units, although in absolute values, PS exposure was less than 3.0 percent positive cells in both RBC units. These results are consistent with those reported in a recent study, which considered the effect of long-term storage of RBCs on the activities associated with the ATP-dependent aminophospholipid, showing that the percentage of cells exposing PS remained low throughout the 49 days of storage, varying between 0.6 and 3.5 percent.³⁸ Some studies suggest that RBCs and lymphocytes that have been modified so that they have PS on their outer leaflet bind more readily to macrophages

than normal RBCs or lymphocytes.^{39,40} Thus, according to the present results showing that PS asymmetry was lost during storage of irradiated RBCs, the viability of these cells could be decreased.

As also observed in this study, Brugnara and Churchill,²³ with a ¹³⁷Cs source, demonstrated a time- and dose-dependence effect on the external K⁺ level in the RBC units irradiated with 1000, 2000, and 3000 cGy. Additionally, other studies demonstrated that the increase of extracellular K⁺ levels is one of the more apparent effects of ionizing irradiation on the RBC units.^{19,20,22-26,28,41-43} Ramirez and coworkers¹⁹ demonstrated that 3000 cGy is sufficient to increase plasma K⁺ levels from 2.2 mmol per L on Day 0 to 31.0 mmol per L on Day 2 and to 68.0 mmol per L on Day 14. Davey and colleagues²⁴ demonstrated that this dose is sufficient to increase K⁺ levels in RBC units from 1.6 mmol per L on Day 0 to 78.1 mmol per L on Day 42 of storage. Mintz and Anderson²⁵ reported that RBC units irradiated with 3000 cGy and stored for 35 days have extracellular K⁺ levels of 68 mmol per L. Brugnara and Churchill²³ irradiated RBC units with doses of 1000, 2000, and 3000 cGy. Between Days 25 and 30 postirradiation, they observed an external K⁺ level of approximately 105 mmol per L in RBC units irradiated with 3000 cGy. In our study, we observed an extracellular K⁺ level of 2.4, 20.0, 45.0, 54.0, and 55.0 mmol per L on Days 0, 2, 14, 35, and 42 of storage, respectively, in the RBC units irradiated with 3000 cGy (Fig. 2B). Hillyer and colleagues⁴² observed an external K⁺ level of 57.0 mmol per L in the RBC units irradiated with 3500 cGy on Day 42 postirradiation. On Day 42, based on Fig. 2B, we observed extracellular K⁺ levels of 65.0 mmol per L in the RBC units irradiated with 4000 cGy. Therefore, according to the irradiation dose and the period of storage of the irradiated RBC units, the extracellular K⁺ levels obtained in these studies were higher compared to our results. Moreover, in our study we also observed that the increase in plasma-free Hb levels in the irradiated RBC units was less evident compared to K⁺ during the first days of storage. Ramirez and coworkers¹⁹ also showed that the elevation in plasma-free Hb levels provoked by 3000 cGy became more evident only after 15 days of storage. Similarly, Davey and associates²⁴ observed a significant increase in plasma-free Hb levels of 623.1 mg per dL in RBC units irradiated with 3000 cGy and stored for 45 days. Mintz and Anderson²⁵ observed a free Hb level of 314.0 mg per dL in RBC units irradiated with 3000 cGy and stored for 34 days. In our study, a free Hb level of approximately 85.0 mg per dL was observed in the RBC units irradiated with 3000 cGy on Day 34 of storage (Fig. 2A), increasing to 125.0 mg per dL on Day 45 of storage (Fig. 2A). Moore and Ledford,⁴¹ with 4000 cGy, observed a free Hb level of 537.0 mg per dL in RBC units irradiated and stored for 35 days. In our study, we observed a free Hb level of 179.0 mg per dL in the RBC units irradiated with 4000 cGy, on Day 45 of storage

(Fig. 2A). Thus, based on the irradiation dose and the time of storage of the RBC units, the free Hb levels obtained in these studies were higher compared to our results. In all the above-cited studies, the volume of an RBC unit obtained from a single donor was equally divided into a number of blood bags, which varied according to the number of doses tested, and irradiated with a ^{137}Cs source from a blood irradiator. The plasma-free Hb and extracellular K^+ levels obtained in our study also are minor when compared with the data obtained in one recent study that investigated the effects of X-rays produced by a blood irradiator in the RBC units irradiated with 2500 and 3500 cGy, which showed a plasma-free Hb level of 980 mg per dL and an extracellular K^+ level of 105 mmol per L in the RBC units irradiated with 3500 cGy, on Day 28 postirradiation.²⁸ In our study, if we had used RBC units with a larger volume, extracellular K^+ and free-Hb would have been more diluted and therefore these differences would have been greater. Moreover, other factors exist that may influence the biologic response to irradiation such as the dose rate, the energy or type of radiation emitted by the source, the spatial distribution of the absorbed dose over the irradiation volume, and the physical conditions used to store the blood components.^{22,28,43-48} Thus, in addition to volume, the different irradiation conditions used such as those cited above may have contributed to the somewhat conflicting findings of these studies. The typical variable parameters associated with blood irradiators or teletherapy equipment such as the spatial distribution of absorbed dose over the irradiation volumes, the dose rate, the time necessary to complete the entire irradiation process, and the conditions necessary to improve dose homogeneity have been previously discussed in the literature.^{14,15,49,50}

Many facilities are not equipped for on-site irradiation of blood components and, in such cases, the irradiated blood units may need to be stored for some period of time before transfusion. This is the case when blood irradiation is performed with a teletherapy unit, for example. Thus, in this situation, the patient may receive blood with a three times higher extracellular K^+ level compared to nonirradiated blood, according to the results obtained here 15 hours after irradiation with 4000 cGy. For this after-irradiation period, there was no significant difference in plasma-free Hb between irradiated and control RBC units. In addition, for the same period of time, the irradiation caused no significant change in the structure of the phospholipid bilayer of the RBC membrane compared to control. Therefore, considering that the increase of the extracellular K^+ level in RBC unit in the first 15 hours postirradiation has no clinical significance at the doses tested, the use of a cobalt teletherapy machine produces no significant change in the *in vitro* parameters as long as the time between irradiation and use does not exceed 15 hours. Moreover, FDA guidelines call for a 28-day

maximum storage period for RBCs after irradiation, irrespective of the day of storage on which the irradiation was performed, but the total storage time cannot exceed that for nonirradiated RBC units.^{12,29} According to the results obtained in this study, ^{60}Co gamma rays cause less damage to RBCs compared to the X-rays or gamma rays derived from a ^{137}Cs source, indicating that the use of ^{60}Co gamma-rays agrees with the period of 28 days' maximum storage for RBCs after irradiation recommended by these guidelines. Thus, considering that a minimum gamma irradiation dose of 2500 cGy may be required to completely inactivate T cells in RBC units when a ^{60}Co source is used,¹⁸ and both the damage caused to the RBC and the spatial distribution of absorbed dose over the irradiation volume obtained in this study, we recommend a dose of 3000 cGy when cobalt teletherapy is used to blood irradiate. As shown by the isodose curves obtained for the phantom (Fig. 1), with this recommendation we guarantee a minimum of 2500 cGy to the whole volume, even in a possible cold point situated in an 82 percent isodose curve.

To standardize the process of blood irradiation by teletherapy units, our group has proposed an electronic and/or mechanical system for the storage of blood bags during irradiation.¹⁵ The device, equipped with two thermal compartments, allows the simultaneous irradiation of PLTs, granulocytes, and RBC units while maintaining a constant temperature for each of these blood components (2-4°C for RBCs and 20-24°C for PLTs or granulocytes). To minimize the dose distribution over the volume, the device is rotated in the radiation field at rotation rate of 2 r.p.m. by means of a computer-controlled stepping motor. We used this system to irradiate the blood bags to minimize the dose heterogeneity over the irradiation volume and to keep constant the temperature of the RBC units during the irradiation process (from 2 to 4°C). The homogeneity of the dose obtained (from 92% to 104%) over the irradiation volume was important to best determine the dose-versus-biologic effect relationship established in this study. We observed that the rotation rate of 2 r.p.m., necessary to guarantee dose homogeneity over the irradiated volume, did not cause additional damage to the irradiated RBC. Thus, regarding the biologic effects investigated, we have validated the use of this system to store blood bags during the irradiation process with a teletherapy unit.

Despite the important role of teletherapy as an alternative means for blood irradiation, little attention has been paid to the quality of the blood irradiated with this equipment. This study permitted us to determine the damage caused to RBCs by 3000 and 4000 cGy and provided a better understanding of the relationship between dose and biologic effect produced in these cells when irradiated with a cobalt teletherapy unit. According to the results obtained in this study, we do not exclude the necessity of better evaluating the RBC storage lesion after

irradiation, such as in vitro metabolic derangements and other biochemical changes, to better delineate the general quality of RBCs during storage after irradiation with a ^{60}Co source.

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