Extracellular potassium concentrations in red blood cell suspensions after irradiation and washing

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BACKGROUND: Extracellular potassium concentration \([K^+e]\) increases with duration of red blood cell storage. Sometimes red blood cells (RBC) are washed before transfusion to infants to reduce \([K^+e]\) of these components. AABB standards permit storage of washed RBCs at 4°C for 24 hours. The \([K^+e]\) of washed RBCs during storage is not known. Experiments were performed to provide those data.

STUDY DESIGN AND METHODS: One day after outdating, 26 RBC units were washed without irradiation or before or after irradiation (25 Gy), and \([K^+e]\) was measured for 24 hours. \([K^+e]\) was measured also immediately before transfusion of 29 nonoutdated irradiated and washed RBC units.

RESULTS: After washing, \([K^+e]\) increased in a time-dependent fashion. \([K^+e]\) increased more rapidly in preparations of irradiated than nonirradiated RBCs. \([K^+e]\) was less after washing after irradiation (1.6 ± 0.3, 2.4 ± 0.3, 3.0 ± 0.3, 3.6 ± 0.3, 4.2 ± 0.4, 5.3 ± 0.5, 8.6 ± 1.0, and 14.3 ± 1.3 mEq/L at 0, 1, 2, 3, 4, 6, 12, and 24 hr; mean ± SD) than washing before irradiation \((p < 0.001)\). The increase in \([K^+e]\) during the first 6 hours after washing after irradiation was linear \((0.61 ± 0.08\text{ mEq K}^+/\text{L/hr})\). The probability of a unit of RBCs having a \([K^+e]\) greater than 5 mEq per L is 0.0 to 0.2 percent 3 hours after washing irradiated RBCs and 0.0 to 1.1 percent 6 hours after washing nonirradiated RBCs.

CONCLUSIONS: \([K^+e]\) increases after washing irradiated and nonirradiated packed RBCs. After irradiation and washing, the \([K^+e]\) for the initial 6 hours can be predicted from the \([K^+e]\) immediately after washing. There is a low probability that a unit of RBCs would have a \([K^+e]\) greater than 5 mEq per L during 6 hours of storage at 4°C after washing if the cells are not irradiated and for 3 hours if the cells are irradiated.
MATERIALS AND METHODS

Experiment 1
Determination of potassium concentrations immediately ("0" hours) and at 6, 12, and 24 hours after 1) washing nonirradiated RBCs, 2) washing irradiated RBCs, and 3) irradiating washed RBCs. When units were irradiated first, washing followed 24 hours thereafter; when units were washed first, irradiation followed immediately. Processing was completed for all units within 48 hours after outdating.

Before initiating these experiments, we did not have data from which we could estimate the number of units of blood that would be required to determine if any differences existed among these three groups (washed without irradiation, washed after irradiation, and washed before irradiation). We planned to examine 5 to 7 units per group to provide the initial data, which we would then use to determine the number of units to be tested. The data from the initial 19 units, however, provided a valid comparison.

Nineteen units of RBCs processed in citrate phosphate dextrose adenine (CPDA) or additive solution (AS)-1 were processed within 48 hours after their outdate. Each unit was assigned (not randomly allocated) to one of the three groups, depending on personnel available to perform the required processing. After being washed, units were stored at 2 to 6°C throughout the period during which we collected samples for analysis of potassium concentrations (see below).

Potassium concentrations were compared at each time point between groups by analysis of variance, followed by Student-Newman-Keuls test for multiple comparisons, and within each group at the various time points by repeated measures analysis of variance, followed by Student-Newman-Keuls test for multiple comparisons. Statistical significance was accepted at p value less than or equal to 0.05.

Experiment 2
Determination of potassium concentrations during the first 6 hours after irradiation and washing of blood. At completion of Experiment 1, we found (see Results) that washing RBCs after irradiation provided lower K+ concentrations than did washing before irradiation, but that the concentrations of potassium 6 to 24 hours after processing were substantial. Consequently, we determined the potassium concentrations during the period we thought the component would most likely be administered: within 4 hours of washing after irradiation and to define the period during which the potassium concentration would likely remain below 5 mEq per L. This value was chosen because it is the one cited frequently by clinicians as the maximum value not causing concern. We also evaluated the potassium concentration at 6 hours after washing after irradiation to allow comparison with data from the similar group in Experiment 1. We used the data from Experiment 1, for the similar group, at 6 hours after irradiation and washing, assuming that the increase in potassium concentration was linear, to determine the number of units to be evaluated. We determined that data from 7 units should be sufficient to demonstrate differences in potassium concentration among the various times after processing and provide a reasonable estimate of the probability of a unit of irradiated and washed blood having a potassium concentration of greater than 5.0 mEq per L at each evaluated time point.

Seven units of RBCs stored in CPDA or AS-1 were irradiated (see below) 1 day after their outdate and washed (see below) on the following day. All units were stored at 2 to 6°C throughout their in-date storage period as well as during the period in which we processed these units and collected samples for analysis of potassium concentration (see below).

Potassium concentrations were compared at 0, 1, 2, 3, 4, and 6 hours, by repeated-measures analysis of variance, followed by Student-Newman-Keuls test for multiple comparisons. The data at 6 hours from this group were compared with the data from the similar group in Experiment 1 by unpaired t test. Statistical significance was accepted at a p value of less than or equal to 0.05. For each unit of RBCs, a linear regression equation was fitted for the relationship of potassium concentration and time after washing.

We determined the total amount of extracellular potassium contained in each unit at each time point by the formula

$$\text{Total K}^+ (\text{mEq}) = (\text{unit weight} / 1.05) \times ((1 - \text{hematocrit}) / 100) \times [\text{extracellular } K^+]$$

and compared these data by repeated-measures analysis of variance followed by Student-Newman-Keuls test for multiple comparisons.

Experiment 3
Determination of potassium concentrations in units of irradiated and washed blood at the time of their administration. Experiments 1 and 2 were performed with units of RBCs 1 day after they exceeded their maximum allowable period of storage. To assess whether irradiating and washing units of blood that had not exceeded their maximal allowable storage life produced similar potassium concentrations after washing, we measured the potassium concentration at the time of transfusion of units collected in CPDA or AS-1 that had been irradiated and washed for clinical use. These units were for infants of less than 1 years of age, undergoing complex cardiac surgical procedures and likely to require rapid, massive transfusion. Inasmuch as the time between washing and
transfusion could be neither controlled nor predicted, we were unable to perform an a priori power analysis to determine the number of units to examine. We chose to examine 20 units, assess whether the data were sufficient, and estimate the number of additional measurements that would be required. The interval between washing and transfusion for the first 20 units varied from 1 to 12 hours; only 4 units were transfused between 1.5 and 2.5 hours and 3 units between 3.5 and 4.5 hours. Thus, we collected data from an additional 10 units to provide sufficient data. After irradiation followed by washing, all units were stored at 2 to 6°C until their issue from the blood bank, at which time they were stored in a cooler until transfused. We have previously determined that the temperature of blood stored in these coolers remains below 7°C for at least 6 hours.

Units of blood were grouped in hourly intervals (0.5-1.49, 1.5-2.49, 2.5-3.49, 3.5-4.49, and 5.5-6.49 hr) and the data compared with data from Experiment 2 by unpaired t test. A linear regression equation was fitted for the relationship of potassium concentration and time after washing for the units evaluated in Experiment 2. This was accomplished in two different ways. In Method 1, we took the sample mean and standard deviation (SD) for each time period of the 7 units as if it were the true population mean and SD and then computed the probability that a unit would have a potassium concentration greater than 5 mEq per L, Pr(K > 5) as

\[ Pr(K > 5) = \Phi((5 - \text{mean})/(SD)), \]

where \( \Phi \) is the standard normal cumulative distribution function; that is, \( \Phi(x) \) is the probability that a normal random variable with mean 0 and SD 1 is less than x. This method yielded a single probability.

The approach of Method 1 assumes that the experimental data mean and SD are those of the true population. Method 2 does not make this assumption and thus provides a more conservative approach. The 95 percent confidence limits for the population mean and population SD were computed for each time point for all units. The possible population means and SDs within these limits were varied in increments of 0.01, and the combination used to compute the probability that the potassium concentration would exceed 5 mEq per L, Pr(K > 5) as

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Washing, irradiation, and measurement of potassium concentration. All washing was performed in a standard manner, with a cell processor (Model 2991, Cobe Laboratories, Lakewood, CO) with a 1-L 0.9 percent NaCl wash. Irradiation was performed in a standard manner, exposing all units to 25 Gy (cesium-137 for 4.34 sec) with an irradiator (Model 143-68 A, JL Shepard, San Fernando, CA). For Experiments 1 and 2, blood bags were then spiked to allow for repeated collection of samples for measurement of potassium concentration in the samples by an analyzer (Synchron Clinical System LX20 Pro, Beckman Coulter, Fullerton, CA), calibrated every 8 hours and checked with control serum every 4 hours. For Experiment 3, potassium concentration was measured with a blood gas analyzer (Gem Premier Plus, Instrumentation Laboratory, Lexington, MA; calibrated every morning and every 8 hr) at the time of clinical administration.

All data are presented as means ± SD. In all cases, significance was accepted at p value less than or equal to 0.05.

**RESULTS**

**Experiment 1**

In each group (washing without irradiation, washing after irradiation, and washing before irradiation), the potassium concentration increased significantly with time after processing (Fig. 1). For all groups, the potassium concentration at each time point differed significantly from that at all other times (all p values < 0.001, except 6 hr vs. 12 hr in the washed after irradiation, where p < 0.01).

There were significant differences of potassium concentrations among the groups after washing (Fig. 1). Immediately after washing (time = 0 hr) the units that were washed after irradiation had a slightly, but signifi-

![Fig. 1. Extracellular potassium concentrations in units of blood 6, 12, 18, and 24 hours after washing without irradiation (●), washing before irradiation (■), or washing after irradiation (▲). At all times from 6 to 24 hours, values among groups were significantly different (all p values < 0.001) between groups. Data are means ± SD.](image)
significantly higher potassium concentration (1.7 ± 0.5 mEq/L) than did the units that were not irradiated (1.2 ± 0.1 mEq/L; p < 0.05), but did not differ from the units that were washed before irradiation (1.3 ± 0.2 mEq/L; p = 0.13). For all other times after processing (6, 12, and 24 hr), the potassium concentrations significantly differed for all comparisons among the three groups (all p values < 0.001), with nonirradiated units always having the lowest potassium concentration and the units irradiated after washing always having the highest concentrations.

**Experiment 2**

The extracellular potassium concentrations of each unit of RBCs irradiated before washing varied linearly (all $r^2 \geq 0.99$) with time during the first 6 hours after washing (Fig. 2). The mean ± SD value of the individual slopes of the relationship between potassium concentration and time after washing was 0.61 ± 0.08 mEq K per L per hour. Values at all time points differed significantly from values at all other times (all p values < 0.001; Fig. 3). Immediately after washing potassium concentration was 1.63 ± 0.27 mEq per L, increasing to 3.01 ± 0.34 mEq per L at 2 hours, 3.60 ± 0.34 mEq per L at 3 hours, and 4.24 ± 0.37 mEq per L at 4 hours. Owing to the low volume of extracellular fluid in these units (hematocrit, 75.5 ± 4.1%), the total amount of extracellular potassium was not large: 0.11 ± 0.03 mEq immediately after washing, increasing to 0.20 ± 0.05 mEq at 2 hours, 0.24 ± 0.06 mEq at 3 hours, 0.28 ± 0.07 at 4 hours, and 0.35 ± 0.09 mEq at 6 hours (p < 0.001 for all comparisons between all time points: 0, 1, 2, 3, 4, and 6 hr).

The potassium concentration from the units of Experiment 2 6 hours after washing (5.3 ± 0.5 mEq/L) did not differ from the potassium concentration in the units of Experiment 1 at the same time after similar processing (5.6 ± 0.9 mEq/L; p = 0.45).

**Experiment 3**

One of the 30 units tested was discovered subsequently not to have been irradiated and was excluded from analysis. The potassium concentrations measured in units of RBCs that were irradiated and washed during their normal storage period (i.e., not outdated) did not differ from those obtained from units that were similarly processed 1 day after the expiration of their storage period (all p values > 0.05; Fig. 3). Potassium concentrations increased with duration after washing (linear regression $r^2 = 0.68$). The duration of time between the collection of the unit of blood and the time at which the unit was irradiated and washed (i.e., storage time before irradiation and washing) did not appear to affect the absolute value or rate of increase of potassium concentration after washing (Fig. 4). An insufficient number of units (3) were transfused more than 6 hours after washing to allow for analysis of the effect of this duration on extracellular potassium concentration [K$^+$].

The probability of any single unit of RBCs having an extracellular potassium concentration greater than 5 mEq per L

The probabilities that a unit of washed RBCs would have a potassium concentration greater than 5 mEq per L during the first 6 hours after washing after irradiation or for 24 hours after washing nonirradiated cells are shown in Table 1. The more conservative Method 2, by design,

![Fig. 2. Extracellular potassium concentrations in 7 units of blood 1, 2, 3, 4, and 6 hours after washing after irradiation. The coefficient of determination of the linear regression lines (---) for each unit equaled or exceeded 0.99.](image1)

![Fig. 3. Extracellular potassium concentrations in 7 units of blood 1, 2, 3, 4, and 6 hours after washing after irradiation. Values at each time points differed from values at every other time point (all p values < 0.001). Data are means ± SD.](image2)
yielded higher probabilities than did Method 1 (except when the mean potassium value exceeded 5 mEq/L). Both methods indicated exceedingly low probabilities that a unit of washed RBCs that had not been irradiated would have a potassium concentration exceeding 5 mEq/L within the first 6 hours after washing. Both methods also indicated exceedingly low probabilities that a unit of RBCs washed after irradiation would have a potassium concentration greater than 5 mEq/L within 3 hours of washing.

**DISCUSSION**

The major findings of our experiments are: 1) extracellular potassium concentration increases rapidly in washed RBC preparations in a time-dependent fashion; 2) irradiating RBCs results in a more rapid increase in extracellular potassium concentrations after washing than in RBC preparations that have not been irradiated; 3) washing RBCs after irradiation results in lower potassium concentrations than if washing precedes irradiation; 4) the probability of a unit of irradiated and washed RBCs having a potassium concentration exceeding 5 mEq/L increases with duration after washing, but remains low for 2-3 hours.

Potassium concentration increases in blood stored for extended periods (days to weeks) in CPDA-1 and AS-1. When blood is irradiated and stored in these solutions for similarly extended periods potassium concentration increases more rapidly than when it is not irradiated, owing to irradiation-induced damage of the RBC membrane. Many institutions are able to irradiate blood shortly before its release, and some also wash these units to decrease the potassium concentration. There are few data, however, to describe the potassium concentration in the immediate period after irradiation and washing. Our finding that irradiation followed by washing will result in potassium concentrations of less than 5 mEq/L in nearly all units for at least 3 hours provides that information. The increase of potassium concentration with time after washing was remarkably linear for each individual unit, with all coefficients of determination equal to or greater than 0.99. This suggests that the radiation-induced membrane injury, at least during the period of measurement, was stable. The similarity of the slopes of the regression equation for this relationship for each unit (0.61 ± 0.08 mEq K/L/hr) suggests that one can predict the eventual potassium concentration during the first 6 hours after washing, by measuring the potassium concentration immediately after washing.

Strauss correctly noted that for most circumstances, the total amount of potassium transfused, which generally is not large, is likely to be more important than the concentration of potassium in the component transfused. In assessing the impact of the potassium concentration or amount infused, in most circumstances the volume is sufficiently small and infused sufficiently slowly that it is completely mixed with the patient’s blood volume. Consequently, for most clinical circumstances transfusion results in little if any increase of in vivo serum potassium concentration. In hypokalemic, normovolemic, stable
adults, with presumably normal cardiac output, infusion of 20 to 40 mEq potassium in 1 hour increases mean plasma potassium concentration by 0.5 to 1.1 mEq per L, and in hypokalemic children aged 2 months to 11 years an infusion of 0.25 mEq potassium per kg per hour increased mean plasma potassium concentration by 0.6±0.5 mEq per L. (range, 0.1-1.6 mEq/L). Assessment of changes of plasma potassium require care, inasmuch as increases of plasma potassium concentration decrease rapidly, falling by half within 15 minutes, likely as a result of redistribution. As one would predict, the higher concentration of potassium in older stored blood, compared with fresh blood has minimal effect when diluted in the prime of the cardiopulmonary bypass circuit. There are, however, some circumstances when the potassium concentration of the transfused preparation may be of clinical importance. One would anticipate that as the volume of transfused blood increases and represents an increasing fraction of the patient’s blood volume, the potential impact of a high potassium concentration increases.

These conditions were modeled by Brown and coworkers finding that rapid blood transfusion in hypovolemic children, with an assumed low cardiac output, can result in plasma potassium concentrations exceeding 8 mEq per L. Their mathematical model was supported by their retrospective comparison of serum potassium concentrations in children who had undergone cardiopulmonary resuscitation with and without rapid blood transfusion. All children who had rapid blood transfusion had plasma potassium concentrations exceeding 6 mEq per L, whereas only one-third of those without rapid transfusion had similarly high serum potassium concentrations. In addition, when blood is transfused directly into the heart, or the superior vena cava, the transfused blood will not have an opportunity to mix fully with the patients entire blood volume and an elevated potassium concentration is likely to reach the coronary arteries. These are possible explanations for the numerous and continued case reports of hyperkalemic arrhythmias, including cardiac arrest, after transfusion of blood with an elevated potassium concentration. It is our view that the clinical circumstances are the most important consideration in making the decision as to whether to wash irradiated cells before they are transfused. The purpose of our investigation was to provide data that would assist those making the clinical decision as to whether reduction of the transfused potassium load by RBC washing is appropriate. Transfusion of a single unit of irradiated washed RBCs 3 hours after washing will provide approximately 0.24 mEq potassium, whereas transfusion 24 hours after irradiation of 1 unit of unwashed irradiated RBCs will provide approximately 1.5 mEq potassium.

There are aspects of our experiments that deserve discussion. In Experiment 1, we did not randomly allocate the blood units to the three groups. Although we believe it unlikely, this could have had an influence on the comparison of potassium concentrations among the three groups. We studied the effects of only one level of radiation, 25 Gy. We chose this dose because it is a common dose of irradiation used for this purpose. RBC membrane injury and potassium leakage is proportional to the dose of radiation, thus lesser doses would likely have resulted in lesser increases in potassium and greater doses in greater increases. For our bench studies, we processed and examined units of blood that were just beyond their approved maximum storage time. It would appear, however, that these data should apply to units within their normal storage life, inasmuch as our data from 29 in-date units do not appear to differ from the data obtained from older units studied in a more rigorous manner. We evaluated a relatively few number of units of blood. Nevertheless, the comparisons were highly statistically significant, and the SDs were small. Our statistical analysis of the probability of a unit of blood having a potassium concentration greater than 5 mEq per L, however, would likely have yielded a smaller range had we evaluated a greater number of units.

In conclusion, we have found that after washing, extracellular potassium concentration increases rapidly in RBC preparations stored at 2 to 6°C. After irradiation and washing, the extracellular potassium concentration for the initial 6 hours can be predicted from the initial potassium concentration. There is a very low probability that a unit of washed RBCs would have a potassium concentration greater than 5 mEq per L during the initial 6 hours of storage at 4°C after washing if the cells are not irradiated and for 3 hours if the cells are irradiated.

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REFERENCES


