J Anesth (2007) 21:42–46
DOI 10.1007/s00540-006-0460-3

Journal of
Anesthesia

Electrostatic field can preserve red blood cells in stored blood preparations

Tomoki Nishiyama[1] and Dobun Hayashi[2]

[1] Department of Anesthesiology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[2] Department of Translational Research for Healthcare and Clinical Science, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Abstract
Purpose. During the storage of red blood cell concentrates (CRCs), red blood cells are progressively destroyed and free hemoglobin and potassium concentrations increase. In this study, we focused on an electrostatic field that maintains food freshness without freezing, even at less than the freezing point. We hypothesized that the storage of CRCs under an electrostatic field could keep red blood cells in better condition than conventional storage.
Methods. Each of 15 packs of 2-day-old CRCs, preserved in MAP (mannitol, adenine, glucose, phosphate, and citrate) solution (MAP-CRC) was divided into 4 smaller equal-size packs and stored at 4°C in a newly developed refrigerator that can generate an electrostatic field. Each group was exposed to a 0-, 500-, 1500-, or 3000-volt (V) electric field for 30 days. Concentrations of free hemoglobin, total haptoglobin, sodium (Na), and potassium (K), and the pH, were measured in the supernatant.
Results. Haptoglobin was not detected. The Na concentration decreased with time but was significantly lower in the 0-V than in the 500-, 1500-, and 3000-V groups. K and free hemoglobin concentrations increased with time, with significantly higher values in the 0-V than in the 500-, 1500-, and 3000-V groups. The pH decreased in the 500-, 1500-, and 3000-V groups, while it did not change in the 0-V group. The pH decrease was smaller in the 500-V than in the 1500- and 3000-V groups.
Conclusion. Storing MAP-CRC in an electrostatic field of 500 to 3000 V could decrease hemolysis in the preparation. Considering the lower pH decrease, 500 V might be the field of choice.

Key words Stored red blood cells · Electric field · Hemolysis · Free hemoglobin

Address correspondence to: T. Nishiyama, 3-2-6-603 Kawaguchi, Kawaguchi, Saitama 332-0015, Japan
Received: June 13, 2006 / Accepted: September 20, 2006

Introduction

During surgery or in emergency cases, massive blood transfusion is sometimes necessary. However, in such cases, free hemoglobin increases [1], which can induce renal tubular damage by sludging, decreasing renal blood flow [2,3]. In addition, massive blood transfusion induces an increase in the serum potassium (K) concentration, increasing the risk of cardiotoxicity. During the storage of red blood cell (RBC) concentrates (CRCs), RBCs are progressively destroyed and free hemoglobin and K concentrations increase [4].

In order to prevent the destruction of RBCs in CRCs, some preservative solutions have been developed [5–7]. We previously found that some additive agents could inhibit the destruction of RBCs, i.e., inhibit hemolysis in the stored blood [8].

Recently, we have focused on the preservation environment electrostatic fields that maintain freshness of food. Our previous study suggested that the storage of CRCs under an electrostatic field could prevent the destruction of RBCs, but not platelets [9]. The present study was performed to confirm our preliminary results in RBCs.

Materials and methods

After receiving approval from the Research Committee of the University of Tokyo Hospital, we obtained 15 packs of 2-day-old CRCs preserved in MAP (mannitol, adenine, glucose, phosphate, and citrate) solution (MAP-CRC) from the Japan Red Cross Society (Tokyo, Japan). Each pack of MAP-CRC was divided into four small packs of equal amounts and stored at 4°C in a newly developed refrigerator which can generate an electrostatic field in its interior (Japanese patent No. 2003-324247; Feel Technology, Shimane, Japan).

Each group was exposed to a 0-, 500-, 1500-, or 3000-volt (V) electric field for 30 days.

Blood samples were drawn from each pack before treatment (day 0), and every 5 days during 30 days of storage. Blood was centrifuged for 10 min at 3000 g to obtain supernatant. The supernatant concentrations of free hemoglobin and total haptoglobin, sodium (Na), and potassium (K), and the pH, were measured. Free hemoglobin concentration was measured by enzyme-linked immunosorbent assay (detection limit, 10 mg·l^{-1})[8]. Total haptoglobin concentration was measured by immunonephelometry (detection limit, 10 mg·l^{-1})[8]. Na, K, and pH were measured with the ABL 505 (Radiometer, Copenhagen, Denmark).

Statistical analysis was performed with two-way repeated measures analysis of variance, followed by the Contrasts as a post-hoc test. A P value of less than 0.05 was considered to be statistically significant.

Results

Haptoglobin was not detected in any packs. The Na concentration decreased with time in all groups. However, Na was significantly lower in the 0-V than in the 500-, 1500-, and 3000-V groups (Fig. 1). K and free hemoglobin concentrations increased with time, with significantly higher values in the 0-V group compared to those in the 500-, 1500-, and 3000-V groups (Figs. 2, 3). The pH decreased in the 500-, 1500-, and 3000-V groups, while it did not change in the 0-V group. The pH decrease was smaller in the 500-V than in the 1500- and 3000-V groups (Fig. 4).

Discussion

In the present study, the storage of MAP-CRC at 500- to 3000-V electric fields inhibited the decrease in Na concentration and the increase in K and free hemoglobin concentrations more than conventional preservation conditions (0 V). These results confirmed the data of our preliminary study [9].

During hemolysis, haptoglobin binds to free hemoglobin in blood. Therefore, we expected that free haptoglobin, (calculated from total haptoglobin and free hemoglobin [1]), would decrease during the study period. However, total haptoglobin was not detected in the present study. This was quite different from findings in our previous study, also using MAP-CRC [4]. We do not know the reason for this discrepancy between the two studies. In our previous study, the MAP-CRC were not irradiated, while the preparations in the present study received radiation. This might have had an effect on haptoglobin, because this was the only difference between the MAP-CRC samples used in the two studies.

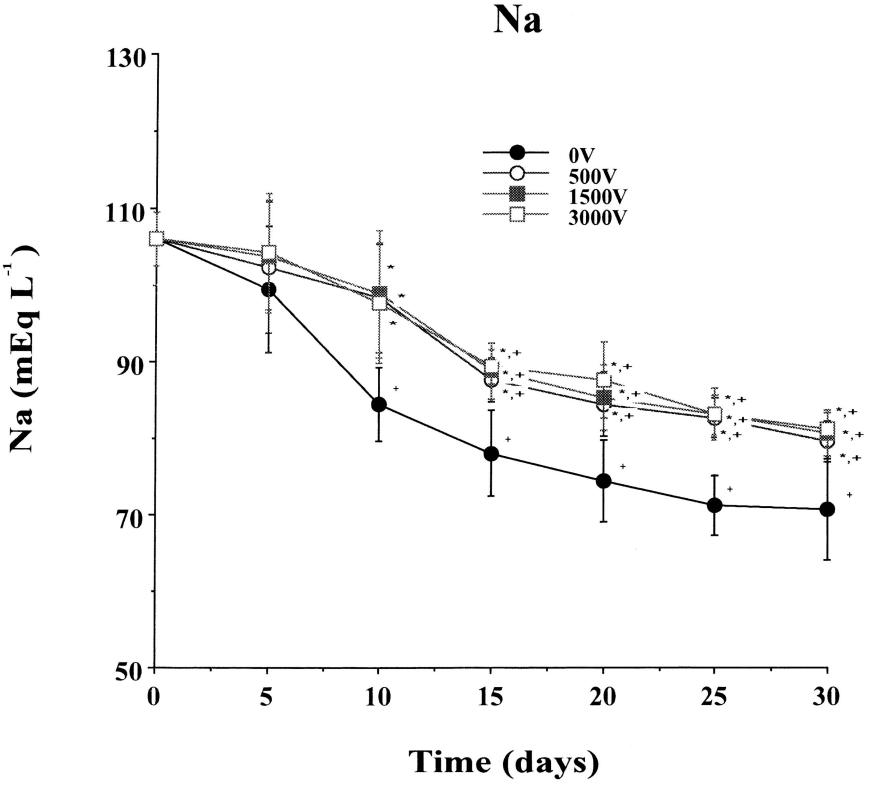

Fig. 1. Supernatant sodium concentrations; means ± SD; *P < 0.05 vs 0 V, ^{+}P < 0.05 vs 0 day

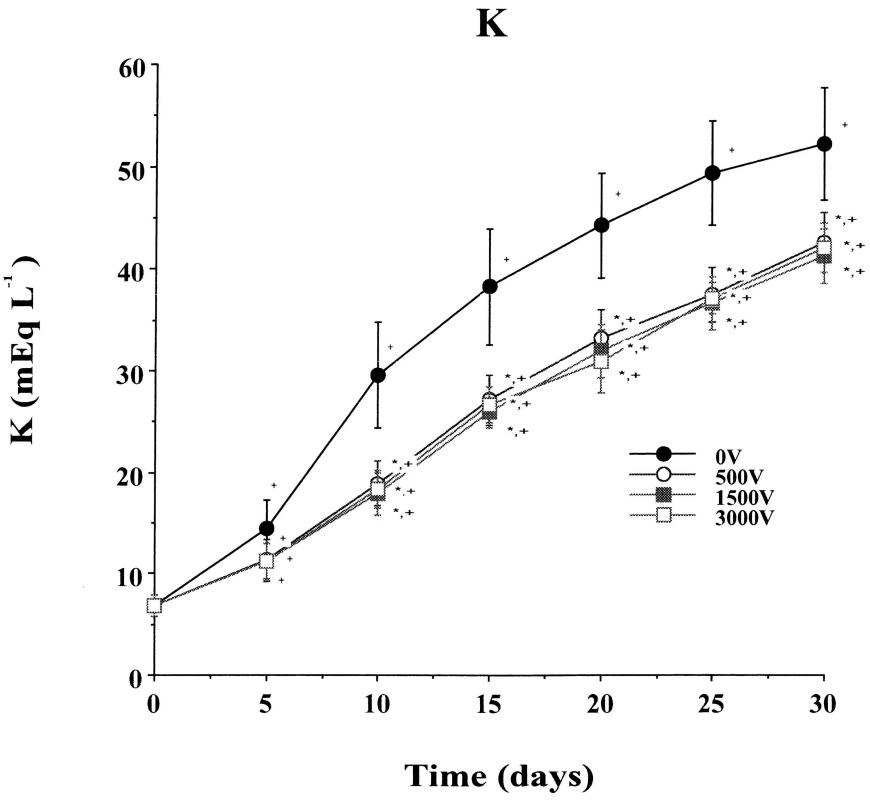

Fig. 2. Supernatant potassium concentrations; means ± SD; $^{*}P < 0.05$ vs 0 V, $^{+}P < 0.05$ vs 0 day

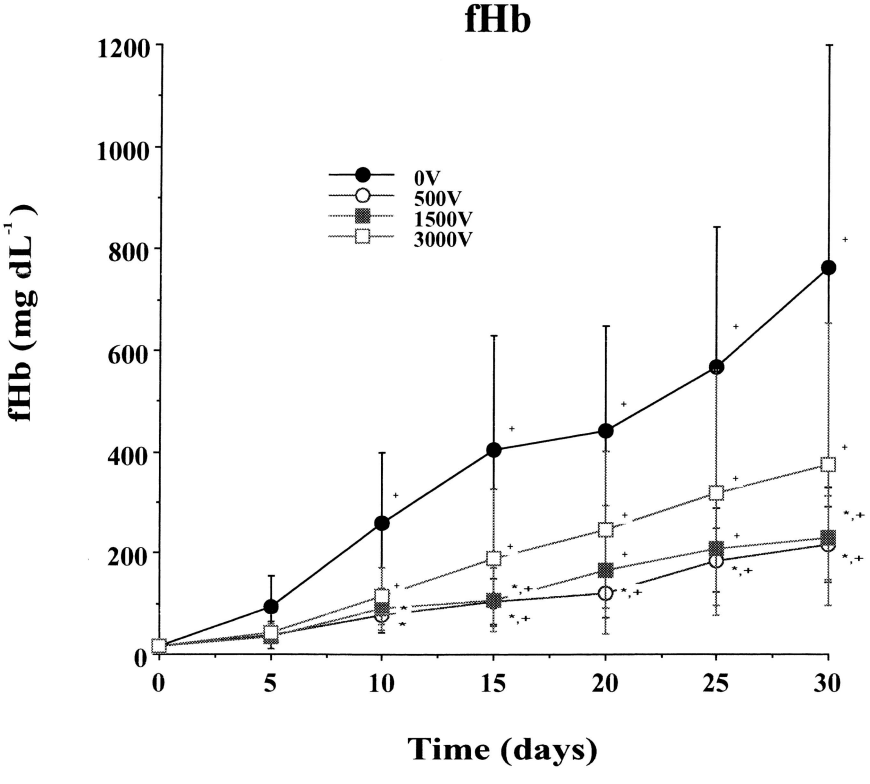

Fig. 3. Supernatant free hemoglobin (*fHb*) concentrations; means ± SD; $^{*}P < 0.05$ vs 0 V, $^{+}P < 0.05$ vs 0 day

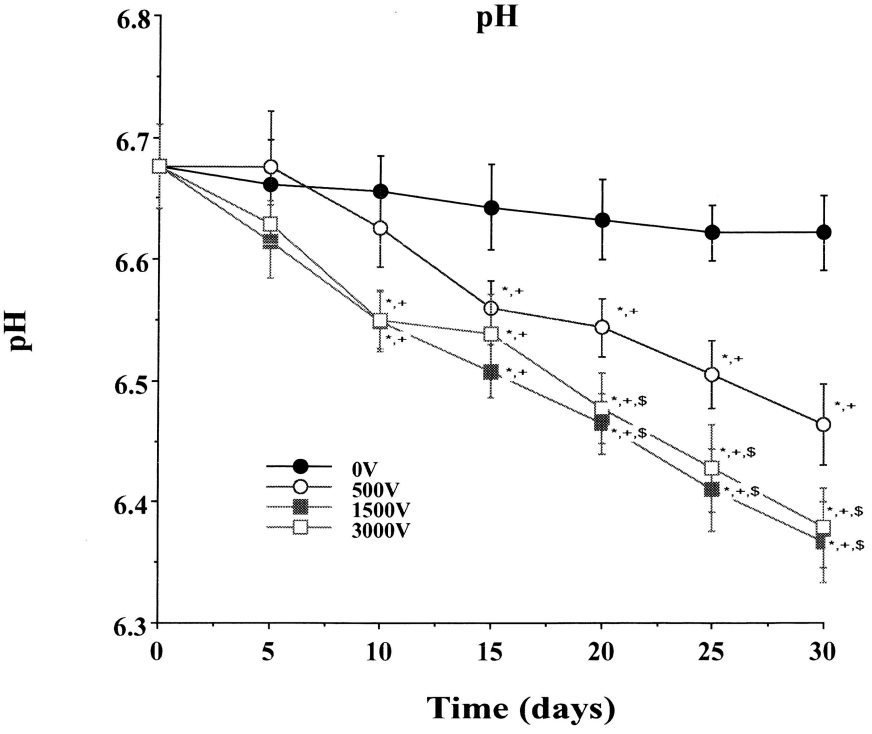

Fig. 4. Supernatant pH; means ± SD; $^{*}P < 0.05$ vs 0 V, $^{+}P < 0.05$ vs 0 day, $^{\$}P < 0.05$ vs 500 V

To preserve RBCs in stored blood, additive solutions, such as SAGM™ [6], ADSOL™, OPTISOL™, or MAP [7], have been used. The MAP solution is routinely used in Japan because it was found to preserve RBCs better than citrate-phosphate glucose solution [7]. However, during the storage of MAP-CRC, progressive hemolysis is still evident, as shown by the increase of supernatant free hemoglobin and K concentrations [4]. Proteases or hydroxy radicals derived from leukocytes may be one of the causative factors of hemolysis [10]. Leukocytes have already been reduced in MAP-CRC [11]. In one of our previous studies, the addition of haptoglobin, a free hemoglobin scavenger, or ulinastatin, a protease inhibitor, decreased hemolysis in the stored MAP-CRC [8].

In the present study, we focused on an electrostatic field as a tool for the preservation of MAP-CRC, because this is effective for the preservation of fresh food. We recently developed a new refrigerator that allows for the application of a stable electrostatic field. The electrostatic field is generated by placing a conductive electrode in the refrigerator. An electrostatic field may provide micro-vibration energy at the molecular level; thus, the cell membrane may be well-preserved. Our previous animal study showed that the function of heart, liver, and kidney was better preserved in electrostatic fields of 100 and 500 V than at 0 V [12]. Therefore, similar preservative effects on RBCs were expected and were confirmed from the present results.

We did not find any electric power strength-dependent effects on hemolysis in the range of 500 to 3000 V. Our previous study to investigate organ preservation also showed no electric power strength-dependency between 100 and 500 V [12]. Therefore, higher voltages might not be necessary. In the present study, the pH decrease was larger in the 1500- and 3000-V groups than in the 500-V group. This was suspected to be due to the exchange between the outward current of K^+ and inward current of H^+ through the damaged RBC membrane, as the K concentration was greater in the 0- and 500-V groups than in the 1500- and 3000-V groups. However, to confirm this hypothesis, the supernatant K concentration would have to be higher at 500 V than at 1500 and 3000 V, but it was not. Therefore, further study is necessary to elucidate the mechanism of pH decrease in a higher electrostatic field. In addition, we need to find a method to inhibit pH decrease. In the current study conditions, it was better to use 500 V than 1500 or 3000 V to maintain the pH.

The storage temperature is also an important factor to preserve RBCs. MAP-CRC is usually stored at 4°C. Therefore, we performed this study at 4°C. However, stored blood can be preserved for longer when frozen under 0°C; however, if it is frozen, the added glycerol should be washed out before transfusion that is complicated [13]. If we could keep MAP-CRC without freezing under 0°C, we would not need a complicated washout procedure and could expect better preservation of RBCs. In our preliminary study, water was not frozen at under 0°C in an electrostatic field. Therefore, it might be possible to keep MAP-CRC, not frozen, at

under 0°C. This possibility should be investigated in the future to find the best combination between storage temperature and electric power.

In conclusion, storing MAP-CRC in an electrostatic field of 500 to 3000 V could decrease hemolysis in the preparation. Considering the smaller pH decrease, 500 V might be better than 1500 and 3000 V.

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