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Immunosuppressive Effects of Packed Red Blood Cells on Monocytes are Related to Both Storage Time and Storage Solution

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Abstract

BACKGROUND—Reduced monocyte function is associated with adverse outcomes from critical illness. PRBC are thought to impair monocyte function but relationships between PRBC storage solution and monocyte suppression are unknown. This study was designed to test the hypothesis that immunosuppressive effects of PRBC on monocytes are related to both storage time *and* preservative solution.

STUDY DESIGN AND METHODS—Monocytes from healthy adult donors were co-cultured with PRBC that had been stored in AS-1, AS-3, or CPD-only for 7, 14, or 21 days. Cells were then stimulated with LPS and their supernatants assayed for TNF α and IL-10. Transwell experiments were performed to evaluate the role of cell-to-cell contact. Monocyte mRNA expression was quantified by RT-PCR.

RESULTS—LPS-induced TNF α production capacity was reduced compared to controls for all groups, but CPD-only PRBC suppressed monocyte function more than PRBC stored in AS-1 ($p = 0.007$) and AS-3 ($p = 0.006$). IL-10 production was preserved or augmented in all groups. Longer storage time was associated with reduced TNF α production capacity for AS-1 and AS-3 groups but not CPD. Preventing cell-to-cell contact did not eliminate the inhibitory effect of PRBC on monocyte responsiveness. PRBC exposure was associated with decreased LPS-induced *TNFA* mRNA expression ($p < 0.05$ for all groups).

CONCLUSIONS—CPD-only PRBC suppressed monocyte function more than PRBC stored with additive solutions. TNF α production was reduced even in the absence of cell-to-cell contact and was impaired at the mRNA level. Further work is needed to understand the role of preservative solutions in this process.

Keywords

packed red blood cells; monocyte; transfusion; immunosuppression; preservative

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Introduction

The monocyte is a key driver of the innate immune response and its ability to respond to a challenge is crucial during critical illness. While acute critical illness is typically characterized by hyperinflammation, patients often survive this initial pro-inflammatory surge only to develop a subsequent period of reduced innate immune responsiveness. This phenomenon is characterized by a reduced capacity of a patient's whole blood to make the pro-inflammatory cytokine tumor necrosis factor (TNF)- α upon *ex vivo* stimulation with lipopolysaccharide (LPS). Reductions in *ex vivo* LPS-induced TNF α production capacity have been associated with increased risks of nosocomial infection and mortality in critically ill adults and children in a variety of ICU settings including sepsis, trauma, and cardiopulmonary bypass.¹⁻⁷ High levels of the anti-inflammatory cytokine interleukin (IL)-10 have been associated with this form of immune suppression in *ex vivo* and *in vivo* studies of critical illness-induced immune suppression,^{1, 5, 8-10} but the relationships between these two important cytokines remain unclear.

Mechanisms underlying this innate immune suppression are likely to be multi-factorial, involving patient-related factors as well as immunomodulatory treatments received in the ICU. Transfusion of packed red blood cells (PRBC) is a commonly employed treatment in adult and pediatric intensive care units.^{11, 12} However, PRBC transfusion has been identified as an independent risk factor for increased mortality in a variety of critically ill patient populations, including trauma patients, adults with acute respiratory distress syndrome (ARDS), and critically ill children.¹³⁻¹⁶ Reasons for this may include alteration of immune function as evidenced by higher risks of nosocomial infection in critically ill patients who have received PRBC transfusion.¹⁷⁻²¹ Also, intriguing *in vitro* evidence suggests that exposure to stored red cells may reduce the responsiveness of innate immune cells and/or promote the production of anti-inflammatory mediators such as interleukin (IL)-10.^{22, 23}

Transfusion of PRBC with longer storage time has been associated with increased risks of mortality and nosocomial infection compared to transfusion of fresher PRBC, suggesting that older PRBC units may be more immunosuppressive.²⁴⁻²⁶ Limited *in vitro* data support this notion.¹⁹ PRBC transfused in the U.S. are stored in commercially available anti-coagulant solutions such as citrate-phosphate-dextrose (CPD) alone or anti-coagulant plus adenine-containing additive solutions such as AS-1 or AS-3, the most commonly used solutions at our institution (**Table 1**). These additive solutions provide substrate for ongoing production of ATP and are designed to maintain red cell viability. Many institutions, however, preferentially use CPD-only units for infants.²⁷ The concern that older PRBC products seem to be more immunosuppressive suggests that changes related to storage may play a role in PRBC-induced immune suppression. However, it is unknown whether red cell storage in the presence of additive solutions is protective of innate immune function.

We therefore performed *in vitro* experiments to test the hypothesis that monocytes exposed to stored PRBC will demonstrate decreased pro-inflammatory cytokine production capacity as a function of both red cell storage age and preservative solution type. We further hypothesize that monocyte function will be most impaired following exposure to PRBC stored in CPD-only compared to AS-1 or AS-3.

Materials and Methods

Blood collection and monocyte isolation

This study was approved by the Institutional Review Board at Nationwide Children's Hospital. After written informed consent was obtained, up to 120 ml of blood was drawn in EDTA tubes from healthy adult volunteers. Subjects with current diagnoses of anemia,

pregnancy, or malignancy; recent blood donation; a history of solid organ or stem cell transplant; or current use of immunosuppressive medications including: systemic corticosteroids, calcineurin inhibitors, azathioprine, mycophenolate, aspirin (within the past 48 hours), or non-steroidal anti-inflammatory drugs (within the past 24 hours) were excluded.

Monocytes were isolated from whole blood by density gradient centrifugation with Lymphocyte Separation Medium (Mediatech, Manassas, VA) followed by positive selection with anti-CD14 magnetic beads (Miltenyi Biotec, Auburn, CA). We have previously reported monocyte purity of $\geq 98\%$ using this method.²⁸ Recovered monocytes were re-suspended in culture media (RPMI [Mediatech] with 10% fetal bovine serum and 1% penicillin/streptomycin) to a concentration of 2 million monocytes per milliliter for use in each of the described experiments.

PRBC exposure and LPS stimulation

For each replicate of experiments, segments from nine units of donated PRBCs of differing storage solutions and ages were obtained from the blood bank at Nationwide Children's Hospital. The nine segments, each from an individual unit, contained leukoreduced PRBC that had been stored in AS-1, AS-3, or CPD for 7, 14, or 21 days (± 1 day) respectively. Two hundred microliters of PRBC (or equal volume of media for control wells) were added to 800 μ l of media containing 1.6 million monocytes in sterile, pyrogen-free, 12-well plates. This volume relationship approximates that seen in a 20 ml/kg PRBC transfusion, assuming a typical blood volume of 80 ml/kg of body weight. After incubating for four hours at 37°C, LPS 1 ng/ml (phenol-extracted from *Salmonella abortus equi*, Sigma, St. Louis, MO) was added to each well. After an additional four hours of incubation at 37°C, the supernatant from each well was collected, centrifuged and stored at -80°C for subsequent cytokine analysis. The cellular fractions were lysed in Trizol (Invitrogen, Carlsbad, CA) and stored at -80°C for mRNA quantification. For each set of experiments, a duplicate plate was created from which the supernatant and cellular fractions were harvested prior to the addition of LPS (but after four hours of incubation with PRBC or control media) in order to evaluate pre-stimulation cytokine production and mRNA expression. Levels of TNF α and IL-10 were measured by chemiluminescence using the *Immulin* automated chemiluminometer (Siemens Healthcare Diagnostics, Deerfield, IL) with lower limits of detection for TNF α and IL-10 of 5 pg/ml. Experiments were performed five times with a different monocyte donor and set of PRBC segments (from nine different units) used for each replicate. Endotoxin- and pyrogen-free reagents and labware were used for all experiments. Cell isolation and stimulation experiments were all conducted using sterile technique in a tissue-culture hood.

Preservative-only experiments

To insure that observed effects were not due to the preservative solutions themselves in the absence of red cells, experiments were repeated using 200 microliters of media (for control wells) or 200 microliters of media plus preservative solution in concentrations that approximate those present in clinical CPD, AS-1, or AS-3-stored PRBC units. No PRBC were used in these experiments. Preservative solutions were sterilely drawn from empty blood bags obtained from the Red Cross.

Transwell experiments

To determine if effects of PRBC exposure on monocyte function are dependent on cell-to-cell contact, the experiments were repeated in tissue culture plates using transwells with a pore size of 0.4 microns. This pore size is sufficiently large to permit diffusion of hemoglobin, but is much smaller than a typical red blood cell (6 – 8 microns). Isolated monocytes in media were plated in 12 well plates as previously described. Transwells were

sterilely placed in the wells and the PRBC products were placed in the upper chambers. After four hours at 37°C, 1ng/ml of LPS was added to the lower (monocyte-containing) chambers for an additional four hours. Media from the lower chambers were collected, centrifuged, and stored at -80°C for subsequent quantification of cytokine production and free hemoglobin. Free hemoglobin concentrations were determined by ELISA (Bethyl Laboratories, Montgomery, TX).

Quantification of monocyte mRNA expression

To determine the effects of stored PRBC exposure on monocyte mRNA expression, quantitative real-time polymerase chain reaction (PCR) was used to evaluate the expression of genes coding for TNF α and IL-10. Monocyte lysates from the control, 7-day, and 21-day wells from the PRBC co-incubation experiments described above were used for these analyses.

RNA was extracted from the monocyte lysates and measured using real-time, quantitative PCR as previously described.⁵ Gene expression was quantified in terms of Relative Copy Number (RCN), having been normalized to the expression of housekeeping genes GAPDH and CAP (cyclic AMP-accessory protein)-1.

Statistical analyses

To account for baseline variation in the response to LPS stimulation for each individual donor's monocytes, cytokine levels are expressed as a percentage of media-only controls for each experiment. One-way analyses of variance were used with Bonferroni post-test analyses to compare stimulated cytokine production within PRBC preservative groups and between preservative groups and controls. Two-way analyses of variance were used with Bonferroni post-test analyses to compare stimulated cytokine production among preservative groups. Data are expressed as mean (SEM). $P < 0.05$ was considered to be statistically significant throughout. Data analyses were performed using Prism5 software (GraphPad, La Jolla, CA).

Results

PRBC exposure and monocyte function

Monocytes incubated with stored PRBC produced less TNF α upon LPS stimulation compared to monocytes incubated with media alone ($p < 0.0001$ CPD vs control, $p = 0.02$ AS-1 vs control, $p = 0.003$ AS-3 vs control; **Fig. 1A**). Production of the anti-inflammatory cytokine IL-10 was preserved (**Fig. 1B**). Exposure to CPD-stored PRBC resulted in decreased *ex vivo* LPS-induced TNF α production compared to exposure to PRBC stored in additive solutions ($p = 0.007$ vs AS-1; $p = 0.006$ vs AS-3). Similarly, *ex vivo* LPS-induced IL-10 production was also lower after exposure to CPD-preserved PRBC compared to AS-1 and AS-3 groups ($p = 0.003$ vs AS-1; $p = 0.0004$ vs AS-3), though was not different from controls (**Fig. 1B**). There was no effect of storage time on TNF α production capacity for the CPD groups, though older PRBC suppressed monocyte function more than younger PRBC in the AS-1 group (21 day, $p < 0.05$) and the AS-3 group (14 day and 21 day, $p = 0.009$). TNF α and IL-10 levels were undetectable (< 5 pg/ml) in all wells prior to stimulation with LPS.

Ex vivo LPS-stimulated TNF α and IL-10 production by monocytes exposed to preservative solutions alone were not significantly different from controls (**Fig. 1 C and D**).

Transwell experiments

When cell-to-cell contact was prevented by the use of transwells, there remained a significant effect of both storage time and storage solution on the degree of monocyte dysfunction, though the inhibitory effect of PRBC on TNF α production capacity was more modest (**Fig. 2A**). As expected, concentrations of free hemoglobin in the lower chamber of the transwells were low (< 10 ng/ml) in control wells. Among wells incubated with PRBC, free hemoglobin concentrations in the lower chambers were not significantly different between storage solution types (CPD: 727 ($\pm$ 128) ng/ml, AS-1: 422 ($\pm$ 71) ng/ml, AS-3: 979 ($\pm$ 325) ng/ml, $p = 0.18$) or storage durations ($p > 0.05$ for all).

Effects of PRBC co-incubation on monocyte mRNA expression

In order to gain mechanistic insight into the effects of PRBC co-incubation on monocytes, we evaluated the capacity of monocytes to produce mRNA coding for TNF α and IL-10 after pre-incubation with stored PRBC. After four hours of co-incubation, prior to LPS stimulation, *TNFA* and *IL10* mRNA expression levels were the same or higher than those seen in control wells regardless of PRBC storage age or storage solution (see Online Supplement). To understand if the observed reduction in TNF α production capacity was the result of impaired *TNFA* transcription or translation, we also measured monocyte mRNA expression after four hours of PRBC exposure followed by LPS stimulation (**Figure 3**). Expression of *TNFA* mRNA was significantly reduced compared to controls in all three preservative groups ($p = 0.02$, 0.04 , and 0.03 for CPD, AS-1, and AS-3 respectively). Notably, co-incubation with 21-day old PRBC stored in AS-1 and AS-3 led to statistically significant reductions in LPS-induced *TNFA* levels compared to controls whereas the effects of 7-day old PRBC were less impressive. LPS-induced *IL10* expression was not statistically significantly different from controls in any group except for a modest reduction in the AS-3 group.

Discussion

Our results confirm those of others who have shown that exposure to PRBC blunts the TNF α response to LPS stimulation while preserving the anti-inflammatory IL-10 response as a function of PRBC storage time.²³ Our data go on to suggest, for the first time, that storage solution may be an important factor in this process, with PRBC stored in additive solutions (AS-1 or AS-3) demonstrating less suppression of monocyte function compared with CPD-stored PRBC. In addition, we show that these immunosuppressive effects of co-incubation with PRBC are only partially ameliorated by prohibiting cell-to-cell contact and are not related to the concentration of free hemoglobin present. Lastly, we demonstrate that the reduction in LPS-induced TNF α production capacity is mediated at the level of mRNA.

Critically ill patients, though typically presenting with pro-inflammatory conditions, can experience a subsequent anti-inflammatory response characterized by increased plasma levels of anti-inflammatory cytokines (e.g. IL-10) and a decreased ability of immune cells to respond appropriately to a new inflammatory challenge.^{1, 7, 8} Data from our laboratory and others suggest that impaired responsiveness of the innate immune system after the onset of critical illness or injury, as measured by reduced TNF α production capacity, is associated with increased risks of nosocomial infection and death.^{1-6, 29} While the anti-inflammatory cytokine IL-10 has been implicated as a potential contributor to this phenomenon,^{1, 5, 8-10} specific mechanisms of critical illness-associated immune suppression remain unclear. It is likely that therapies received in the ICU may contribute to suppression of monocyte function.

PRBC transfusion has been identified as an independent risk factor for adverse outcomes in critically ill adults and children.¹³⁻¹⁵ Longer storage age of PRBC has also been linked to mortality,²⁴ increased organ dysfunction,^{30, 31} and increased infection risk^{18, 25, 32} following transfusion. Experimental studies have suggested that innate immune suppression may play a role.

Biedler *et al* incubated aliquots of fresh donated whole blood with allogeneic, leukoreduced PRBC that had been stored for 24-26 days and demonstrated increased production of IL-10 following stimulation with LPS,²² though TNF α production capacity was not reduced. Mynster *et al* incubated freshly donated whole blood with allogeneic, leukoreduced PRBC of varying storage times, followed by LPS stimulation.²³ They demonstrated reduced LPS-induced TNF α production capacity as a function of longer storage time, along with an increase in IL-10 production. In order to evaluate the effect of soluble mediators, Karam *et al* incubated supernatants from leukoreduced PRBC of varying storage times with whole blood in media before stimulating with LPS. In their model, co-culture with supernatants from the oldest red cells resulted in decreased LPS-induced production of the pro-inflammatory cytokines IL-6 and TNF α , with no change in IL-10 production. All of the above studies used laboratory-based PRBC storage protocols that utilized additive solutions. Our data corroborate the finding that, for PRBC stored with additive solution, longer storage time is associated with a greater degree of innate immune suppression. We are unaware of any study that has previously evaluated the effects of clinically-banked PRBC of varying storage solution content on innate immune function.

As a group, the CPD-only PRBC suppressed monocyte function more than AS-1 or AS-3 PRBC regardless of storage time. While AS-1 and AS-3 PRBC became progressively more immunosuppressive with increasing storage time, we did not find a significant effect of storage time when evaluating CPD-only PRBC. This is potentially clinically relevant given that many institutions, including ours, preferentially provide CPD-only PRBC to infants.²⁷ Other populations at our institution that preferentially receive CPD-only PRBC include open heart surgery patients < 4 years of age and children receiving extracorporeal membrane oxygenation (ECMO) support. This practice has, in part, been driven by concerns for adenine or mannitol-induced hepatic and renal dysfunction that have not been borne out in clinical trials.^{33, 34} Our findings raise the possibility that our youngest patients may be receiving more potentially immunosuppressive transfusions than older children who receive PRBC stored in AS-1 or AS-3.

The mechanisms underlying PRBC-mediated monocyte suppression are currently unclear. The inhibition of leukocyte responsiveness shown by Mynster was not seen when leukocytes were incubated with supernatants from PRBC. This effect was seen only after treatment with cell-containing suspensions. Our transwell experiments support the notion that cell-to-cell contact may play a role in modulating the immune response, in that LPS responsiveness was partially restored when cell-to-cell contact was prevented. It should be noted, however, that a statistically significant effect persisted in the CPD and AS-3 groups, implying that a soluble mediator(s) is likely to be at least partially responsible. Free hemoglobin has been shown to augment immune responsiveness,³⁵ while hemoglobin/haptoglobin complexes are cytotoxic to innate immune cells.³⁶ It seems unlikely, however, that hemoglobin can account for our results given that concentrations in the monocyte compartments were not significantly different between groups.

Red cell storage may release other potentially biologically relevant mediators including free iron, hydrogen ions, and potassium.^{37, 38} Previous studies have suggested that various PRBC storage solutions can promote differences in red cell membrane lipid, protein, and microvesicle profiles.^{39, 40} Additionally, it has been recently shown that erythrocyte-derived

microvesicles may have the ability to suppress LPS-induced TNF α production by innate immune cells.⁴¹ It is therefore possible that microvesicles created from red cells under variable storage conditions may differentially alter the innate immune response in a way that could explain our findings. We view this as an attractive target for future study.

When we evaluated monocyte mRNA expression in our model, we found reduced expression of *TNFA* mRNA in cells exposed to stored PRBC, along with preserved or elevated levels of *IL10* expression. These reductions in *TNFA* mRNA suggest that the lower TNF α protein production capacity seen after PRBC co-incubation is due to reduced *TNFA* gene transcription or increased mRNA turnover rather than impaired translation.

Our study has several important limitations. First, our experimental model, while using volume ratios that approximate clinical transfusion, does not fully replicate the cell numbers and immunobiology of transfusion *in vivo*. The interactions between donor PRBC, storage solutions, vascular endothelium, and a variety of host leukocytes are likely to be far more complicated than those in our isolated-monocyte model. We were, however, able to evaluate the specific effects of stored PRBC on a crucial driver of the innate immune response and offer mechanistic insights into this interaction. Our model also allowed us to use clinically available PRBC units without the need for crossmatching, as there would be no red cell antigen-antibody interaction. We did not measure the concentrations of constituent elements of the preservative solutions within each PRBC segment, nor did we quantify the percentage of reticulocytes within donor units. We carried out this study, however, with segments from our clinical blood bank and performed experiments with five replicates such that our study should reflect the PRBC content that patients are likely to receive clinically. While the segments from PRBC units may not exactly reflect the content of their parent PRBC bags, our approach allowed us to use a clinical blood product in a way which was conservative of blood resources and cost effective. Additionally, while we used leukoreduced PRBC, it is possible that the few remaining donor leukocytes in the PRBC segments contributed in some way to our findings. We chose to limit our analyses to the outdate (21 days) of CPD blood. It is certainly possible that different effects of storage age may have been seen with PRBC units older than 21 days. It should be noted that our model utilized monocytes from healthy adults. Additional work is warranted to evaluate the effects of PRBC on monocytes from critically ill adults and children using *in vitro* and *in vivo* approaches. Lastly, while TNF α and IL-10 appear to be important mediators in the setting of critical illness-induced monocyte dysfunction, other cytokines and cell types certainly may play a role in PRBC-induced immune modulation and are deserving of future study.

In conclusion, we have shown in an *in vitro* model of red cell transfusion that PRBC have an immunosuppressive effect on healthy monocytes as a function of storage time and storage solution. Additive solutions, such as AS-1 and AS-3, fared much better than CPD in preserving monocyte responsiveness, though older PRBC were more immunosuppressive in these groups. These effects were only partially cell-to-cell contact dependent and were not associated with increased concentration of free hemoglobin. It is possible that PRBC stored in CPD-only may carry hidden risks to recipients that have not been previously considered when evaluating transfusion strategies. Further work is necessary to more fully elucidate mechanisms of PRBC transfusion-related innate immune suppression and to determine its clinical relevance. Today, however, we feel strongly that the type of preservative solution should be analyzed as a co-variable along with storage age in current and future clinical trials of blood transfusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Ex vivo LPS-stimulated monocyte cytokine production following exposure to stored PRBC (A,B) or preservative solution alone (C,D)

(A) Monocytes co-cultured with banked PRBC of varying storage times and preservative solutions produced less TNF α upon *ex vivo* LPS stimulation compared to control (media only) monocytes ($p < 0.0001$ CPD vs. control, $p = 0.02$ AS-1 vs. control, $p < 0.003$ AS-3 vs. control). Impairment of TNF α production was greatest for CPD-preserved compared to AS-1 or AS-3-preserved groups. An effect of storage time was seen for AS-1 and AS-3 groups but not for CPD. (B) *Ex vivo* LPS-stimulated IL-10 production was not different between PRBC-exposed monocytes and controls. IL-10 production was lower after co-culture with CPD-preserved PRBC compared to AS-1 or AS-3-preserved PRBC. Cytokine production levels by control monocytes (LPS stimulation without PRBC exposure) were as follows: TNF α : 48,525 ($\pm 7,832$) pg/ml and IL-10: 207.5 (± 27) pg/ml. TNF α and IL-10 levels were low (< 50 pg/ml and < 5 pg/ml, respectively) in supernatants prior to LPS stimulation in all groups. (C, D) No differences in *ex vivo* LPS-induced TNF α (C) or IL-10 (D) production were seen following exposure to preservative solutions alone. Data are expressed as mean (SEM) of percentage of media only controls. * $p < 0.05$ vs. controls; ** $p < 0.01$ vs. controls; # $p < 0.05$ vs CPD.

Figure 2. Ex vivo LPS-stimulated monocyte cytokine production following co-culture with stored PRBC in 0.4 micrometer transwells

Monocytes were co-cultured with PRBC in transwells to prohibit cell-to-cell contact. (A) Exposure to PRBC solution stored in CPD or AS-3 resulted in less monocyte *ex vivo* LPS-stimulated TNF α production compared to control monocytes ($p = 0.003$ CPD vs. control, $p = 0.02$ AS-3 vs. control). TNF α production was lower for CPD-preserved PRBC compared to AS-1-preserved PRBC. (B) *Ex vivo* LPS-stimulated IL-10 production was higher after exposure to AS-1-preserved PRBC compared to controls and CPD-preserved PRBC. IL-10 production was not different among CPD, AS-3 and controls. Data are expressed as mean (SEM) of percentage of media only controls. * $p < 0.05$ vs. controls; ** $p < 0.01$ vs. controls.

Figure 3. Monocyte mRNA expression following co-culture with stored PRBC and stimulation with LPS

(A) LPS-induced expression of mRNA coding for TNF α was decreased in monocytes exposed to PRBC compared to control (media only) monocytes ($p = 0.02$ CPD vs. control, $p = 0.04$ AS-1 vs. control, $p = 0.03$ AS-3 vs. control). (B) LPS-induced expression of *IL10* was not different among CPD-preserved groups, AS-1 preserved-groups and controls. *IL10* expression tended to be lower after exposure to AS-3-preserved PRBC ($p = 0.09$ vs. control). Data are expressed as mean (SEM) of relative copy number (RCN). * $p < 0.05$ vs. controls.

Table 1
Approximate concentrations of preservative solution constituents in final PRBC products

Data are expressed as mg/100ml. Calculations assume 450 ml donor blood volume and 63 ml CPD with a hematocrit of 45%, concentrated to a hematocrit of 72% before the addition of 100 ml additive solution (standard blood manufacturing practice).

	CPD	AS-1	AS-3
Sodium chloride	0	900	410
Dextrose	1610	2630	1530
Adenine	0	27	30
Trisodium citrate	1660	443	1031
Sodium phosphate	140	12	288
Mannitol	0	750	0
Citric acid	206	17	59

Adapted from the American Association of Blood Banks and the American Red Cross Circular of Information.