Transfusion of fresh but not old stored blood reduces infarct size and improves cardiac function after acute myocardial infarction in anemic rats*

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Objectives: **We recently demonstrated that transfusion of fresh blood to 100 g/L hemoglobin in anemic animals offers cardioprotection after acute myocardial infarction. The objective of this study was to compare the cardioprotective effects of fresh vs. stored blood when transfused in anemic rats after acute myocardial infarction.**

Study Design: **Randomized animal study.**

Setting: **University laboratory.**

Subjects: **Male Sprague-Dawley rats weighing 200–300 g.**

Intervention: **Myocardial infarction was induced by coronary artery ligation in 49 male Sprague-Dawley rats weighing 200–300 g, 38 of which were anemic (80–90 g/L) and 11 with normal hemoglobin levels. Anemic animals were randomized to receive fresh blood (within 4 hrs), stored blood (7 days), or no transfusion immediately after myocardial infarction.**

Measurements and Main Results: **At 24 hrs after myocardial infarction, cardiac function, infarct size, and apoptosis were determined. Erythrocyte ATP, 2,3-DPG, hemoglobin, and free hemoglobin levels in the supernatant were determined. Transfusion with fresh but not stored blood significantly decreased infarct size and myocardial apoptosis in anemic rats when compared to anemic animals not undergoing transfusion. Cardiac function and survival were significantly improved in the anemic animals undergoing fresh blood transfusion compared to control anemic animals. Analysis of stored red blood cells showed reductions of intracellular ATP and 2,3-DPG levels and free hemoglobin was increased in the supernatant.**

Conclusions: **The prolonged storage of blood negates the beneficial effects of fresh blood transfusion, which include reductions in infarct size and myocardial apoptosis, and improvements in cardiac function and short-term survival after acute myocardial infarction in this animal model. (Crit Care Med 2012; 40:740–746)**

Key Words: anemia; apoptosis; blood transfusion; myocardial infarction; storage lesion

In patients with acute myocardial infarction (MI), anemia is both prevalent (1, 2) and a predictor of adverse outcomes (2–5). Red blood cell (RBC) transfusion is the only available therapeutic intervention for the acute treatment of anemic patients. However, the minimum hemoglobin (Hb) threshold for transfusion and optimal target Hb, as well as the overall effectiveness of RBC transfusion in the treatment of acute coronary syndromes, remain widely disputed (1, 6, 7). Given that the myocardium has a basal oxygen extraction ratio of 55%–70%, the heart has little capacity to further increase oxygen extraction (8), making it vulnerable to anemia and, furthermore, an appropriate model system to quantify the effects of transfusion during MI. To this end, we recently demonstrated that transfusion of fresh blood to increase Hb from 80 g/L to a final Hb level of 100 g/L in animals after acute MI increased survival, decreased infarct size, and improved cardiac function (9).

Currently, RBC concentrates may be stored up to 42 days, during which a series of well-defined biochemical and corpuscular changes to RBCs occur, along with the accumulation of bioactive substances in storage media (10, 11). It is possible that the conflicting results reported on the benefits of transfusion in acute coronary syndromes (1, 6, 7) may be partly related to differences in the quality of the blood product as a result of prolonged storage.

Several recent reviews of clinical studies examining the association of storage duration with morbidity and mortality have summarized the conflicting and inconclusive evidence (12–14). A number of confounding factors, including heterogeneity of populations with respect to comorbidities, inconsistent transfusion interventions using blood products of different storage durations, mixtures of blood products including leukoreduced and nonleukoreduced, and different preservative solutions, and varied clinical outcome measures such as infection, length of stay, and mortality, limit the interpretation of these clinical studies. Of note, however, no clinical studies have addressed the effects of blood storage and

***See also p. 983.**

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Supported by the CIHR, Canadian Blood Services, Hema Quebec, Bayer Partnership Fund, and the Anemia Institute for Research and Education, and Heart and Stroke Foundation of Ontario (HSFO). Dr. Feng is a HSFO Career Investigator.

Drs. Hu and Xenocostas contributed equally to this work.

Dr. Xenocostas has been a consultant for and has received educational grants from Ortho Biotech, Canada, and Janssen. The remaining authors have not disclosed any potential conflicts of interest.

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DOI: 10.1097/CCM.0b013e3182376e84

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transfusion after myocardial ischemia. We hypothesized that prolonged storage would negate the beneficial effects of blood transfusion in the anemic setting during acute MI. To test this hypothesis, we investigated the effects of fresh vs. stored blood transfusion on clinically relevant outcomes after acute MI using an animal model to objectively measure the effects of transfusion and blood storage on cardiac function and tissue injury. Results from our study support the use of fresh blood in the setting of anemia during acute MI and may provide insight in the design of future clinical trials to study the effects of fresh vs. older blood transfusion in anemic patients with acute MI.

MATERIALS AND METHODS

Experimental Animals and Induction of Anemia. The experiments were conducted using male Sprague-Dawley rats (200–300 g). All animals were provided water and food *ad libitum*, and they were housed in a temperature- and humidity-controlled facility with 12-hr light and dark cycles. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1996). Experimental protocols were approved by the Animal Use Subcommittee at the University of Western Ontario.

To induce anemia, rats were fed an iron-deficient diet (10–20 ppm; TestDiet 5859, Richmond, IN) previously described by Strube et al (15) in combination with phlebotomy (2–3 mL) from the jugular vein twice weekly to reach target Hb levels of 80–90 g/L (9, 16). Immediately after phlebotomy, rats were infused with an equal volume of 10% pentastarch (Bristol-Myers Squibb, Montreal, Quebec).

Experimental Design and Transfusion. Rats were randomly assigned to 4 experimental groups: group 1, normal Hb 140–150 g/L, MI (n = 11); group 2: Hb 80–90 g/L, MI (n = 13); group 3: Hb 80–90 g/L, MI with fresh blood transfusion to Hb levels of 100 g/L (n = 11); and group 4: Hb 80–90 g/L, MI with stored (7 days old) blood transfusion to Hb levels of 100 g/L (n = 14). Transfusion was performed in groups 3 and 4, immediately after coronary artery ligation surgery, to increase the Hb level to 100 g/L. Donor blood was collected from syngeneic rats and stored as previously described (9, 16). Briefly, rats were anesthetized with an intramuscular injection of ketamine (60 mg/kg) and xylazine (10 mg/kg), and the carotid artery was cannulated to retrieve the maximum amount of blood. Blood was collected into a 20-mL syringe with 3 mL of CPDA-1 (citrate-phosphate-dextrose-adenine; Baxter, Toronto, Ontario) and injected into a sterile blood collection bag (Fenwal; Baxter, Toronto, Ontario). The whole blood was kept at 4°C for <4 hrs in the fresh blood group and 7 days in the stored blood group. A 7-day storage period was based on our previous study that showed equivalent biochemical changes and deformability to 29-day stored human RBCs in CPDA-1 (17). Right before transfusion, the stored nonleukoreduced blood was drawn from the collection bag and centrifuged at 500*g* for 5 mins at 4°C. The supernatant was removed to achieve a hematocrit of 70%. Total volume of packed RBCs infused to achieve 100 g/L Hb was approximately 3 mL within 30 mins. Thirty minutes after transfusion, the Hb levels in the recipient rats were measured using a 10-μL blood sample obtained from the saphenous vein by spectrophotometry with Hb assay kit (Pointe Scientific, Canton, MI).

Induction of MI. The MI was induced by ligation of the left descending coronary artery as described in our previous reports (9, 16, 18). Briefly, rats were anesthetized with intramuscular injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). Animals were then intubated and artificially ventilated. A left thoracotomy was performed to expose the heart and the left anterior descending coronary artery was ligated by positioning a 6-0 silk suture between the pulmonary artery outflow tract and the left atrium. The lungs were thereafter hyperinflated using positive end-expiratory pressure and the thorax was immediately closed. Animals were caged individually after each surgical operation.

Hemodynamic and Infarct Size Measurements. Hemodynamic measurements were performed to assess *in vivo* cardiac function 24 hrs after coronary artery ligation (9). Rats were re-anesthetized with ketamine and xylazine, and a polyethylene catheter (PE-50) was inserted into the right carotid artery to record the arterial pressure. The catheter was then advanced retrograde into the left ventricle (LV) for recording of LV pressures. Heart rate, arterial pressure, LV systolic pressure, LV end-diastolic pressure, and the maximal positive and minimal negative first derivatives of LV pressure ($+dP/dt_{max}$ and $-dP/dt_{min}$; parameters that represent contractility and diastolic relaxation, respectively) were analyzed by PowerLab Chart software (ADInstruments, Colorado Springs, CO).

After hemodynamic measurements were obtained, infarct size was determined using Evans blue and 5% triphenyltetrazolium chloride according to our previous reports (9, 16, 19). The proportions of the LV that were normally perfused, at risk, or infarcted were calculated by volumetric analyses and expressed as a percent of total LV weight. Infarct size (%) was expressed as the weight of infarcted LV divided by the weight of LV at risk.

Caspase-3 Activity. Caspase-3 activity was measured in heart tissues from the peri-infarct area using a caspase-3 assay kit (BIOMOL, Plymouth Meeting, PA) (16). Briefly, heart tissue homogenates were incubated in the presence of caspase-3 substrate Ac-DEVD-AMC, with or without the inhibitor Ac-DEVD-CHO. Fluorescence intensity (excitation at 360 nM, emission at 460 nM) was measured using a fluorescent microplate reader (SpectraMaxM5; Molecular Devices, Sunnyvale, CA), and activity was extrapolated from a standard curve. Data were expressed as the amount of AMC substrate cleaved per μg of protein per hour.

Terminal Deoxynucleotidyl Transferase d-UTP Nick-End Labeling Staining. Terminal deoxynucleotidyl transferase d-UTP nick-end labeling (TUNEL) was used to localize and quantitatively assess cells undergoing apoptosis using the In Situ Cell Death Detection Kit (Roche, Indianapolis, IN) on paraffin heart sections (16, 19). As a counterstain, cells were stained with Hoechst 33,342. Fifteen separate fields were examined using a fluorescent microscope (Observer D1; Zeiss, Oberkochen, Germany) at ×630 magnification to quantify the number of TUNEL-positive nuclei. Images were obtained by a laser confocal microscope (LSM 510 Meta; Zeiss). Data were expressed as the average percentage of TUNEL-positive nuclei indentified by two independent observers.

Blood Biochemistry. Intracellular ATP was measured in fresh and stored blood samples using a bioluminescence kit (Sigma, St. Louis, MO). Erythrocytic 2,3-DPG concentrations in fresh and stored blood samples were measured using an ultraviolet absorbance assay (Roche, Laval, Quebec) as per manufacturer's instructions. Supernatant Hb was quantified using a colorimetric plasma-free Hb assay (Catachem, Oxford, CT).

Statistics. Data are expressed as the means ± SEM. Survival was analyzed by Kaplan-Meier method using log-rank test. Infarct size and hemodynamics were analyzed using one-way analysis of variance followed by Bonferroni test, which was limited to four comparisons of interest. The biochemical measurements were analyzed by the Student *t* test; $p < .05$ was considered statistically significant.

RESULTS

Body Weight and Hemoglobin

All animals had a similar body weight and Hb at baseline. Animals fed an iron-deficient diet in combination with phlebotomy had significantly decreased Hb levels as compared to the standard diet group by the end of a 2-wk period before surgery ($p < .01$), with mean Hb levels reaching the target level of 80–90 g/L (Table 1). After blood transfusion, the Hb level was increased to 100.5 ± 0.4 g/L in the anemic animals.

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Table 1. Body weight and hemoglobin at baseline and before surgery

Groups	Body Weight (g)		Hemoglobin (g/L)	
	Baseline	Before Surgery	Baseline	Before Surgery
Hb 140–150 g/L (n = 11)	180.7 ± 2.8	274.2 ± 3.1	143.0 ± 1.8	144.1 ± 1.3
Hb 80–90 g/L (n = 13)	181.2 ± 2.7	273.6 ± 3.8	144.1 ± 1.5	83.3 ± 0.6[a]
Hb 80–90 g/L + fresh blood transfusion (n = 11)	183.6 ± 3.5	272.4 ± 3.9	145.1 ± 1.7	82.2 ± 1.3[a]
Hb 80–90 g/L + stored blood transfustion (n = 14)	182.1 ± 2.4	276.4 ± 3.6	144.8 ± 2.0	83.3 ± 0.4[a]

Data are mean ± SEM. Fresh blood transfusion indicates transfusion of fresh blood to reach hemoglobin (Hb) of 100 g/L. Stored blood transfusion indicates transfusion of 7-day stored blood to reach Hb of 100 g/L. Body weight and Hb were similar among all groups at baseline. Before surgery, Hb was significantly lower in anemic groups compared to Hb 140–150 g/L group.

[a]$p < .01$).

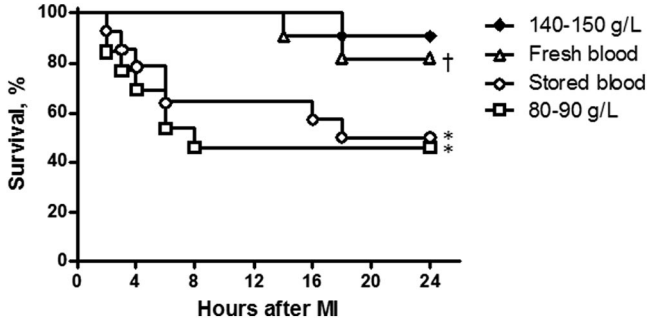

Figure 1. Effects of transfusion of fresh and stored blood on survival in anemic rats after myocardial infarction (*MI*). Transfusion was performed in rats with hemoglobin (*Hb*) levels of 80–90 g/L immediately after MI to increase Hb levels to 100 g/L using fresh (<4 hrs) or stored (7 days) red blood cells. Survival was monitored for 24 hrs after surgery. n = 11–14 rats per group. *$p = .017$ and 0.026 for Hb 80–90 g/L and stored blood vs. Hb 140–150 g/L, respectively. †$p = .046$ vs. Hb 80–90 g/L.

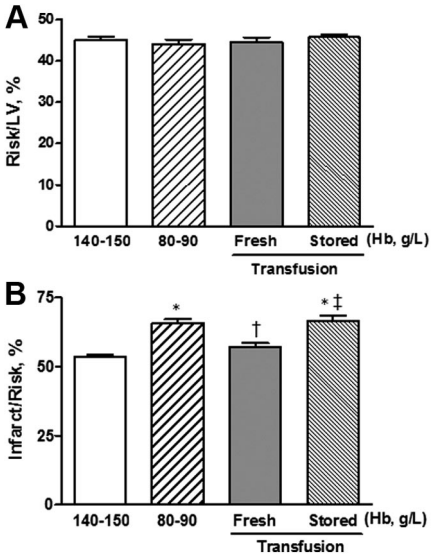

Figure 2. Effects of transfusion of fresh and stored red blood cells on infarct size after myocardial infarction in anemic rats. *A*, Percent weight of risk area weight to total left ventricular (*LV*) weight shows similar ischemic areas among all groups (p = not significant). *B*, Infarct size is expressed as the percentage of weight of infarct area to weight of LV at risk. Data are mean ± SEM from 6–9 rats per group. Overall infarct size comparison by one-way analysis of variance was significant ($p < .05$). *$p < .05$ vs. hemoglobin (*Hb*) 140–150 g/L. †$p < .05$ vs. Hb 80–90 g/L. ‡$p < .05$ vs. fresh red blood cell transfusion.

Effects of Transfusion of Fresh and Stored Blood on Survival After MI in Anemic Rats

After MI, the animal survival rates were monitored for 24 hrs. Survival in the untransfused anemic group (6/13) was significantly decreased after MI compared to the normal Hb group (10/11) ($p = .017$; Fig. 1). Transfusion of fresh blood (9/11) but not stored blood (7/14) significantly increased animal survival after MI compared to the untransfused anemic group ($p = .046$; Fig. 1).

Effects of Transfusion of Fresh and Stored Blood on Infarct Size After MI in Anemic Rats

Twenty-four hours after MI, the area at risk-to-LV weight ratios were not significantly different between any groups, indicating that a similar degree of myocardial ischemia was induced in all groups (P = not significant; Fig. 2*A*). However, the infarct-to-area at risk weight ratios were significantly increased in the untreated Hb 80–90 g/L compared to the those of normal Hb group ($p < .05$). Transfusion of fresh, but not stored, blood significantly decreased infarct size to the same level as that of the normal Hb group ($p < .05$; Fig. 2*B*).

Effects of Transfusion on Cardiac Function After MI in Anemic Rats

Cardiac function was determined at 24 hrs after MI. Heart rate was not significantly different among all groups (P = not significant; Table 2). The mean arterial pressure was significantly lower in the untreated anemia group compared to that of the normal Hb group ($p < .05$). Transfusion of fresh blood, but not stored blood, significantly increased mean arterial pressure to similar levels as those of the normal Hb group ($p < .05$; Table 2). LV systolic pressure was decreased in the untreated anemia group compared to the normal Hb group ($p < .05$) and was maintained by transfusion of fresh, but not stored, blood in the anemic animals ($p < .05$; Table 2). LV end-diastolic pressure was increased in the untreated anemia group compared to the normal Hb group ($p < .05$; Table 2) and was not significantly affected by transfusion of fresh or stored blood in the anemic animals (P = not significant; Table 2). LV $+dP/dt_{max}$ and $-dP/dt_{min}$, which represent cardiac contractility and relaxation, respectively, were significantly lower in the untreated anemic rats compared to the normal Hb rats ($p < .05$; Fig. 3*A* and 3*B*). Transfusion of fresh, but not stored, blood in anemic rats significantly increased LV $+dP/dt_{max}$ and $-dP/dt_{min}$ compared to the untreated anemic group ($p < .05$; Fig. 3*A* and 3*B*). These results suggest that transfusion of fresh, but not stored, blood improves cardiac function in anemic animals after MI.

Effects of Transfusion of Fresh and Stored Blood on Myocardial Apoptosis After MI

Myocardial apoptosis within the peri-infarct area was analyzed by TUNEL

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Table 2. Changes in heart rate, mean arterial pressure, left ventricular systolic pressure, and left ventricular end-diastolic pressure 24 hrs after myocardial infarction

Groups	Heart Rate (bpm)	Mean Arterial Pressure (mm Hg)	Left Ventricular Systolic Pressure (mm Hg)	Left Ventricular End-Diastolic Pressure (mm Hg)
Hb 140–150 g/L (n = 8)	334.1 ± 4.3	108.8 ± 3.1	133.0 ± 2.9	4.8 ± 1.5
Hb 80–90 g/L (n = 6)	324.5 ± 14.5	81.2 ± 3.1[a]	109.3 ± 3.0[a]	11.3 ± 1.3[a]
Hb 80–90 g/L + fresh blood transfusion (n = 9)	342.2 ± 8.2	105.6 ± 3.5[b]	128.2 ± 3.0[b]	8.6 ± 1.4
Hb 80–90 g/L + stored blood transfusion (n = 7)	338.4 ± 10.9	89.0 ± 3.0[a,c]	104.5 ± 1.4[a,c]	9.1 ± 1.4

Data are mean ± SEM . Fresh blood transfustion indicates transfusion of fresh blood to reach hemoglobin (Hb) of 100 g/L. Stored blood transfusion indicates transfusion of 7-day stored blood to reach Hb of 100 g/L.

Overall comparison by one-way analysis of variance was significant in mean arterial pressure, left ventricular systolic pressure, and left ventricular end-diastolic pressure ($p < .05$).

[a]$p < .05$ vs. Hb 140–150 g/L; [b]$p < .05$ vs. Hb 80–90 g/L; [c]$p < .05$ vs. fresh red blood cell transfusion.

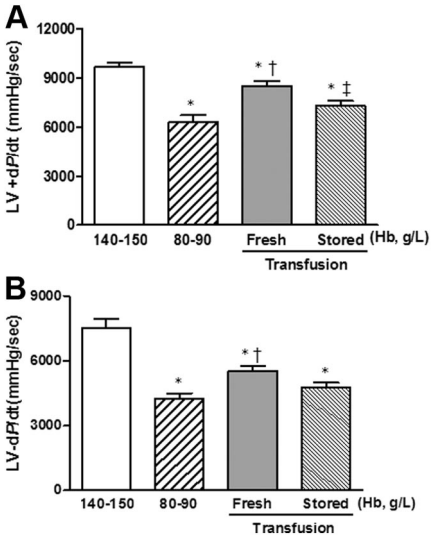

Figure 3. Effects of transfusion of fresh and stored red blood cells on left ventricular (*LV*) contractility (+*dP/dt*) and relaxation (−*dP/dt*) 24 hrs after myocardial infarction in anemic rats. *A*, LV +d*P*/dt$_{max}$. *B*, LV −d*P*/dt$_{min}$. Data are mean ± SEM from 6–9 rats per group. Overall comparison by one-way analysis of variance was significant ($p < .05$). *$p < .05$ vs. hemoglobin (*Hb*) 140–150 g/L. †$p < .05$ vs. Hb 80–90 g/L. ‡$p < .05$ vs. fresh red blood cell transfusion.

staining and caspase-3 activity. Representative images of TUNEL staining for normal Hb, untreated anemia, and transfusion with fresh blood or stored blood groups are shown in Figures 4*A*, 4*B*, 4*C*, and 4*D*, respectively. Quantitative analysis showed that the number of TUNEL-positive cells and caspase-3 activity in the peri-infarct region were significantly increased in the untreated anemic group compared to the normal Hb group ($p < .05$). Transfusion of fresh, but not stored, blood significantly decreased TUNEL positivity and caspase-3 activity in the anemic animals ($p < .05$; Fig. 4*E* and 4*F*).

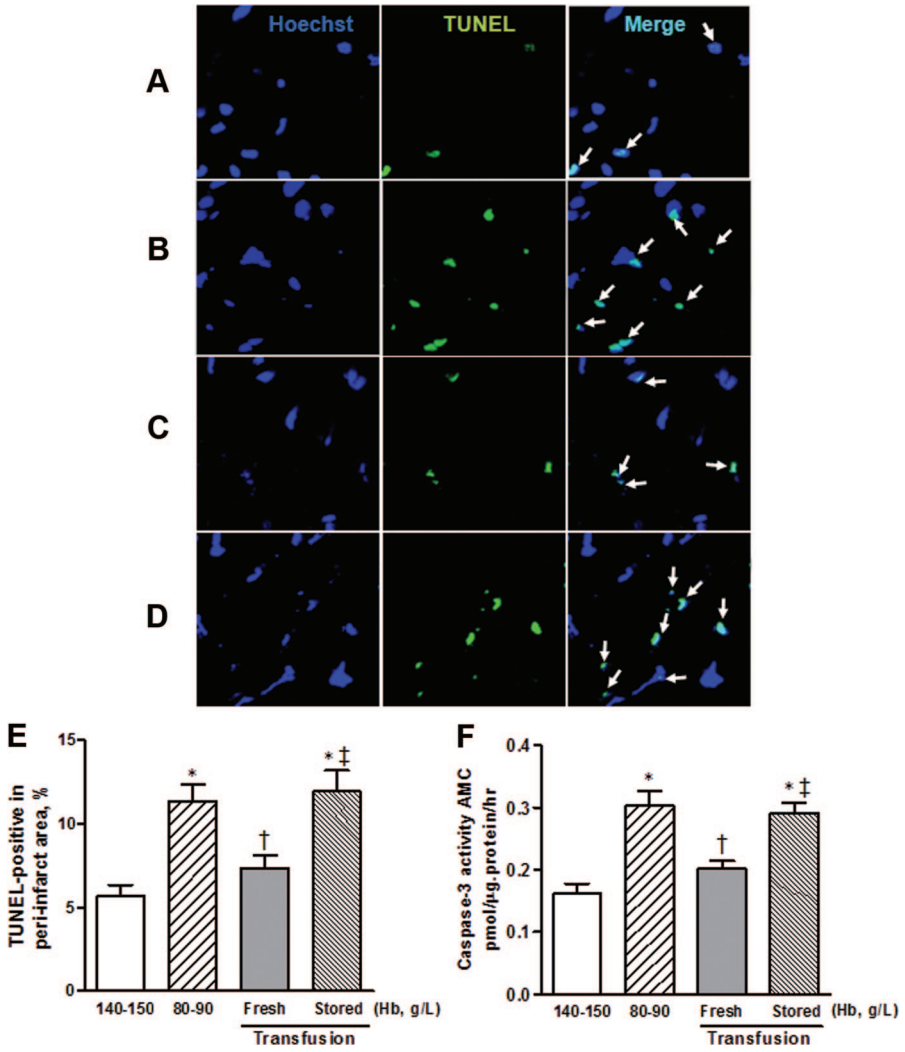

Figure 4. Effects of transfusion of fresh and stored red blood cells (*RBCs*) on myocardial apoptosis in peri-infarct area 24 hrs after myocardial infarction in anemic rats. *A–D*, Representative confocal images of terminal deoxynucleotidyl transferase d-UTP nick-end labeling (*TUNEL*) staining from normal hemoglobin (*Hb*) (*A*), Hb 80–90 g/L (*B*), Hb 80–90 g/L plus fresh RBC transfusion (*C*), and Hb 80–90 g/L plus stored RBC transfusion (*D*). TUNEL-positive nuclei stained in *green* with Hoechst (*blue*) as a nuclear stain. *White arrows* indicate positive signal. *E*, Quantitative analysis of TUNEL-positive nuclei. *F*, Myocardial caspase-3 activity. Data are mean ± SEM from 6–7 rats per group. Overall comparison by one-way analysis of variance was significant ($p < .05$). *$p < .05$ vs. Hb 140–150 g/L. †$p < .05$ vs. Hb 80–90 g/L. ‡$p < .05$ vs. fresh RBC transfusion.

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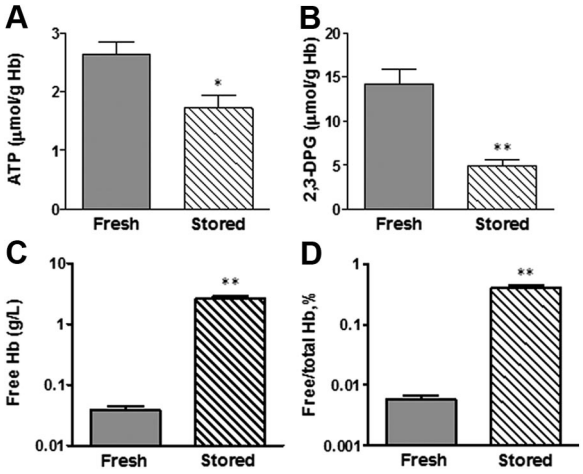

Figure 5. Blood biochemistry of fresh (<4 hrs) vs. stored (7 days) blood. *A*, Red blood cell ATP. *B*, Red blood cell 2,3-DPG. *C*, Supernatant-free hemoglobin (*Hb*). *D*, Supernatant-free Hb-to-total Hb ratio. Data are mean ± SEM, n = 6–7. *$p < .05$. **$p < .001$ vs. fresh red blood cells.

These results suggest that fresh, but not stored, blood transfusion inhibits myocardial apoptosis in anemic animals after MI.

Effects of Storage on Blood Biochemistry

ATP and 2,3-DPG levels were significantly decreased in the stored compared to fresh RBCs ($p < .05$; Fig. 5*A* and 5*B*). Free Hb and percentage of free to total Hb in the storage medium were also significantly increased after storage ($p < .01$; Fig. 5*C* and 5*D*). These changes are consistent with previously described storage-induced lesions in RBCs (17).

DISCUSSION

In this study, we used an animal model of anemia and myocardial ischemia to measure organ injury and the response to transfusion of fresh and stored RBC products. Using the same model, we previously demonstrated the harmful effects of anemia (Hb <100 g/L) after irreversible myocardial ischemia on LV function and mortality, and we found that transfusion with fresh blood was therapeutically beneficial (9). The present study showed that the beneficial effects of transfusion are limited to fresh blood only, and we demonstrated for the first time to our knowledge that stored blood is ineffective at salvaging ischemic myocardium.

Several investigators have reported a deleterious effect of RBC storage on tissue oxygenation after transfusion in animal models. In a hemorrhagic shock model, van Bommel et al (20) found that transfusion of rat RBCs stored for 28 days showed significantly reduced deformability and did not improve microvascular tissue PO_2 when compared to fresh (<5 days stored) RBCs. This result was attributed to the occlusion of the microcirculation by nondeformable cells, a phenomenon we observed using intravital microscopy to monitor stored fluorescently labeled transfused rat RBCs adhering in capillaries of striated muscle (21). In a xenogenic transfusion model, an isovolemic exchange transfusion of human blood into rats decreased microvascular Po_2 in animals receiving stored blood (22). Although we did not directly measure myocardial microvascular Po_2,our results are consistent with the reported negative effects of stored blood on tissue oxygenation.

Several studies also have examined the association of RBC storage times and clinical outcomes in cardiac patients, but none in the setting of anemia and acute MI. A large retrospective study conducted by Koch et al (23) showed that transfusion of older blood (mean storage time, 20 days) as compared to newer blood (11 days) in patients undergoing cardiac surgery was a predictor for increased mortality and a number of complications, although more patients in the older blood treatment group had LV dysfunction and peripheral vascular disease at baseline. A more recent study in pediatric patients undergoing bypass surgery also found that storage time of RBCs used for priming the cardiopulmonary bypass circuit was an independent risk factor for postoperative morbidity (24). Other retrospective studies (25–27) failed to identify any significant associations between blood storage and morbidity after cardiac surgery. Lelubre et al (12) performed a systematic review of the literature, but a number of confounding factors makes it difficult to identify any relationship between age of transfused RBCs and outcomes in adult patients. A meta-analysis by Vamvakas (28) also concluded that available clinical studies do not support an association of transfusion of old RBCs and increased morbidity and mortality.

The mechanisms for the observed differences in efficacy of fresh vs. stored blood in our study remain uncertain. Prolonged storage results in the well-described "storage lesion" in human (10, 11), rat (17), and mouse (29) blood. In the present study, we confirmed the previously described decline in RBC ATP and 2,3-DPG, and we also observed increased free Hb concentrations in the storage medium. However, we did not directly address which of the constellation of changes contributes to the deleterious effects observed.

In our studies ATP levels declined from 2.64 and to 1.73 µmol/g Hb after 7 days of storage, a level sufficient to achieve RBC survival of >70% 24 hrs after transfusion (17). Recently, ATP synthesis by the glycolytic pathway in RBCs has been shown to act as a potent vasodilator when released from RBCs under hypoxic conditions, binding to purinergic receptors and stimulating nitric oxide production in endothelial cells (30, 31). In stored rat blood, ATP levels were reduced, suggesting an impairment of glycolytic ATP production. The loss of deformability that occurs during prolonged storage could also reduce the ability of RBCs to release ATP (17). Interestingly, ATP-replete human blood transfused in rats improved microvascular Po_2 (22). Thus, it is possible that the observed reductions in ATP in stored rat blood may result in the inability of transfused RBCs to release ATP and regulate local blood flow, thereby reducing tissue O_2 delivery and increasing infarct size.

The free Hb concentrations measured in the stored blood in this study are within clinically acceptable levels for human blood transfusion. Plasma-free Hb scavenges nitric oxide 1000-fold faster than the Hb in RBCs (32, 33). Because endothelium-derived nitric oxide is a crucial regulator of coronary circulation (34), this potent nitric oxide scavenging

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ability of free Hb may also contribute to the increased tissue injury seen after stored blood transfusion via increased vasoconstriction. Clinical use of free Hb solutions has shown to be associated with MI and mortality in hemorrhagic shock (35).

Recent work by Hod et al (36) using a mouse transfusion model showed that the deleterious effects of stored blood are mediated by proinflammatory cytokines released by monocytes/macrophages and tissue iron deposition. More importantly, they demonstrated that neither the supernatant nor RBC membranes (cell ghosts) could elicit similar inflammatory responses. The acute release of cytokines and iron from monocytes/macrophages could also contribute to myocardial tissue injury and apoptosis observed in our study.

The present study was limited to examining the acute effects of transfusion in relatively small number of animals per group. The long-term effects of RBC transfusion remain to be determined. We chose a single target Hb of 100 g/L based on our previous work that showed this to be the optimal level for improving cardiac function and survival (9, 16). Extrapolation from the current animal study to human population in terms of transfusion threshold and target should be cautioned because transfusion in patients with Hb levels >80 g/L failed to show any benefits and actually appeared to be deleterious in some clinical studies (7, 37, 38). However, the age of blood used in these clinical studies was not reported. Thus, it is possible that transfusion of the aged blood is responsible for the detrimental effects observed in these studies (7, 37, 38).

The findings of the present study suggest that the changes that occur during blood storage are significant enough to abrogate the beneficial effects of transfusion on myocardial salvage after MI. Our study supports the use of fresh blood in the setting of anemia during acute MI. This insightful information may stimulate studies on blood storage to define "fresh" and "older" human blood and may help in the design of future clinical trials to study the effects of fresh vs. older blood transfusion in anemic patients with acute MI.

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