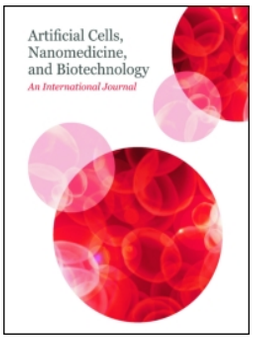

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Polymerized Placenta Hemoglobin Improves Cardiac Functional Recovery and Reduces Infarction Size of Isolated Rat Heart

Tao Li and Jin Liu
West China School of Pharmacy, Sichuan University, Laboratory of Anesthesiology and Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, China

Qian Yang
Department of Medicinal Chemistry, School of Pharmacy, Chengdu Medical College, Chengdu, China

Wei Wu, Pu Zhang, Jing Yang, Jing Li and Wensheng Zhang
Laboratory of Anesthesiology and Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, China

Chengmin Yang
Institute of Tianjin Union Biotechnology Development, Tianjin, China

Abstract: *Objective*: To investigate the cardioprotective effect of polymerized human placenta hemoglobin (PolyPHb) to rat heart subjected to 8-hour hypothermic storage and 2-hour normothermic reperfusion. *Methods and Results*: Isolated rat hearts were perfused with Langendorff model; after 30 minutes of baseline, the hearts were arrested and stored by St. Thomas' solution (STS) without (STS group) or with 0.5 gHb/dL PolyPHb (PolyPHb group) at 4°C for 8 hours, then reperfused for 2 hours. Compared with STS group, PolyPHb in STS greatly improved the recovery of left ventricular developed pressure (LVDP), maximum LVDP increase and decrease rate ($\pm$dp/dt), coronary flow rate (CF). Also, both the cardiac enzyme release, including creatine kinase (CK) and lactate dehydrogenase (LDH), and myocardial infarction size were significantly reduced in PolyPHb group. *Conclusion*: Our study demonstrated that the PolyPHb was beneficial to improving cardiac functional recovery and reducing myocardial infarction of 8-hour hypothermic stored rat heart.

Keywords: Hemoglobin-based O2 carriers, hypothermic storage, cardiac function, myocardial infarction, ischemia/reperfusion injury

INTRODUCTION

Despite advances in medical science and clinical care, maintaining organ viability after donation until transplantation is still a challenge for optimal graft function and survival [16].As a widely used method in clinical practice, hypothermic storage can effectively reduce the metabolic rate and oxygen (O_2) consumption of donor heart [14], thus preserving cardiac function and decreasing ischemia/reperfusion (I/R) injury after being transplanted to the recipient. However, with increasing numbers of organs obtained from older and more marginal donors, improvement of preservation techniques is urgently required [7,12].

Polymerized human placenta hemoglobin (PolyPHb), one type of hemoglobin-based O_2 carriers (HBOCs), was initially developed by the research group of Professor Chengmin Yang to treat patients in emergency (e.g. trauma and hemorrhagic shock) [10,11]. However, in our preliminary experiment, we found that PolyPHb also had potential protective effects for rat hearts against I/R injury. PolyPHb utilizes the placenta blood discarded in

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Address correspondence to Jin Liu, MD, Department of Anesthesiology, West China Hospital, 37 Wainan Guoxue Road, Chengdu 610041, China. E-mail: scujinliu@gmail.com

the past, and allows transporting more O_2 to the hypoxia tissues owing to its higher O_2 affinity than adult peripheral blood Hb [10], as well as a lower viscosity and smaller mean diameter than human red blood cells, suggesting PolyPHb may be helpful to microcirculation perfusion and thereby alleviate myocardial I/R injury [13,19].(McNeil et al. 2001; Standl et al. 2003) Based on this hypothesis and our preliminary experiment, we designed this study to investigate whether PolyPHb provided a cardioprotective effect to isolated rat hearts after 8-hour hypothermic storage and 2-hour normothermic reperfusion.

MATERIALS AND METHODS

The present study was performed in adherence with the *Guidelines on the Use of Laboratory Animals* published by the National Institutes of Health and approved by local Animal Care and Use Committees.

PolyPHb Preparation

PolyPHb was prepared as we previously described [10]. Briefly, Hb from fresh human placenta blood (donated by Tianjin Union Stem cell and Genetic Engineering Ltd., Tianjin, China) was purified and viral inactived by heat treatment, then intra- and inter-molecularly cross-linking were performed by using pyridoxal phosphate (PLP) and glutaraldehyde (GDA), respectively. After that, ultrafiltration and molecular sieve chromatography were performed to harvest PolyPHb with molecular weight range from 64 KD to 600 KD. The prepared PolyPHb solution was added into STS to a final concentration of 0.5 gHb/dL.

Heart Hypothermic Storage

Isolated rat hearts were perfused using a Langendorff apparatus (Radnoti, Monrovia, CA) as described previously [4]. Briefly, 20 male Sprague-Dawley rats (250-300g) were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and heparin (500 IU). The hearts were quickly excised and perfused with Krebs-Henseleit buffer (KHB: 120.0 mM NaCl, 4.5 mM KCl, 20.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 10.0 mM glucose, pH 7.4, 37°C) at a constant pressure of 100 cmH_2O. A thin-wall latex balloon was inserted into the left ventricle (LV) through the left atrium to continuously monitor the LV function, including heart rate (HR), left ventricular developed pressure (LVDP), maximum LVDP increase (+dp/dt) and decrease rate (−dp/dt), and with LV end-diastolic pressure (LVEDP) at 10mmHg (ADInstruments Pty Ltd., Bella Vista, NSW, AUS). After 30 minutes of baseline, 2 mL and 10mL of cold St. Thomas' solution (STS: 10 mEq/L Na^-, 20 mEq/L K^-, 20 mEq/L Cl^-, 277.8 mM glucose) without (STS group, n = 10) or with 0.5 gHb/dL PolyPHb (PolyPHb group, n = 10) were used to arrest and store the rat hearts, respectively. After 8 hours of 4°C storage, the hearts were reperfused with KHB for 2 hours. Coronary flow rate (CF) was calculated from the coronary effluent, sampling time, and cardiac wet weight. Figure 1 shows the schematics of the experimental protocol.

Cardiac Enzyme Release Measurement

The coronary effluents at the end of 30-minute baseline and 2-hour reperfusion were collected; then the release of cardiac enzyme, including creatine kinase (CK) and lactate dehydrogenase (LDH), were measured by Olympus

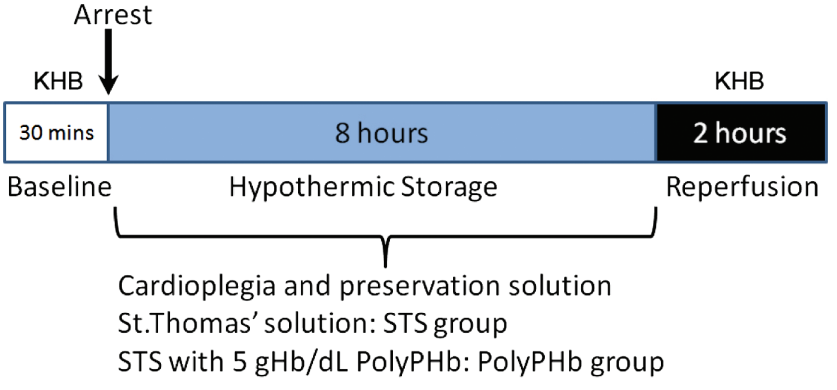

Figure 1. The schematics of the experimental protocol. After 30 minutes of baseline, hearts were arrested with and stored in STS without (STS group) and with 5 gHb/dL PolyPHb (PolyPHB group) at 4°C for 8 hours, then reperfused with KHB for 2 hours. STS: St. Thomas' solution, KHB: Krebs-Henseleit buffer.

AU5400 autoanalyser (Olympus Diagnostics, Melville, NY) within 2 hours.

Myocardial Infraction Size Determination

Myocardial infraction size in PolyPHb group and STS group hearts were estimated by 2,3,5-triphenyltetrazolium chloride (TTC) staining. Briefly, after reperfusion, the hearts were weighed, frozen, and cut into 2-mm-thick slices parallel to the atrioventricular groove. The slices were thawed and stained by incubation in 1% TTC solution in phosphate buffer (0.1M) at 37°C for 10 to 20 minutes, then fixed in 4% paraformaldehyde solution. After fixation, the myocardial infraction size was determined by Image-Pro Plus 4.5 software.

Statistical Analysis

All values in the text and figures were presented as mean ± SEM. The recovery of HR, LVDP, ±dp/dt and CF were analyzed by 2-factor ANOVA with repeated measures. The myocardial infarct size and the release of CK and LDH were analyzed by unpaired 2-tailed Student *t* test (SPSS 13.0 software). P values <0.05 were considered statistically significant.

RESULTS

Cardiac LV Function Recovery

In this study, no significant differences of LVDP, ±dp/dt, HR and CF were seen at baseline (data not shown). During reperfusion, there was no significant difference of the recovery of HR in 2 groups (Figure 2a); however, the recovery of LVDP in PolyPHb group after 2-hour reperfusion (57.9949 ± 2.10%) was significantly higher than that in STS group (21.44 ± 2.92%, $P<0.01$) (Figure 2b). The recovery of ±dp/dt was also greatly elevated in PolyPHb group (+dp/dt: 54.72 ± 5.95%, −dp/dt: 47.55 ± 4.31%)as compared with STS group (+dp/dt: 22.45 ± 6.23%, $P<0.01$; −dp/dt: 25.10 ± 5.06%, $P<0.01$) (Figure 2c and 2d). The similar result was observed when analyzing CF recovery; in the PolyPHb group, the recovery of CF (80.28 ± 7.85%) was better than that in the STS group (65.82 ± 7.91%), but did not have statistical significance (Figure 2e).

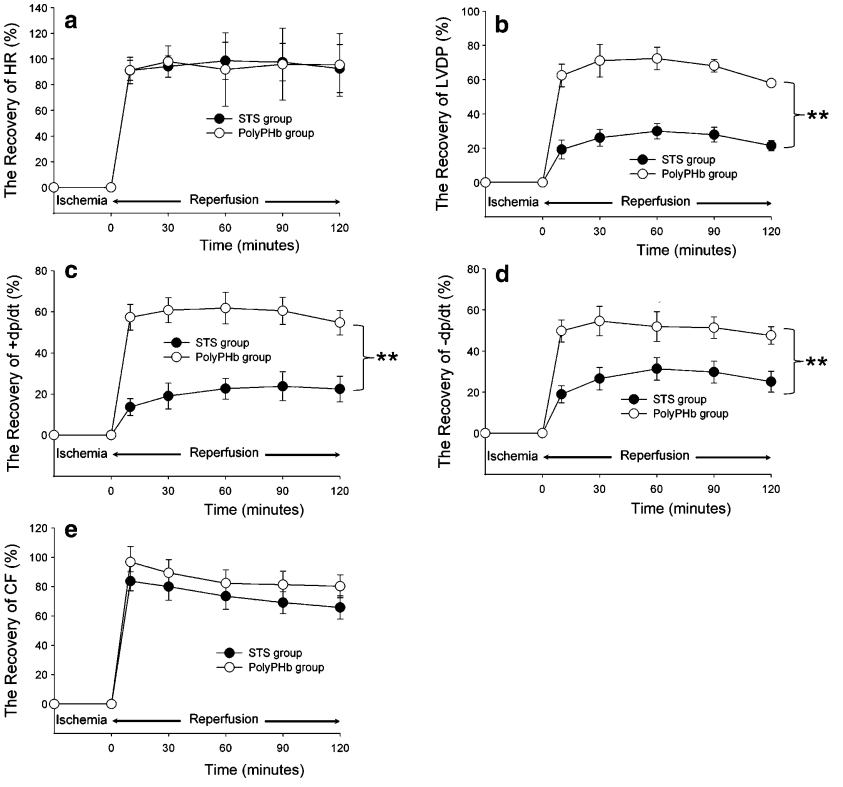

Figure 2. The recovery of HR (a), LVDP (b), ±dp/dt (c and d), and CF (e) during 2-hour reperfusion. Values were presented as mean ± SEM (n = 8 to 9). **P <0.01 vs STS group. HR: heart rate, LVDP: left ventricular development pressure, ±dp/dt: maximum LVDP increase and decrease rate, CF: coronary flow rate.

Cardiac Enzyme Release

At 30-minute baseline, the levels of CK and LDH release were low and showed no significant difference between 2 groups (data not shown). But after reperfusion, both of them were triggered to release at relatively high levels in the STS group (462.75 ± 72.92 IU/L/g wet heart for CK release and 278.75 ± 33.62 IU/L/g wet heart for LDH release), the burst of cardiac enzyme release were markedly attenuated in PolyPHb group (142.46 ± 41.8264 IU/L/g wet heart for CK release, $P < 0.05$; and 57.50 ± 16.02 IU/L/g wet heart for LDH release, $P < 0.05$ vs SC group) (Figure 3).

Myocardial Infraction

As shown in Figure 4, after 8-hour hypothermic storage and 2-hour normothermic reperfusion, the size of myocardial infraction in STS group hearts was 36.39 ± 3.77%, which was greatly reduced to 12.12 ± 1.61% in PolyPHb group hearts ($P < 0.01$ vs SC group).

DISCUSSION

HBOCs were initially developed as blood substitutes [1,3]. However, there are several problems associated with infusion of HBOCs, such as renal toxicity, coronary and cerebral vasospasm, gastrointestinal side effects, chest and abdominal pain [8,20], which have placed HBOCs in a disadvantageous situation in clinical trials. Therefore, scientists around the world started to investigate the alternative clinical uses of HBOCs. In recent years, some animal studies of HBOCs have indicated their potential cardioprotective effects in I/R hearts [1,2,5].

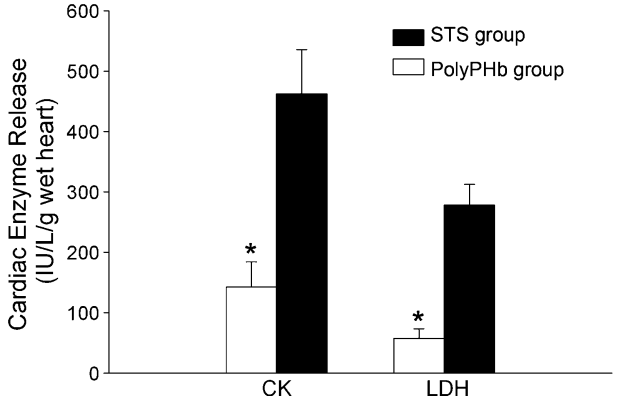

Figure 3. The total CK and LDH release of 2 group hearts after 2-hour reperfusion. Values were expressed as mean ± SEM (n = 8 to 9). *$P < 0.05$ vs STS group. CK: creatine kinase, LDH: lactate dehydrogenase.

In the present study, we added the PolyPHb into STS to assess its protective effect for isolated rat hearts after 8-hour hypothermic storage and 2-hour normothermic reperfusion. The results demonstrated that PolyPHb in STS greatly elevated the contractile performance of I/R heart as compared to STS group without PolyPHb. The cardiac enzyme release (CK and LDH) and myocardial infarction were significantly reduced in the PolyPHb group, which further proved the protective effect of PolyPHb. In fact, this protective effect might be exerted not only during hypothermic storage, but also in the period of cardiac arrest, for that STS with PolyPHb was also employed as the cardioplegic solution. Three reasons were taken into account. First, O_2-rich PolyPHb could preload the myocardium with O_2 during heart arrest. Second, in static cold storage it was hard to diffuse O_2 into cardiac tissue, thus PolyPHb might only provide limited protection. Last, blood cardioplegia was commonly adopted in cardiac surgery, and considerable evidence supported its superior myocardial protective effect as compared with crystalloid cardioplegia [6]. In this experiment, the choice of STS rather than University of Wisconsin (UW) solution, one of the best preservation solutions, was mainly because UW solution was in limited clinical use for heart transplantation [17] and we did not clearly understand the interaction between PolyPHb and the colloid component in UW solution.

HBOCs have been demonstrated to promote vasoconstriction and augment systemic hypertension due to scavenging of NO,but this effect can be reduced by intra- and intermolecular cross-linking and conjugation of the Hb molecule to prevent abluminal movement [15,18]. In

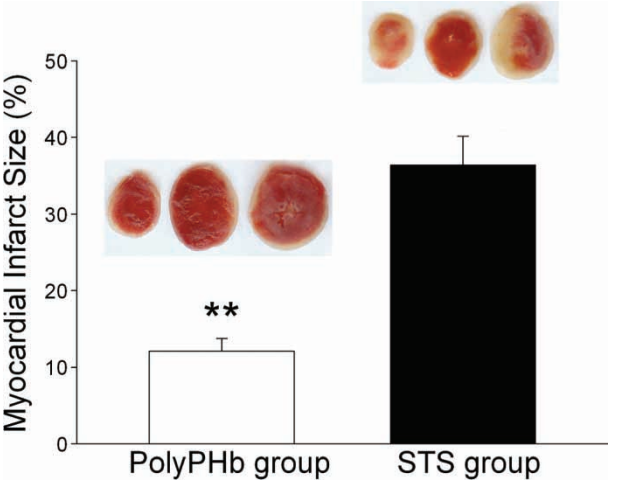

Figure 4. Myocardial infarct size determined by TTC staining. Red staining areas indicate viable tissue and non-stained pale areas indicate infarct tissue. Representative TTC-stained myocardial sections were shown at the top. Values were presented as mean ± SEM (n = 5, 5 to 6 slices per heart). **$P < 0.01$ vs STS group.

our study, PolyPHb was intra- and intermolecular cross-linked by PLP and GDA, respectively. Moreover, the concentration of PolyPHb (0.5 gHb/dL) in STS was considered high enough to preclude dimerization [3], thus further avoiding deterioration of the cardiovascular system.

In conclusion, the results of our study suggest that PolyPHb in STS is beneficial to the recovery of cardiac function and can greatly reduce the cardiac enzyme release and myocardial infarct size, thus providing a protective effect to isolated rat hearts against I/R injury. These findings may reveal the alternative clinical use of HBOCs in heart transplantation and other cardiac surgeries.

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