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Beneficial effects of novel cross-linked hemoglobin YQ23 on hemorrhagic shock in rats and pigs

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ABSTRACT

Background: To overcome the problems of previously reported hemoglobin-based oxygen carriers, we developed a stabilized nonpolymeric cross-linked tetrameric hemoglobin solution (YQ23). The aims of this study were to investigate the oxygen carrying and releasing properties of this novel hemoglobin-based oxygen carrier and to determine whether it has beneficial effects for hemorrhagic shock.

Methods: Using a hemorrhagic shock model in Sprague–Dawley rats and mini-pigs, we tested the effects of infusing 0.1, 0.3, and 0.5 g/kg YQ23 on animal survival, tissue oxygen delivery (DO_2) and consumption (VO_2), hemodynamics parameters, and liver, renal, and cardiac function.

Results: YQ23 infusion increased the survival rate of rats and pigs with severe hemorrhagic shock in a dose-dependent manner. Moreover, it improved the hemodynamic parameters, cardiac output, DO_2 and VO_2, and the mitochondrial respiratory function of vital organs. Among the three doses of YQ23, 0.5 gHb/kg YQ23 achieved a similar beneficial effect as whole blood.

Conclusions: This study indicated that the novel cross-linked tetrameric hemoglobin YQ23 has good oxygen carrying and releasing properties and exhibits beneficial effects on hemorrhagic shock in rats and pigs by improving the oxygen carrying and delivery function of blood, which maintains organ function.

Introduction

Traumatic hemorrhagic shock is a common clinical syndrome that is often observed in civilian and military situations and is a major cause of premature death for injured soldiers.[1] Effective resuscitation of shock is an essential prerequisite for follow-up therapy. The common resuscitation fluids for hemorrhagic and hypovolemic shock are volume expanders such as lactated ringer's solution and hydroxyethyl starch, which lacks oxygen carrying capacity or cell protection properties.[2-5] Therefore, it is important to develop resuscitation fluids with oxygen carrying and cell protective functions.

Packed red blood cells and whole blood have been touted as superior resuscitative agents in the treatment of hemorrhagic shock[6,7]; however, blood is not always available in wartime or emergency scenarios. Cross-matching blood types are also a

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major concern for these methodologies.[8] Modified hemoglobin is an ideal blood substitute because it does not require cross-matching, and there is minimal risk of bacterial or viral infections.[9,10] Therefore, many companies and laboratories have spent significant effort developing hemoglobin-based oxygen carriers (HBOCs).[11,12] Results from preclinical and clinical studies have showed that these HBOCs have beneficial effects for traumatic, hypovolemic, and hemorrhagic shock.[13,14]

Despite the advancements made in HBOCs in the last decade, there are several major deficiencies with the previously reported HBOCs. First, most are polymeric hemoglobin, which readily converts to methemoglobin. Methemoglobin does not bind oxygen and, therefore, cannot oxygenate tissues.[15] Second, previously reported HBOCs often contain an unacceptably high percentage of dimeric hemoglobin. The presence of dimeric hemoglobin can cause severe renal injury[16], making these HBOCs unsuitable for human use.

To overcome these problems, a novel bovine-derived, stabilized nonpolymeric cross-linked tetrameric hemoglobin solution, YQ23, was developed. The cross-linked tetrameric hemoglobin has an average molecular weight of 65 kDa with <5% dimeric and methemoglobin. The aim of this study was to determine whether YQ23 exhibited beneficial effects on hemorrhagic shock and organ protection.

Materials and methods

Preparation of cross-linked hemoglobin (YQ23)

YQ23 products were obtained from New B Innovation Limited. The YQ23 had undetectable or low levels of dimeric hemoglobin (32 kDa) and methemoglobin (4.8%), phospholipid, DNA impurities, and protein impurities. The concentration of YQ23 product was 6.2 g/dL, and its pH range was 7.2-7.8. The osmolality and viscosity (at 37°C) were >250 mOsm/kg and 0.9 cP, respectively. The p50 value was approximately 40 mm Hg. Other information regarding the YQ23 product is shown in patent no. US7,932,356 B1, US 8,048,856 B1, and PCT/US12/46130.

Animal preparation and management

This study was approved by the Research Council and Animal Care and Use Committee of the Research Institute of Surgery, Daping Hospital, Third Military Medical University (Chongqing, P.R. China). The protocol conformed to the guidelines of the ethical use of animals. Efforts were made to minimize animal suffering and to reduce the number of animals used.

The study included 248 Sprague–Dawley rats (weight: 240-280 g) and 30 mini-pigs (weight: 20-30 kg) of both sexes that were fasted for 12 h but allowed water *ad libitum* before experimentation. On the day of the experiment, rats were anesthetized with sodium pentobarbital (≤50 mg/kg, intraperitoneal injection). Pigs were first anesthetized with ketamine by intramuscular injection and then by sodium pentobarbital until they were unresponsive to a needle stimulus.

Catheters were inserted into the right femoral artery and femoral vein for blood pressure measurement, blood withdrawal, and for drug administration and resuscitation. After completing surgical procedures, rats and pigs were allowed to stabilize for 10 min (Baseline). The severe hemorrhagic model was established by 50% blood volume phlebotomy from the right femoral arterial catheter within 40 or 60 min (40 min for rats and 60 min for pigs).

After establishing the hemorrhagic shock model, rats and pigs were randomly divided into five groups that received the following treatments: lactated ringer's solution (LR), whole blood, or YQ23 0.1, 0.3, and 0.5 gHb/kg. The total volume of fluid infusion in each group was equivalent to two times the volume of whole blood removed. For the LR group, only LR equivalent to two times the volume of whole blood removed was administered; for whole blood group, one volume of LR plus one volume of whole blood were administered. LR was infused first, followed by whole blood; for the three YQ23 groups, LR equivalent to one volume of blood loss was given first, followed by administration of different doses of YQ23, and then LR was given to compensate to two times the volume of blood loss. The infusion rates in rats were 25 mL/h for LR, 20 mL/h for whole blood, and 5 mL/h for YQ23; the infusion rates in pigs were 600 mL/h for LR, 500 mL/h for whole blood, and 400 mL/h for YQ23. Hemodynamics, cardiac output (CO), oxygen delivery (DO_2), oxygen consumption (VO_2), tissue blood flow, and liver and renal function were observed during and after YQ23 infusion.

Parameters measurements

24-h survival in rats

Eighty rats were divided into five groups as described previously, with 16 rats in each group. Animals were observed for 24 h after model establishment, and at the end of observation, animals were sacrificed by cervical dislocation.

Hemodynamic parameters in rats

Forty rats were divided into five groups as described previously ($n = 8$ per group). Except for femoral artery and vein catheters, a third catheter was inserted into the left ventricle of the heart for hemodynamic monitoring via the right carotid artery. Mean arterial blood pressure (MAP) and hemodynamic parameters, including left intraventricular systolic pressure (LVSP) and maximal change rate of left intraventricular pressure ($\pm dp/dt_{max}$), were determined at baseline, the end of shock, 10 min, 30 min, 1 h, 2 h, and 3 h after YQ23 infusion with a polygraph physiological recorder (SP844; Power Laboratory, ADInstruments, Sydney, Australia).[17,18]

Cardiac function, and tissue DO_2 and VO_2 in rats

In this experiment, another 40 rats were divided into five groups ($n = 8$ per group). A thermodilution catheter was inserted into the left ventricle via the right carotid artery to measure CO and the right external jugular vein was catheterized for ice water injection.[19,20] Other instrumentation and YQ23 infusion were the same as in previous experiments. CO and heart rate (HR) were measured using a CO analyzer (Power Laboratory, ADInstruments) and cardiac index (CI) and stroke index (SI) were calculated at baseline, end of

shock, 10 min, 30 min, 1 h, 2 h, and 3 h after YQ23 infusion. Blood samples from the femoral artery and vein were collected for blood gas analysis (Phox plus L; NOVA Biomedical, Waltham, MA) at baseline, the end of shock, 30 min, 1 h, 2 h, and 3 h after YQ23 infusion. The CI and SI were calculated using the following equations: $S = K \times W^{2/3}$ and CI = CO/S, SI = CI/HR, where S is the body surface expressed in square centimeter, K is 9.1 and W is body weight expressed in grams. The DO_2 and VO_2 were calculated using the following equations: $DO_2 = CI \times 13.4 \times Hb \times SaO_2$ and $VO_2 = CI \times 13.4 \times Hb \times (SaO_2 - SvO_2)$, where Hb is hemoglobin, SaO_2 is arterial oxygen saturation, and SvO_2 is venous oxygen saturation.

Transcutaneous partial pressure of oxygen, tissue blood flow, and mitochondrial function in vital organs and liver and kidney function in rats

Eighty-eight rats were divided into five groups ($n = 8$ per group per time point as described in the following, all five groups share the same baseline). Animals were treated identically as described in "Animals Preparation and Management." Transcutaneous partial pressure of oxygen and the tissue blood flow of the liver, kidney, and brain were measured by a laser Doppler system (Periflux System 5000; Perimed, Stockholm, Sweden) at baseline, end of shock, 30 min, 1 h, 2 h, and 3 h after beginning YQ23 administration. After blood flow measurements at 1 h and 3 h, blood samples were collected to measure liver and kidney function including blood urea nitrogen (BUN), serum creatinine (SCR), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) using a biochemical analyzer (LX-20; Beckman Coulter Inc, Brea, CA). Rats were then sacrificed, and liver, kidney, and brain tissues were collected. Tissues were prepared as described previously for respiratory control rate (RCR) measurements using a mitochondrial function analyzer (Strathkelvin 782, North Lanarkshire, Scotland).[21-23]

Hemodynamics, cardiac function, and tissue DO_2 and VO_2 in pigs

Thirty pigs were divided into five groups ($n = 6$ per group). The establishment of the hemorrhagic shock model and fluid resuscitation regimes were described in the section "Animal Preparation and Management." In addition to these procedures, the right carotid artery was exposed and isolated, and a catheter was inserted into the left ventricle to measure hemodynamic parameters (HR, MAP, LVSP, $\pm dp/dt_{max}$) at baseline, end of shock, 30 min, 1 h, 2 h, and 3 h after beginning YQ23 administration. A Swan Ganz catheter was inserted into the right atrium to measure CO at the same time points. Blood samples were collected from the femoral artery and vein to measure the blood gas indices (SaO_2, SvO_2, and Hb) with the blood gas analyzer (ABL 800 FLEX; Radiometer, Copenhagen, Denmark) to calculate the DO_2 and VO_2. The CI, SI, DO_2, and VO_2 were determined as described in previous sections on rats, except K was 9.0. On completion of measurements, all catheters were removed, and the incisions were closed. Animals were observed for 24 h after YQ23 administration. At the end of observation, animals were euthanatized through bolus injection of 10% KCl at 0.3-0.5 mL/kg.

Statistical analyses

Survival was analyzed by Kaplan–Meier survival analyses and the log-rank test. Data (blood loss, MAP, LVSP, $\pm dp/dt$ max, CO, CI, SI, tissue blood flow, and RCR) were presented as the mean ± standard deviation (SD) of n observations. The statistical differences of the data among groups were analyzed by two factor analyses, followed by the *post-hoc* Tukey test (SPSS v15.0; SPSS Inc, Chicago, IL) for multiple comparisons between two groups. $P < 0.05$ was considered significant.

Results

Beneficial effects of YQ23 on hemorrhagic shock in rats

Overall survival and survival rates

The 24-h survival rates in the 0.1, 0.3, and 0.5 gHb/kg YQ23 groups were 37.5%, 50%, and 75%, respectively, which were significantly higher than the LR group (18.8%). Among the three groups of animals receiving YQ23, the 24-h survival rate in 0.5 gHb/kg YQ23 group was also higher than the whole blood group (62.5%). YQ23 given at the doses of 0.1, 0.3, and 0.5 gHb/kg all prolonged the survival time of hemorrhagic shock rats, and the effect of 0.5 gHb/kg YQ23 on overall survival was similar to that of whole blood (Fig. 1A and B).

Changes in hemodynamic parameters

After hemorrhagic shock, the hemodynamic parameters of rats including MAP, LVSP, and $+dp/dt_{max}$ decreased significantly compared with baseline. After resuscitation with LR, whole blood, or YQ23, MAP, LVSP, and $\pm dp/dt_{max}$ recovered. The improvement of these parameters in the 0.3 and 0.5 gHb/kg YQ23 groups was significantly better than in the LR group. The extent to which 0.5 gHb/kg YQ23 improved hemodynamic parameters was comparable or superior to that of whole blood. It was reported that in the hemorrhagic shock model, resuscitation with other HBOCs caused a 30%-40% increase in blood pressure because of their vasoconstriction effects.[24] After infusing YQ23, MAP only increased to a level similar to normal blood pressure even at the highest dosage (0.5 gHb/kg). These results suggested that YQ23 did not cause obvious vasoconstriction in animals (Fig. 1C-F).

Changes in cardiac function parameters, tissue DO_2 and VO_2, and blood gases

Cardiac function

After hemorrhagic shock, the CO, CI, and SI decreased significantly. After administration of LR, whole blood, or YQ23, these parameters showed obvious recovery. The improvement of these parameters after resuscitation with 0.5 gHb/kg YQ23 was significantly better than those in animals treated with LR alone (Fig. 2A-C).

Tissue DO_2 and VO_2

Tissue DO_2 and VO_2 in rats decreased significantly after hemorrhagic shock. The DO_2 and VO_2 after infusion with 0.1 or

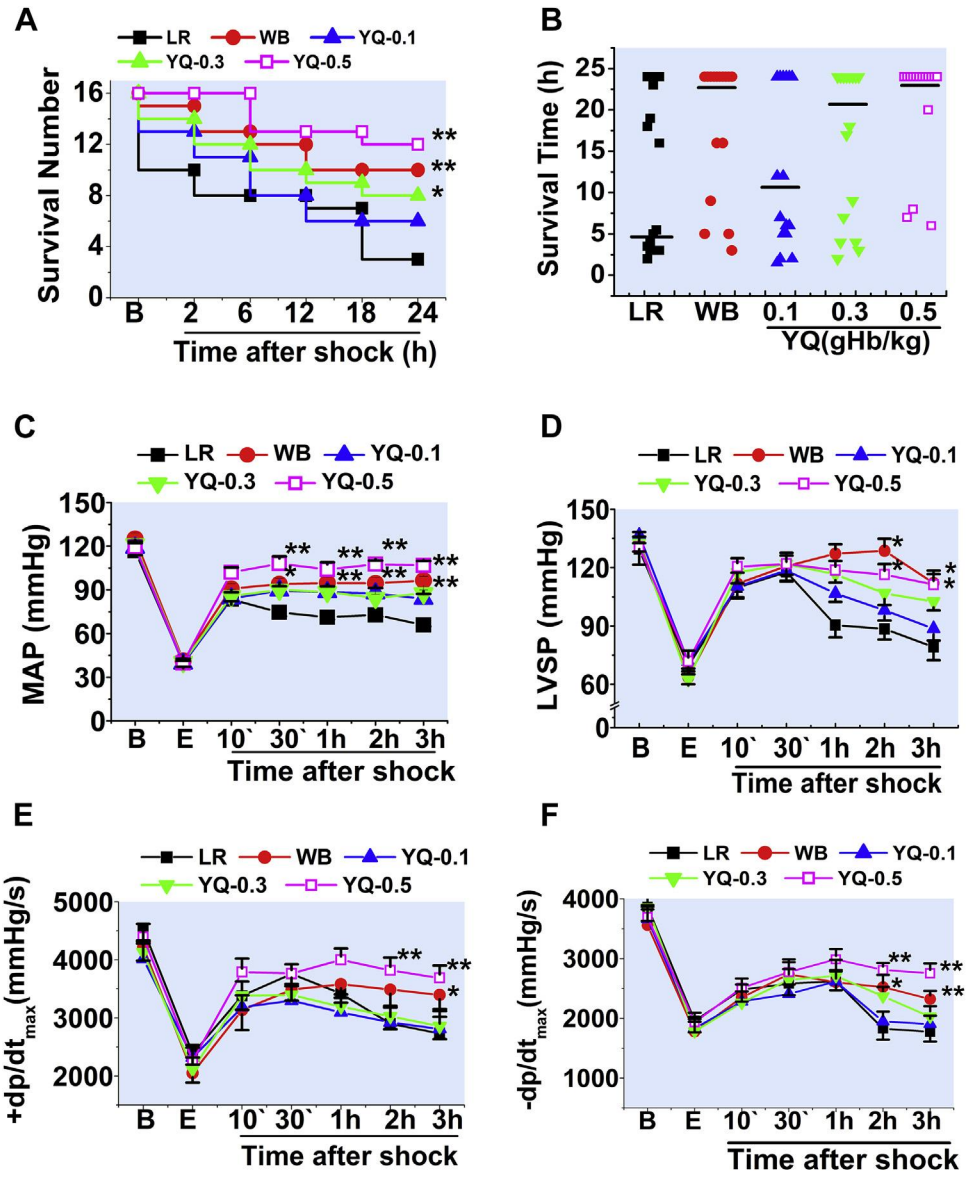

Fig. 1 – Effects of YQ23 on the overall survival, survival time, and hemodynamic parameters after hemorrhagic shock in rats. (A) Number of surviving animals at different time points after YQ23 administration ($n = 16$ per group). (B) Survival time ($n = 16$ per group). (C) MAP ($n = 8$ per group). (D) LVSP ($n = 8$ per group). (E, F) The maximum rate of left ventricular pressure (dp/dt_{max}; $n = 8$ per group). B = baseline; E = end of shock; WB = whole blood. YQ23 0.1, 0.3, and 0.5: YQ23 0.1, 0.3, and 0.5 gHb/kg, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the LR group. (Color version of figure is available online.)

0.3 gHb/kg YQ23 were higher than animals treated with LR; the DO_2 in the 0.5 gHb/kg YQ23 group was similar to the whole blood group and the VO_2 in the 0.5 gHb/kg YQ23 group was lower than in the whole blood group (Fig. 2D and E). The total peripheral resistances in the different groups were similar (Fig. 2F).

Blood gases

SaO_2 increased significantly at 1 h and 2 h after YQ23 infusion, and the extent of increase was superior to LR and comparable with whole blood infusion. YQ23, whole blood, and LR had no obvious effects on blood carbon dioxide pressure, blood oxygen pressure or blood pH, and there were no differences among groups (Table 1).

Changes in blood flow and mitochondrial function in vital organs, transcutaneous partial pressure of oxygen, hemorheology, and liver and kidney function in rats

Blood flow to liver, kidneys, and brain and mitochondrial function

Administration of YQ23 at 0.3 and 0.5 g/kg significantly increased blood flow to the liver, kidneys, and brain in rats under hemorrhagic shock compared with LR infusion. The improvement in blood flow to these organs after YQ23 treatment (0.3 and 0.5 gHb/kg) was similar to whole blood, whereas infusion of 0.5 gHb/kg YQ23 showed better effects on liver blood flow compared with whole blood (Fig. 3A-C). RCR is an indicative of mitochondrial function and reflects the

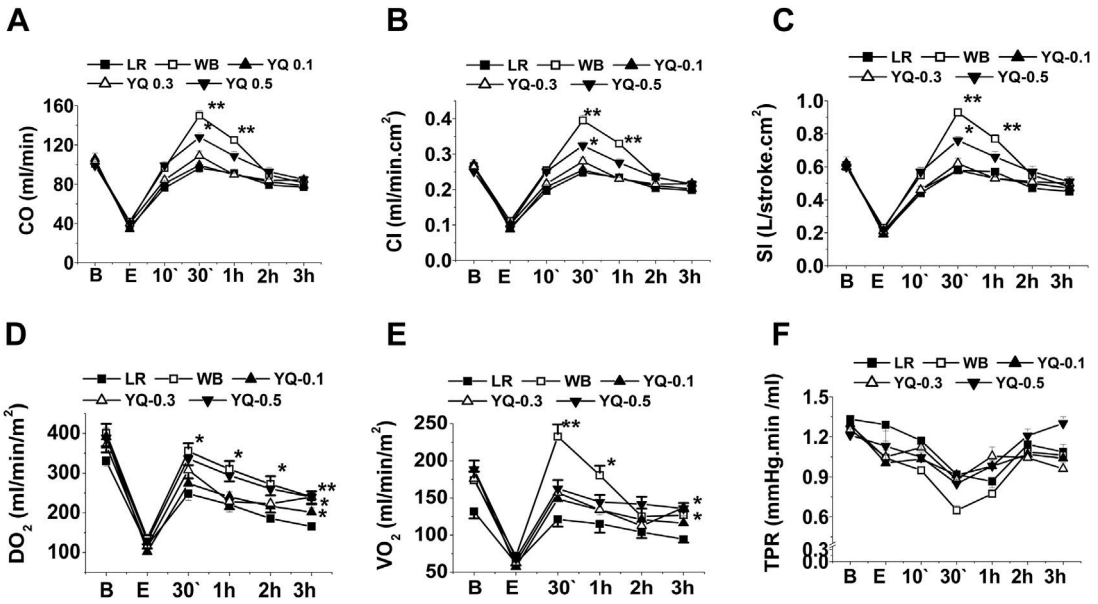

Fig. 2 – Effects of YQ23 on cardiac function, oxygen delivery, and consumption and vascular resistance after hemorrhagic shock in rats. Data represent the mean ± SD of eight observations. (A) CO. (B) CI. (C) SI. (D) DO_2. (E) VO_2. (F) Total peripheral resistance. B = baseline; E = end of shock; WB = whole blood.YQ23 0.1, 0.3, and 0.5: YQ23 0.1, 0.3, and 0.5 gHb/kg, respectively. *P < 0.05, **P < 0.01, compared with the LR group.

consumed oxygen rate of mitochondria with and without adenosine diphosphate. The RCR of liver, kidney, and brain of rats treated with 0.3 g/kg and 0.5 g/kg YQ23 was higher than the LR group. The improvement in kidney RCR induced by 0.3 and 0.5 gHb/kg YQ23 and liver and brain RCR induced by 0.5 gHb/kg YQ23 were comparable with that of whole blood (Fig. 3D-F).

Transcutaneous partial pressure of oxygen

The transcutaneous partial pressure of oxygen ($PtcO_2$) decreased significantly after establishing the hemorrhagic shock model. Infusion of YQ23 at all three doses increased the $PtcO_2$ quickly and maintained the $PtcO_2$ at a higher level than in the LR group. $PtcO_2$ after the infusion of 0.3 and 0.5 gHb/kg YQ23 was significantly higher than infusion of LR and were comparable to the infusion of whole blood (Fig. 4A).

Liver and kidney function

YQ23 infusion at doses of 0.1, 0.3, or 0.5 gHb/kg led to changes in AST and ALT levels. Similarly, YQ23 infusion altered the levels of BUN and SCR. The levels of BUN and SCR in the YQ23 groups were lower than or equal to those in the LR group. Taken together, these data suggest that YQ23 treatment resulted in less liver and kidney damage compared with LR treatment (Fig. 4B-E).

Beneficial effects of YQ23 on hemorrhagic shock in pigs

Survival rate and survival time

The 24-h survival rates in the LR, 0.1, 0.3, and 0.5 gHb/kg YQ23 and whole blood groups were 33.3%, 50%, 66.7%, 83.3%, and 100%, respectively (Fig. 5A). The survival time in all YQ23 groups was higher than the LR group. The survival time in the YQ23 0.5 gHb/kg group was similar to the whole blood group (Fig. 5B).

Hemodynamic parameters

MAP decreased significantly after hemorrhagic shock, and MAP recovery was observed after infusing LR, whole blood, or YQ23. The MAP increase after infusing 0.5 gHb/kg YQ23 was higher than with whole blood (Fig. 5C). Infusion of 0.3, 0.5 gHb/kg YQ23, or whole blood caused a significant increase in LVSP and $\pm dp/dt_{max}$. LVSP and $\pm dp/dt_{max}$ in the 0.3 and 0.5 gHb/kg YQ23 groups at 3 h after infusion were significantly higher than in the LR group, and the results were similar to those in the whole blood group. LVSP in the 0.3 and 0.5 gHb/kg YQ23 groups was higher than in the LR group, but there was no significant difference between $\pm$ dp/dt_{max} in the 0.1 gHb/kg YQ23 group (Fig. 5D-F).

Cardiac functions, tissue oxygen delivery, and consumption

Infusion of YQ23 at 0.3 or 0.5 gHb/kg resulted in a significant increase in CO, CI, and SI compared with LR treatment. The effects on these parameters after 0.5 gHb/kg YQ23 administration were similar to the whole blood group (Fig. 6A-C). Infusion of YQ23 at 0.3 and 0.5 gHb/kg led to a significant increase in DO_2 and VO_2 compared with LR infusion. Among the three doses of YQ23, 0.5 gHb/kg showed the most significant effect; the VO_2 of this group was similar to that of the whole blood group (Fig. 6D and E). The total peripheral resistances in different groups were similar (Fig. 6F).

Discussion

Blood substitutes are essential for emergency care of critical conditions, especially for combat fields and disaster rescues.

Table 1 – Effects of YQ23 on blood gases after hemorrhagic shock in rats.

Group	Baseline	End of shock	The time of administration			
			30 min	1 h	2 h	3 h
SaO_2 (%)						
LR	88.4 ± 9.0	79.9 ± 9.1	84.7 ± 12.7	79.6 ± 10.8	78.7 ± 11.3	79.9 ± 6.8
WB	94.2 ± 5.7	86.7 ± 11.4	89.9 ± 8.9	85.0 ± 10.1	90.2 ± 8.8*	90.8 ± 6.5**
YQ23 0.1 g	95.4 ± 2.2	88.1 ± 7.1	89.8 ± 9.6	88.0 ± 12.5	88.2 ± 11.5	89.3 ± 12.7
YQ23 0.3 g	96.7 ± 5.8	87.5 ± 8.4	89.6 ± 10.9	84.6 ± 12.1	85.9 ± 11.0	91.0 ± 10.5*
YQ23 0.5 g	94.8 ± 6.3	85.0 ± 8.6	85.2 ± 10.6	89.4 ± 9.2	89.8 ± 7.0*	94.0 ± 3.5**
PO_2 (mm Hg)						
LR	104.6 ± 9.0	119.6 ± 8.7	106.7 ± 12.9	99.8 ± 9.5	97.5 ± 21.2	99.0 ± 11.8
WB	109.0 ± 12.6	112.8 ± 9.0	103.5 ± 16.5	90.2 ± 10.6	89.6 ± 10.5	93.5 ± 20.8
YQ23 0.1 g	103.9 ± 13.3	120.2 ± 18.0	109.5 ± 10.7	97.3 ± 14.5	99.5 ± 19.3	106.2 ± 18.2
YQ23 0.3 g	95.8 ± 14.4	109.9 ± 11.8	91.4 ± 21.9	93.6 ± 17.3	92.4 ± 12.5	101.2 ± 15.0
YQ23 0.5 g	105.3 ± 9.9	114.3 ± 15.5	90.1 ± 8.8	92.4 ± 11.0	96.2 ± 13.0	99.9 ± 8.4
PCO_2 (mm Hg)						
LR	41.2 ± 6.5	36.0 ± 6.4	39.8 ± 4.5	40.8 ± 4.8	39.9 ± 5.8	33.1 ± 7.0
WB	48.4 ± 5.7	40.8 ± 2.0	47.0 ± 3.7	41.8 ± 3.6	35.4 ± 4.1	31.2 ± 4.8
YQ23 0.1 g	45.3 ± 7.6	38.6 ± 5.2	42.6 ± 6.8	39.8 ± 4.1	38.1 ± 7.6	34.4 ± 9.2
YQ23 0.3 g	45.1 ± 4.3	40.5 ± 6.5	45.2 ± 7.7	41.6 ± 6.2	37.3 ± 7.8	33.9 ± 5.5
YQ23 0.5 g	42.5 ± 4.2	37.9 ± 8.7	42.5 ± 5.6	35.0 ± 4.8	34.7 ± 3.2	31.5 ± 4.3
pH						
LR	7.373 ± 0.043	7.379 ± 0.036	7.366 ± 0.030	7.373 ± 0.038	7.390 ± 0.050	7.456 ± 0.057
WB	7.325 ± 0.066	7.357 ± 0.034	7.346 ± 0.040	7.372 ± 0.034	7.394 ± 0.062	7.440 ± 0.075
YQ23 0.1 g	7.342 ± 0.044	7.330 ± 0.065	7.344 ± 0.048	7.339 ± 0.101	7.383 ± 0.068	7.326 ± 0.167
YQ23 0.3 g	7.368 ± 0.051	7.372 ± 0.048	7.401 ± 0.049	7.409 ± 0.057	7.422 ± 0.072	7.445 ± 0.107
YQ23 0.5 g	7.401 ± 0.048	7.398 ± 0.070	7.424 ± 0.069	7.479 ± 0.054	7.468 ± 0.052	7.469 ± 0.082

PCO_2 = blood carbon dioxide pressure; PO_2 = blood oxygen pressure; WB = whole blood.
Data represent the mean ± SD of eight observations.
YQ23 0.1, 0.3, and 0.5: YQ23 0.1, 0.3, and 0.5 g Hb/kg. *$P < 0.05$, **$P < 0.01$, compared with LR group.

HBOCs are the ideal blood substitute, and previous studies have shown that HBOCs have beneficial effects for traumatic shock[24-26]. Philbin *et al.* found that bovine polymerized hemoglobin (HBOC-201) improved the 72-h survival rate (100%) in pigs with a moderately severe hemorrhagic shock compared with 6% hetastarch (88%); in a rabbit acute hemorrhagic shock model, infusion of hemoglobin vesicles resulted in superior resuscitation effects over controls, which included increased MAP and CI, maintenance of high levels of oxygen consumption, and reduced blood glucose levels, without disturbing the micro-oxygenation in the brain, kidneys, liver, and muscles.[27] Despite the favorable resuscitation effects of the previously reported HBOC, high percentages of methemoglobin, renal toxicity, and vasoconstriction responses remain major safety concerns for these products.

In this study, we investigated a novel bovine-derived, stabilized nonpolymeric cross-linked tetrameric hemoglobin, YQ23, which contains lower levels of dimeric hemoglobin and protein impurities. Using two different species for animal models of hemorrhagic shock, we investigated the effects of YQ23 on animal survival, hemodynamic parameters, cardiac function, oxygen delivery and consumption, and organ function. In contrast to previous blood substitute studies on severe hemorrhage, which aimed to replace hemoglobin in a gram-to-gram basis, a tremendously lower dose of YQ23 (0.1-0.5 gHb/kg) was administered to rats and pigs in this study. Our results demonstrated that infusion of 0.1, 0.3, or 0.5 gHb/kg YQ23 improved the overall survival of rats and pigs with severe hemorrhagic shock in a dose-dependent manner (Figs. 1A and B, 5A and B), improved cardiovascular function and the DO_2 and VO_2 (Fig. 2, Table 1) and other organ functions. Among the three doses of YQ23, 0.5 gHb/kg exhibited the best effects, with many parameters appearing similar to the whole blood group. Moreover, no hypernomic increases in MAP were observed in rats or pigs with hemorrhagic shock after YQ23 treatment (Figs. 1C and 6C). These findings suggest that YQ23 has good oxygen carrying and releasing properties, with no obvious *in vivo* vasoconstriction side effects or liver and renal toxicities.

HBOCs exert their resuscitation functions by carrying and delivering oxygen to tissues under hemorrhagic and hypovolemic shock. However, most of the reported HBOCs are polymeric hemoglobin, and thus, are readily converted to methemoglobin which does not deliver oxygen effectively.[28,29] In this study, the methemoglobin level of YQ23 was maintained at a low level (5%). With such a low percentage of

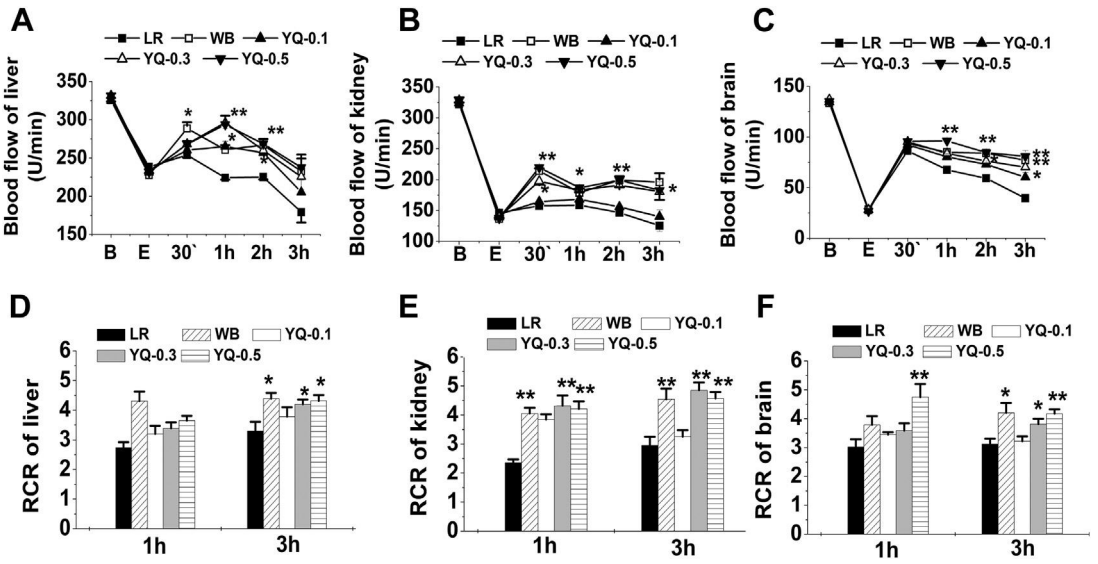

Fig. 3 – Effects of YQ23 on blood flow and mitochondrial function of the liver, kidneys, and brain after hemorrhagic shock in rats. Data represent the mean ± SD of eight observations. (A) Liver blood flow. (B) Kidney blood flow. (C) Brain blood flow. (D) Liver RCR. (E) Kidney RCR. (F) Brain RCR. B = baseline; E = end of shock; WB = whole blood. YQ23 0.1, 0.3, and 0.5: YQ23 0.1, 0.3, and 0.5 gHb/kg, respectively. *P < 0.05, **P < 0.01, compared with the LR group.

methemoglobin, YQ23 infusion to rats and pigs with hemorrhagic shock efficiently restored blood SaO_2, arterial blood oxygen pressure, and tissue DO_2 and VO_2, which reduced damage to vital organs. YQ23 infusion could enhance the oxygen carrying and releasing function of blood and subsequently improve the hemodynamic and cardiac functions of animals with hemorrhagic shock. The incidence of acute kidney injury has been reported in trials using previous generations of HBOCs with an unstable molecular weight and higher percentages of dimers.[30] YQ23 infusion in our study did not result in increased BUN or SCR compared with LR, suggesting that YQ23 did not cause further kidney damage, which may be attributable to the stable structure and low dimer percentage of YQ23.

Previous studies have shown that HBOCs have vasoconstriction effects. Our study found that while YQ23 significantly increased MAP, the total peripheral resistance was not significantly increased, suggesting that YQ23 does not possess or has lower vasoconstriction effects. The increase in MAP by YQ23 may come from improvements in other organ function via DO_2 and VO_2 improvements, not vasoconstriction.

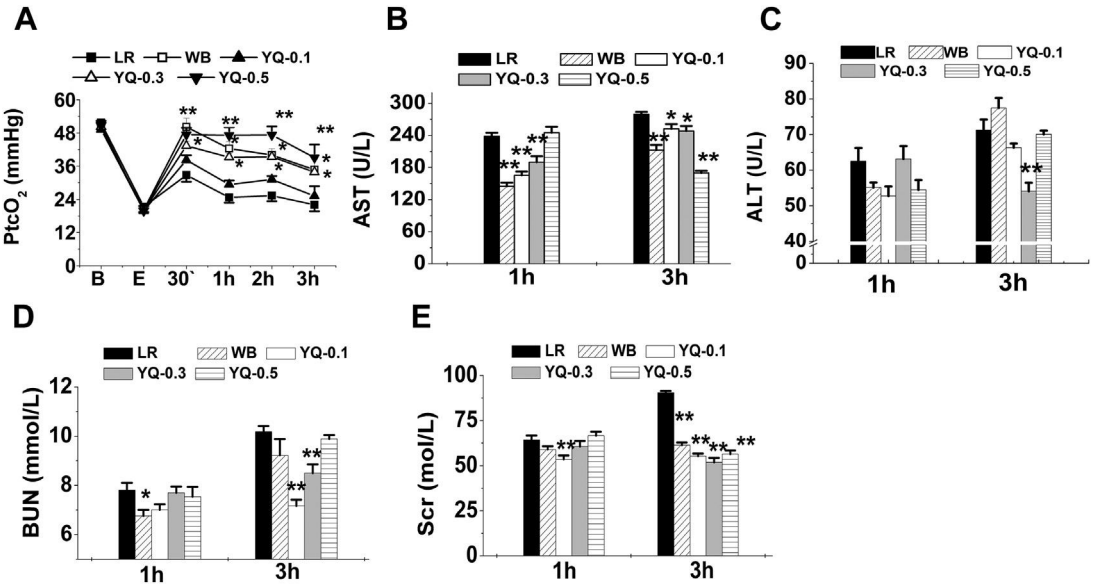

Fig. 4 – Effects of YQ23 infusion on $PtcO_2$, liver, and kidney damage in rats under hemorrhagic shock. Data represent the mean ± SD of eight observations. (A) $PtcO_2$. (B) AST. (C) ALT. (D) Blood urea nitrogen. (E) SCR. WB = whole blood. YQ23 0.1, 0.3, and 0.5: YQ23 0.1, 0.3, and 0.5 gHb/kg, respectively. *P < 0.05, **P < 0.01, compared with the LR group.

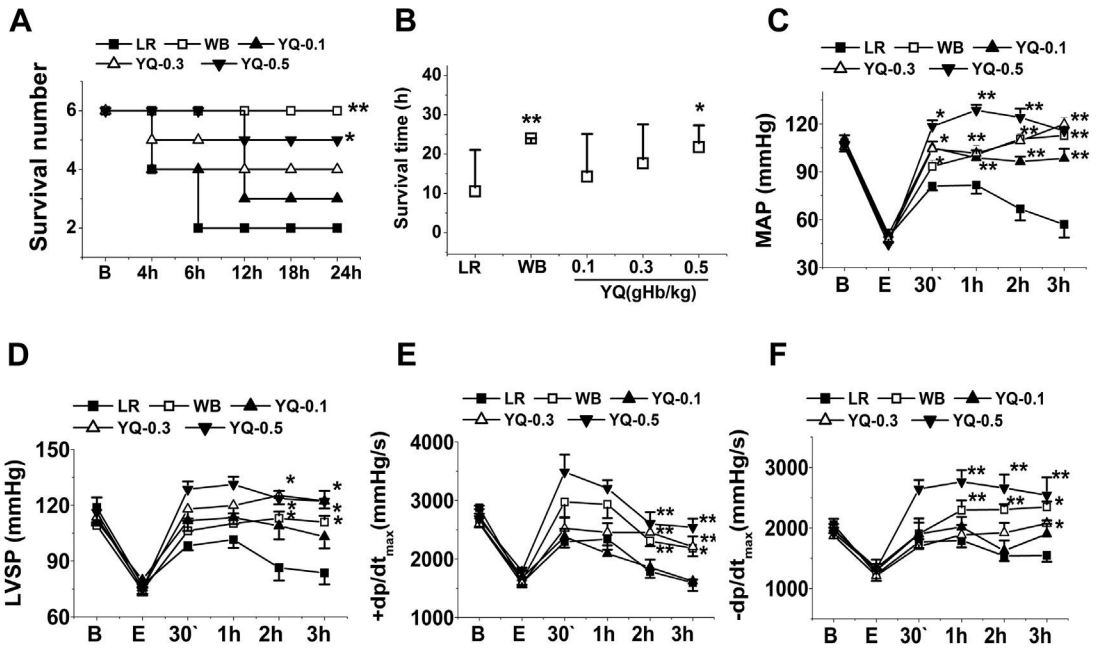

Fig. 5 – Effects of YQ23 on the overall survival, survival time, and hemodynamic parameters after hemorrhagic shock in pigs. Data represent the mean ± SD of six observations. (A) Number of surviving animals at different time points after YQ23 administration. (B) Survival time. (C) MAP. (D) LVSP. (E, F) The maximum rate of left ventricular pressure (dp/dt_{max}). B = baseline; E = end of shock; WB = whole blood. YQ23 0.1, 0.3 and 0.5: YQ23 0.1, 0.3 and 0.5 gHb/kg, respectively. *P < 0.05, **P < 0.01, compared with the LR group.

This study clearly demonstrated the beneficial effects of YQ23 on hemorrhagic shock without any of the adverse effects observed in previously reported HBOCs. Although these findings support YQ23 being a promising HBOC, there are a few limitations to this study. First, whether YQ23 has any effect on coagulation is unclear as heparin was used as an anticoagulant in the animal studies. Second, the effects of YQ23 on other vital organs, except heart, liver, and kidney,

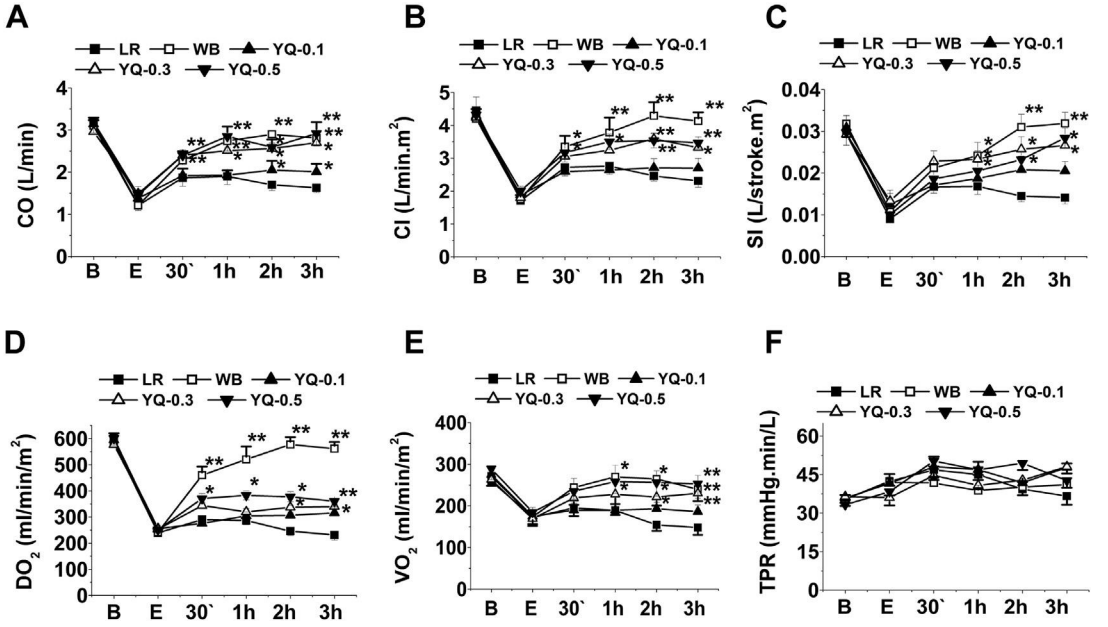

Fig. 6 – Effects of YQ23 on cardiac function, DO_2 and VO_2 after hemorrhagic shock in pigs. Data represent the mean ± SD of six observations. (A) CO. (B) CI. (C) SI. (D) DO_2. (E) VO_2. (F) Total peripheral resistance. B = baseline; E = end of shock; WB = whole blood. YQ23 0.1, 0.3, and 0.5: YQ23 0.1, 0.3, and 0.5 gHb/kg, respectively. *P < 0.05, **P < 0.01, compared with the LR group.

were not studied. Third, although no immediate infusion-related adverse effects were observed in animals during the study, the immunotoxicity and immunogenicity in animals will need further investigation to establish a YQ23 safety profile. Finally, the acute observation time after YQ23 infusion in this study was only 3 h; therefore, the change of some parameters may not have fully emerged during the observation time. The long-term effects of YQ23 will be examined in future work.

Conclusion

In this study, we reported a novel stabilized, nonpolymeric cross-linked tetrameric hemoglobin, YQ23, to be a promising treatment for hemorrhagic shock. Animal studies showed that YQ23 has good oxygen carrying and delivery properties, with no obvious vasoconstriction activity or liver or renal toxicities. YQ23 improved the oxygen carrying and releasing function of blood, as well as the hemodynamic and cardiac functions in rats and pigs under hemorrhagic shock.

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Authors' contributions: L.T., Y.G.M., and Z.Y. carried out the experiments, collected and analyzed the data, and prepared the article. T.F.C., L.S.H., and K.S.Y. prepared hemoglobin-based oxygen carrier samples. L.T., L.L.M., and W.B.L. conceived the study, participated in design and coordination, and edited the article. All authors read and approved the final version of the article.

Disclosures

There is no financial conflict of interest for this study.

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