

Preoperative autologous blood donation using the leap-frog technique for cardiopulmonary bypass surgery in a swine model[★]

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Abstract

BACKGROUND: Allogeneic blood transfusion in humans of the same blood type has been implemented, but studies regarding swine blood type and how to perform allogeneic blood transfusion have been rarely reported.

OBJECTIVE: To investigate the feasibility of preoperative autologous blood donation using the leap-frog technique for cardiopulmonary bypass surgery in a swine model.

METHODS: Sixteen domestic swine were randomly divided into two groups: autologous blood donation and allogeneic blood transfusion. Another four swine were used as blood donors. Two groups of swine underwent cardiopulmonary bypass surgery. The autologous blood donation group received self-transfusion reserved before surgery while the allogeneic blood transfusion group received the same amount of allogeneic blood. Hemoglobin (Hb) concentration and hematocrit (Hct) level prior to and after donation in the autologous blood donation group was recorded. Hb concentration in the two groups was recorded prior to, during, immediately after, and 1 day after the surgery.

RESULTS AND CONCLUSION: The total blood volume of each experimental swine was (2500±428) mL. For the autologous blood donation group, the predicted blood volume of the first donation was (501±86) mL and the actual blood volume was (493±93) mL; in the second donation, the predicted blood volume was (750±128) mL and the actual blood volume was (719±98) mL. There was a significant difference in Hb concentration and Hct level between prior to and after donation in the autologous blood donation group ($P < 0.01$). Hb concentration at 1 day after the surgery was significantly higher in the autologous blood donation group than in the allogeneic blood transfusion group ($P < 0.01$), while no significant difference in Hb concentration existed between these two groups prior to, during, and immediately after the surgery ($P > 0.05$). Compared with prior to surgery, Hb concentration in each group was significantly lower at 1 day after the surgery ($P < 0.01$). The autologous blood donation group exhibited significantly higher survival rate of swine than the allogeneic blood transfusion group ($P < 0.01$). These findings indicate that compared with allogeneic blood transfusion, preoperative autologous blood donation using the leap-frog technique appears to be a safe, effective method with a high survival rate for cardiopulmonary bypass surgery.

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INTRODUCTION

When actual blood loss does not exceed 20% of predicted blood volume, the blood lost is compensated by peripheral vaso-constriction and interstitial fluid entry into the vessels, and arterial pressure is kept within normal range. When acute blood loss exceeds 30% of the systematic blood volume, it is difficult to compensate by the organism, with possible physical signs including hypotension and hypoperfusion^[1]. Swine are often used for cardiac surgery experiment for their similarities with humans in terms of heart anatomical structure, cardiovascular distribution and heart to body weight ratio^[2]. Evidence exists that cardiac surgery is one of the operations in which a large number of red cells are transfused^[3-4]. Allogeneic blood transfusion in humans of the same blood type has been implemented, but studies regarding swine blood type and how to perform allogeneic blood transfusion have been rarely reported. Much blood loss in animal experiments is a primary cause of high mortality rate. The present study compared preoperative autologous blood donation using the leap-frog technique with allogeneic blood transfusion and investigated the feasibility of preoperative autologous blood donation using the leap-frog technique to search for a practical method that can further enhance swine survival rate after complicated surgery.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

This study was performed at the Laboratory Animal Center, Affiliated Hospital of Medical College, Qingdao University, between May and September 2009.

Materials

Twenty domestic swine of either gender, aged 3-4 months, weighing 27-50 kg, were included in this study. Sixteen swine underwent cardiopulmonary bypass surgery and then were randomly divided into two groups with eight swine in each group: autologous blood donation and allogeneic blood transfusion. The remaining four swine were used as blood donors.

Methods

In the autologous blood donation group, autologous blood donation was performed at 2 and 9 days prior to surgery respectively, while in the allogeneic blood transfusion group, allogeneic blood was taken from the other four swine for many times according to surgical progression. Before blood donation, swine were fasted for 8 hours but allowed free access to water. On the day of blood donation, following general anesthesia, swine were intramuscularly administered

12 mg/kg ketamine, 10 mg midazolam, and 0.3 mg scopolamine. Subsequently, the swine were laid in a lateral position, a mixture of ketamine and midazolam (10 mg ketamine and 0.5 mg midazolam in 1 mL mixture) was persistently pumped *via* the veins on the ear edge. Blood donation was performed after approximately 300 mL lactated Ringer's solution was perfused. Prior to puncturation of the saphenous and femoral artery, 50 µg fentanyl was slowly injected *via* the veins. The swine systemic blood volume was 65–70 mL/kg. At 9 days prior to surgery, the first blood donation was performed by taking 20% systemic blood. Hb concentration and Hct level prior to and after blood collection were determined. Autologous blood was collected and stored in a bag containing anticoagulant citrate dextrose solution at 4 °C. After blood collection, four eggs were daily given to each swine in addition to more food. At 2 days after surgery, the blood collected during the first donation was transfused back into the swine and the second blood donation (also 30% of the systematic blood volume) was performed and preserved at 4 °C. After the second blood collection, four eggs per day were continuously given to each swine. During each blood collection, swine vital signs were monitored to prevent hypoxia. One or two swine used for allogeneic blood transfusion were selected for collection of 800–100 mL blood per swine according to swine body mass. The experimental procedures including blood collection and preservation were the same as the autologous blood donation group. Swine blood donated was all transfused back during or after the surgery.

Main outcome measures

Hb concentration and Hct level prior to and after donation in the autologous blood donation group was recorded. Hb concentration in the two groups was recorded prior to, during, immediately after, and 1 day after the surgery.

Design, enforcement and evaluation

This study was designed by Yuan Li and Xu Ping, performed by Yuan Li, Ge Nan, Zheng Xin and Yang Lin-shan, and evaluated by Wang Shi-duan.

Statistical analysis

Statistical analysis was performed by Yang Lin-shan and the measurement data were expressed as Mean±SD. Paired *t* test was used for index comparison between groups at the same time point, as well as for index comparison between prior to surgery and immediately after donation in the autologous blood donation group. A level of *P* < 0.01 was considered statistically significant.

RESULTS

Quantitative analysis of experimental animals

Among 16 swine undergoing cardiopulmonary bypass surgery, four died within 5 hours after the surgery (one from the autologous blood donation group and three from the allogeneic blood transfusion group), and 12 swine from two groups were included in the final analysis at 1 day after the surgery.

Hb concentrations during the cardiac bypass surgery

The total blood volume of each experimental swine was (2500±428) mL. For the autologous blood donation group, the

predicted blood volume of the first donation was (501±86) mL and the actual blood volume was (493±93) mL; in the second donation, the predicted blood volume was (750±128) mL and the actual blood volume was (719±98) mL. There was significant difference in Hb concentration and Hct level between prior to and after donation in the autologous blood donation group (*P* < 0.01) (Table 1).

Table 1 Hemoglobin concentration and hematocrit level prior to and after donation in the autologous blood donation group (x±s, n=7)

Detection time	Hemoglobin concentration (g/L)	Hematocrit level (%)
Prior to donation	129.0±6.55	36.88±1.89
After first donation	114.5±3.78 ^a	33.13±1.73 ^a
After second donation	101.8±6.50 ^a	29.75±2.49 ^a

^a*P* < 0.01, vs. prior to donation

There was no significant difference in Hb concentration prior to donation between the autologous blood donation and allogeneic blood transfusion groups (*P* > 0.05). In the autologous blood donation group, Hb concentration after the second donation was (101.8±6.50) g/L, while in the allogeneic blood transfusion group, the Hb concentration prior to donation was (127.0±7.96) g/L, there was significant difference between these two groups (*P* < 0.01). Hb concentration in the autologous blood donation group was significantly greater than in the allogeneic blood transfusion group at 1 day after the surgery (*P* < 0.01), and there was no significant difference between these two groups prior to, during, immediately after, and 1 day after the surgery (*P* > 0.05). Compared with prior to surgery, Hb concentration in each group was significantly lower at 1 day after the surgery (*P* < 0.01). Precise results are shown in Table 2. After blood transfusion, there was one swine presenting with hemoglobinuria in each group. No transfusion reactions, such as shiver, fever, or hypersensitiveness, were observed during and after blood transfusion in the remaining swine in each group.

Table 2 Hemoglobin concentration during the perioperative period in each group (x±s, g/L)

Group	n	Prior to surgery	During the surgery
Autologous blood donation	7	129.0±6.55	74.1±6.20
Allogeneic blood transfusion	5	127.0±7.96	79.4±2.26

Group	n	Immediately after surgery	1 day after surgery
Autologous blood donation	7	94.3±3.85	115.2±3.97 ^b
Allogeneic blood transfusion	5	91.4±4.41	102.2±2.77 ^{ab}

^a*P* < 0.01, vs. autologous blood donation group; ^b*P* < 0.01, vs. prior to surgery

Survival rate

In the autologous blood donation group, seven swine survived, with a survival rate of 87.5%. In the allogeneic blood transfusion group, five swine survived, with a survival rate of 62.5%. The survival rate was significantly higher in the autologous blood donation group than in the allogeneic blood transfusion group ($\chi^2 = 27.5714, P < 0.01$).

DISCUSSION

Approximately 20% of cardiac surgery patients need allogeneic blood during the perioperative period^[5]. In order to reduce allogeneic blood transfusion, preoperative autologous blood donation has been widely used in countries outside China^[6-10] and satisfactory outcomes have been acquired. To the best of our knowledge, similar results have not been reported in swine model experiment because preoperative autologous blood donation is hard to perform and difficult to manage. The problem of hemodilution exists during the cardiopulmonary bypass surgery^[11-13]. Blood loss caused by open heart surgery and mechanical destruction of blood by extracorporeal circulation unit can lead to anaemia and hypoxemia. Blood transfusion is much more necessary to cardiac surgery in animals because animal experiment conditions are poorer than clinical surgery, which would lead to more blood loss. In the present study, each swine, weighing 27–50 kg, lost approximately 600 mL blood during the perioperative period, thus they would suffer from severe anaemia, which greatly influences the success rate of surgery if blood transfusion was not given. No literature has been retrieved regarding blood type detection or crossmatch prior to blood transfusion in swine as well as adverse effects after blood transfusion. It is difficult to determine whether the animal death was caused by failed surgery or was related to allogeneic blood transfusion. During the swine surgery in which much blood would be lost, such as liver and heart transplantation, swine blood is mostly harvested from slaughterhouse 1–2 days prior to surgery, but heparin (100–200 mg) or sodium citrate must be added to prevent hemagglutination. Evidence exists that blood can be also provided by donor swine^[14]. Precisely, following jugular vein cannula, swine were transfused with a small amount of saline solution. A total of 1 000–1 500 mL swine blood was collected into a sodium citrate pre-added blood-collecting bottle which was connected to the jugular venous duct. After thorough bloodletting, swine often die, and blood type detection and crossmatch are generally not determined prior to bloodletting. Because experimental swine costs much, thus blood collection from experimental swine would increase experimental cost. The present adopted preoperative autologous blood donation using the leap-frog technique, which meets the need of blood use in surgery and also allows the survival of swine, leading to low scientific research costs.

In the present study, the extracorporeal circulation time was (110.94±11.68) minutes. To save blood use, all collected blood was transfused back, which retrieve some red cells to some extent. There was one swine presenting with hemoglobinuria after blood transfusion in each group. The possible cause for the autologous blood donation group is that the mechanical destruction of blood caused by the use of extracorporeal circulation unit increases free hemoglobin, and the possible cause for the allogeneic blood transfusion group is the potential hemolytic reaction caused by allogeneic blood transfusion in addition to increased free hemoglobin. Results from this study demonstrate that Hb concentration was slightly, but not significantly, higher in the autologous blood donation group than in the allogeneic blood transfusion group; however, at 1 day after the surgery, the Hb concentration was significantly higher

in the autologous blood donation group than in the allogeneic blood transfusion group ($P < 0.01$). This occurs because autologous blood transfusion does not need blood type detection and crossmatch, has no rejection, and is conducive to recovery of animals. Evidence exists that preoperative autologous blood donation can lower Hb concentration and Hct level prior to and during surgery compared with control group, but when discharge, Hb concentration and Hct level are higher in the preoperative autologous blood donation group than in the control group^[8].

Modern blood transfusion medicine has become a new subject and blood transfusion has an irreplaceable role in treatment. Xiao *et al*^[15] tried allogeneic blood transfusion in animal experiments, but some problems exist in allogeneic blood transfusion among large- and middle-sized animals in terms of blood matching to prevent transfusion reaction and control blood transfusion-caused transmissible diseases. Allogeneic blood transfusion easily leads to bacterial infection owing to immunosuppression^[9]. Allogeneic blood transfusion for surgical operations in animals has not been widely used. One autologous blood donation and transfusion in surgical operation of miniature pigs can greatly enhance the survival rate of pigs after surgery and it is safe and feasible^[16]. In recent years, we try to retrieve the blood lost and back transfused into animals after screening to save the animals suffering from hemorrhage during the surgery. The method can markedly boost the survival rate of animals, but it is restrained in animal experiments owing to strict sterile operation procedure, complicated operation of blood collection, and high cost.

In the present study, blood loss is calculated according to the blood left in the suction bottle, gauze, dressing, extracorporeal circulation unit, and blood sample^[17-18], approximately 600 mL per pig. One preoperative autologous blood donation cannot satisfy the surgery and more blood lost during one blood collection would cause the hemorrhagic shock of experimental animals. Considering no other surgical wounds during blood collection, 20% systematic blood was collected at 9 days prior to cardiac bypass surgery. During blood collection, a compound solution of sodium lactate solution and hydroxyethyl starch was added to supplement with the lost blood. At 2 days prior to surgery, the collected blood was back transfused into the animals and 30% systematic blood was further collected. Swine vital signs are not markedly influenced because some autologous blood is back transfused. For patients undergoing autologous blood donation using the leap-frog technique, erythropoietin is suggested to accelerate erythropoiesis^[19-20]. In the present study, erythropoietin was not used, but four eggs per day were added. Results from this study demonstrated that swine survival rate was significantly higher in the autologous blood donation group than in the allogeneic blood transfusion group (87.5% versus 62.5%, $P < 0.01$). In the autologous blood donation group, one swine died from anaemia and hypoxemia 2 hours after surgery because much blood was lost prior to extracorporeal circulation and all autologous blood pre-donated prior to surgery was added to prevent too high dilution. In the allogeneic blood transfusion group, three swine died, two because of unable to improve hypoxemia during chest closure and one because of hypoxia caused by nontimely incubation. These findings suggest that the preoperative autologous blood donation is feasible for experiments among large-sized animals.

Further complementary studies involving larger animal numbers are warranted.

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前蛙跳式自体预贮血在猪心脏体外循环手术模型中的应用*

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摘要

背景: 同种异体输血在相同血型的人类中已经得到实现, 但关于猪的血型类型及如何异体输血国内外无确切报道。

目的: 探讨术前蛙跳式自体预贮血在猪心脏体外循环手术模型中的可行性。

方法: 16只家猪随机分组分为2组, 自体预贮血组、同种异体输血组。另4头猪放血供同种异体输血组异体输血应用。两组猪均在体外循环下行心脏手术。自体预贮血组手

术全程仅输自体血, 同种异体输血组手术全程输注等量异体血。记录自体预贮血组放血前后的血红蛋白及血细胞压积, 两组术前基础值、体外循环过程中、体外循环结束后及术后1d的血红蛋白值。

结果与结论: 20只实验猪全身血容量(2500±428) mL; 自体预贮血组第1次预计放血量为(501±86) mL, 实际放血量为(493±93) mL; 第2次预计放血量为(750±128) mL, 实际放血量为(719±98) mL。自体预贮血组猪采血前后血红蛋白计数及血细胞压积差异有显著性意义(P < 0.01)。自体预贮血组术后1d时点血红蛋白数值明显高于同种异体输血组(P < 0.01), 两组其余术前基础值、体外循环过程中、体外循环结束后时点血红蛋白数值差异无显著性意义(P > 0.05); 与术前比较, 两组术后1d时点血红蛋白值

显著低于术前基础值(P < 0.01)。自体预贮血组存活率明显高于同种异体输血组(P < 0.01)。结果说明与同种异体输血相比, 术前蛙跳式自体预贮血安全有效, 术后猪成活率更高。

关键词: 自体预贮血; 猪; 动物; 心脏手术; 同种异体输血; 器官移植

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