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Critical hematocrit and oxygen partial pressure in the beating heart of pigs

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Abstract

In surgical interventions loss of blood is a typical complication. A common therapeutical approach is the substitution of the blood loss volume by plasma substitutes like hydroxyethyl starch, dextrane or gelatine. None of these substitutes showed a sufficient oxygen transport capacity. Therefore the therapy with blood substitutes can negatively affect the functional integrity of all tissues in general and especially of those with high oxygen consumption like e.g. the myocardium. This study was aimed to get information about the minimal hematocrit, known as critical hematocrit (cHct), which guarantees a stable and adequate oxygen tension in the myocardium. The study was performed in pigs (strain: Deutsche Landrasse) as animal model. The animals (n=7, female, 56.9±5.7 kg, 6 months of age) were provided from a regional breeder. The experiments were licensed by the government agency of Dresden, Germany. The Hct was reduced by an isovolemic dilution of blood with infusion of an isotonic gelatine solution (gelatine polysuccinate 4%, B. Braun). Care was taken to guarantee an isovolemic replacement, because either hypo- or hypervolemic blood substitutions can have significant influences on the microcirculation. The blood volume substituted ranged between 3000 ml (minimum) and 7780 ml (maximum). During blood substitution with gelatine polysuccinate the myocardium as well as the skeletal muscle showed an increased pO_2 in all animals until the Hct fell below 15 %. A further blood dilution and concomitant Hct decrease was accompanied by a decrease of the pO_2. The rate of decrease even accelerated when the Hct fell below 10 % and led to an oxygen partial pressure (pO_2) lower than before blood dilution. For the first time data are presented of the tissue oxygen partial pressure (pO_2) in the myocardium of the left ventricle of the beating heart of young pigs approaching and exceeding the limitation of Hct, which results in an ischemia of the myocardium. These data seem to show that the critical Hct of pigs is reached at about 10 %.

Lower Hct values resulted in insufficiency of the circulatory system and in ischemia of the myocardium within a few minutes followed by a collapse of the pumping function of the heart.

1 Introduction

In surgical interventions loss of blood is a typical complication. A common therapeutical approach is the replacement of blood loss by blood substitutes (e.g. solutions of hydroxyethyl starch, dextran or gelatine). None of these substitutes showed an oxygen transport capacity comparable to blood. Therefore the therapy with blood substitutes can negatively affect the function particularly of tissues with high oxygen consumption like the myocardium, the skeletal muscle, and the brain.

In this study we measured the hematocrit (Hct) and the tissue oxygen partial pressure (pO_2) in the beating heart and the skeletal muscles of pigs. The optimal Hct is defined in the literature as maximally effective, when the tissue-pO_2 is at the maximum [1-5]. On the contrary, we wanted to get information about the minimally effective Hct, known as critical hematocrit (cHct) [6-8], guaranteeing a stable and still adequate pO_2 in the myocardium and the skeletal muscle. This information is essential for an effective and optimized substitution therapy.

2 Materials and Methods

2.1 Animals

Physiology and anatomical features of the pig heart (including the coronary vascular system and the coronary collateral vessels) are comparable to the human heart [9]. For this reason, the pig model is frequently used for studies of the blood circulation [10]. We also decided to use pigs (strain „Deutsche Landrasse“) as animal model. The animals (n=7, female, 56.9±5.7 kg, 6 months of age) were provided from a regional breeder. They showed no clinical symptoms of disease and were kept in-house until the experiments started. The experiments were licensed by the government agency of Dresden, Germany.

2.2 Anaesthesia

Anaesthesia was started by intravenous (i.v.) administration of azaperone (6 mg/kg body weight, Stresnil™, Jannsen-Cilag), ketamine 0.1 ml/kg body weight (Ketamin™ 10 %, Sanofi-Ceva), xylazine (0.3 mg/kg body weight, Rompun™ TS, Bayer Vital), diazepam (0.35 mg/kg body weight, Faustan™, Arzneimittelwerk Dresden) and atropine (0.01 mg/kg body weight, Atropinum Sulfuricum™, Eifelfango). For the maintenance of anaesthesia, fentanyl (5-10 µg/kg body weight and hour i.v., Fentanyl, Curamed) and thiopental (2-3 mg/kg body weight i.v., Trapanal™, Byk Gulden) were applied. Additionally, lactated Ringer solution (B. Braun, Melsungen, Germany) was continuously infused (i.v.). After confirmation of surgical tolerance, relaxation was induced by pancuroniumbromide (4 mg/kg body weight i.v., Pancuronium™, Curamed) and subsequently an endotracheal tubus was placed for artificial respiration (50 % O_2, 4.0-4.5 l/min, 16-19 breaths/min). The arterial pO_2 was kept stable within the range of 130-150 mmHg, the arterial pCO_2 within 40-45 mmHg and the pH was constant at 7.4. These parameters and the systolic and diastolic arterial blood pressures (SBP, DBP) were measured via a catheter in the A. carotis communis dextra. In addition, a Swan-Ganz catheter was placed via the V. jugularis interna dextra to continuously measure the cardiac output (CO), the general venous pO_2 and the central venous pressure (CVP) within the pulmonary artery. To control heart activity an ECG was used.

2.3 Continuous reduction of Hct

The Hct was reduced by a continuous replacement of blood by an isotonic gelatine solution (gelatine polysuccinate 4%, B. Braun). Care was taken to guarantee an isovolaemic blood dilution, because a hypo-/hypervolaemic blood substitution could exert a significant influence on the capillary microcirculation [11]. Blood was removed from the right V. jugularis externa

and replaced at the same time by isovolaemic infusion of the blood substitute in the left V. jugularis externa. To quantify the Hct, blood samples were taken successively after every replacement of 50 ml.

2.4 Measurement of pO_2

During blood substitution the pO_2 values in the beating heart left ventricle myocardium as well as in a peripheral muscle (M. femoris sinister) were measured continuously using a disposable invasive flexible microsensor of the Clark type with an outer diameter of 470 µm and a sensitive area near the catheter tip of 7.38 mm^2 (Integra NeuroSciences, Andover, England). The pigs were placed with their back on an operating table covered with a heater mat (37 °C) to prevent a decrease of body temperature. The thorax and the skin in the area of the M. femoris sinister were opened and sensors for pO_2 and temperature (myocardium: 2 pO_2 sensors, 1 temperature sensor; skeletal muscle: 1 pO_2-sensor, 1 temperature sensor) were inserted 4.8+1.1 mm in the myocardium. Insertion depth was validated by necropsy after the death of the animal. The sensors were inserted into the skeletal muscle in parallel to the muscle fibers. To avoid any influence of organ/tissue movement on pO_2 and temperature measurements, the sensors were fixed either by sewing or by fibrin glue. The surface of the heart was kept wet by a first gauze layer saturated with isotonic NaCl-solution and secondly by a sterile blanket put on top of the gauze and covering the opened thorax to prevent cooling and a loss of humidity. The skin wound at the left leg was closed with a suture.

The analysis of pO_2 measurements was started 30 minutes after insertion because directly after insertion the myocardial pO_2 initially decreased and then increased again reaching constant values again after 30 min. Details of the measurement method have been published elsewhere [12-14].

2.5 Statistical analysis

All random samples were characterized by mean values and standard deviations and analyzed by the Student's two-tailed t-test for paired samples. Because of the explanatory character of the study a Bonferroni-adjustment was not applied. Differences smaller than $p=0.05$ were considered significant.

3 Results

One mm^3 of myocardial tissue is assumed to be supplied by approximately 2500 capillaries. This could mean that in our examination the tissue pO_2 of a vascular micro domain was measured [15]. Before the substitution of blood was started, the blood circulation parameters showed no abnormalities (SBP: 87±18 mmHg, DBP: 54±13 mmHg, heart rate (HR): 83±16 beats/minute, CVP: 6.9±3.5 mmHg). The volume of substituted blood ranged between 3000 ml (minimum) and 7780 ml (maximum). Blood substitution was continued successively until the animal died. A mean blood volume of 5254±1672 ml had been substituted when the animal died, where the standard deviation at baseline revealed a high interindividual variance (31.8%).

Figure 1

After blood substitution with gelatine polysuccinate the myocardium as well as the skeletal muscle of all animals showed an increased pO_2. With decreasing Hct pO2 increased in both tissues significantly ($p<0.05$) until the Hct arrived at 15 %. A further Hct decrease was then followed by a decrease of the pO_2. The pO2 dropped steeply when the Hct fell below 10 %

(Fig. 1). Passing the Hct of 10%, the pO2 values did not differ from baseline values, but during the following dilution period the pO2 values approached zero and the pigs died.

In figure 2 the relation between blood pressure and Hct is illustrated. The SBP was unchanged until the Hct approached 10%. In contrast to the pO2 there was no increase of SBP.

Figure 2

DBP was unchanged until Hct fell below 20%, then DBP decreased continuously towards values of 5 mmHg throughout the whole further period of blood dilution.

Figure 3 shows the relation between HR and CVP with the systemic Hct. The median HR diagram showed an increasing heart rate with each 50 ml-blood dilution step until the decreasing Hct arrived at 10 %. A further blood dilution was followed by a decrease of the HR towards zero.

The median CVP diagram showed no significant relation between the CVP and the Hct.

Figure 3

4 Discussion

The replacement of blood by plasma substitutes is an accepted therapeutical approach in acute medicine in cases of excessive blood loss. It is well documented that this type of blood substitution results in an increased pO_2 in the skeletal muscle of mammals and humans [3, 4, 11, 16, 17]. In the study it could be shown for the first time that a well controlled isovolaemic hemodilution with 50 ml of 4% gelatine polysuccinate induced a significant pO_2-increase of

about 50 % in the myocardium of the left ventricle of the beating heart. A pO_2 increase of about 100 % was registered after a blood volume of 100 ml had been replaced.

Both the skeletal muscle and the active myocardium were in our focus. In pigs we could show for the first time that the decrease of hematocrit by hemodilution resulted in an increase of the pO2 in the left ventricle myocardium as well as in the skeletal muscle. However, the skeletal muscle pO_2 increase after hemodilution was not as high as in the myocardium.

In humans and rabbits the capillary Hct and the microvascular oxygen concentration did not change the systemic Hct fell below 20% [18, 19]. It is assumed that the reduction of the Hct and microvascular oxygen transport capacity respectively were compensated by an increase in the microvescular perfusion [20]. In 1988 already, Lindbom et al. [21] reported that a decrease of the capillary Hct in skeletal muscles of rabbits was compensated by an increase of the red blood cell velocity in capillaries. Thus the increase in microvascular perfusion is assumed to be the result of an increased cardiac output [22-25] and resulted in our study in an elevated myocardial and skeletal muscle pO_2 above the baseline values. Unless Hct values less than 10% appeared during hemodilution, the oxygen partial pressure did not decrease in the myocardium and the skeletal muscle. pO2 decreased significantly in these tissues when the Hct fell below 10%.

In a state of anaesthesia the compensatory rise of the cardiac output occurs via an increase of the ventricular stroke volume and of the heart rate.

There are two possible mechanisms discussed as responsible for the increased stroke volume. The reduced blood cell count following dilution leads to a decreased blood viscosity [26-28]. Blood of decreased viscosity can pass the capillary bed faster than blood with a physiological

Hct. This would result in an increased venous blood flow to the heart. Caused by such a raise in ventricular preload the myocardial contractility would increase according to the Frank-Starling-mechanism [16, 29]. In our study there was no additional effect on the preload beside the lowered viscosity as revealed by the constant central venous pressure between 6 to 8 mmHg over the whole period of testing. Beside the effect on the myocardial contractility the lower viscous blood could also induce a direct NO-induced vasodilation and thus cause a reduced afterload of the left ventricle [30].

The increase in the cardiac output is also a result of an increase in heart rate. For the myocardium a recently published study conducted in pigs showed that an increase in the heart rate up to 110 beats/min resulted also in a significant increase in the myocardial pO2 [31]. In our study we could isovolumetrically lower the Hct down to about 10% and, still, the pig organism would compensate a reduced pO2 in the myocardium and the skeletal muscle by an increase of the heart rate. However, the pigs could no longer compensate a higher degree of blood dilution and an Hct below 10%. This resulted in a lethal drop of the pO_2 both in the skeletal and the cardiac muscle.

To document the performance of the circulatory system we measured the systolic and diastolic blood pressure. The pig organism was able to maintain a sufficient performance of the circulatory system until the systemic Hct fell below 15 %. Anaemia to that extent caused by hemodilution could no longer be compensated and resulted in a decrease of the systolic and diastolic blood pressure. This decrease was most pronounced, when the Hct fell below 10 %.

5 Conclusion

In this study, for the first time data are presented on the kinetic of tissue pO2 values in the beating heart of anesthetized normothermal young pigs treated with consecutive steps of isovolaemic hemodilution to gain information about the so called critical hematocrit. These data clearly reveal a critical Hct value of about 10%. Lower Hct values led within a few minutes to an insufficiency of the circulatory system and to an ischemia of the myocardium (and of the skeletal muscle as well) followed by a collapse of the pump function of the heart. Our results confirm data from other groups which declaimed a critical Hct of 10-15 % for rats (17), rabbits (30) and dogs (8).

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Figures

Figure 1. Association between the pO_2 of the left ventricle myocardium (pO_2lV, left figure) / skeletal muscle (M. femoris sinister, pO_2SM, right figure) and the systemic Hct (○ = individual values, ● = mean value (n=7)

Figure 2. Association between blood pressure (systolic blood pressure, SBP, left figure), diastolic blood pressure, DBP, right figure) and systemic Hct (○ = individual values, ● = mean value (n=7)

Figure 3. Association between heart rate (HR, left figure), central venous pressure (CVP, right figure) and systemic Hct; ○ = individual values, ● = mean value (n=7)