Perfusion scheme for long-term preservation of isolated pig liver in an experiment

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Abstract

Background: Recently, the method of machine perfusion of a liver transplant, which has been gaining popularity, justifies the hopes placed on it. At the same time, the existence of many perfusion schemes confirms the need to find the optimal one. The purpose of this study was to experimentally develop a perfusion scheme for long-term preservation of the liver outside the body by perfusion with normothermic oxygenated blood.

Materials and methods: To create a perfusion device, we used a roller blood pump console, a standard pediatric oxygenator, venous and arterial filters, polyvinyl chloride and silicone lines, and a constant-flow-to-pulsating (external pulsator) converter of our own design. In the perfusion scheme after the roller pump, the blood flow was divided into two streams. The first was sent to the oxygenator. Then, having passed the pulsator, the flow was fed through the aortic stump into the hepatic artery. The second flow was directed to the upper soft-walled tank. As an experimental model, 5 pigs weighing up to 20 kg were used. Under general endotracheal anesthesia under conditions of thoraco-laparotomy, the liver was explanted along with a section of the caudal vena cava and aorta. Injection cannulas were inserted into the aorta and portal vein, and a draining cannula was inserted into the distal end of the vena cava. The cannulas were connected to the perfusion machine and perfusion of the organ was started. Sensors for monitoring blood flow volume, pressure, temperature, and blood oxygen saturation were located at different parts of the perfusion scheme.

Results and discussion: All experiments were carried out for 12 hours. The temperature of the perfusate was maintained within the range up to 37°C; volumetric blood flow in the portal vein was 450-500 ml/min; pressure was maintained within 12-24 mm Hg; the pulse rate in the arterial line did not exceed 100 beats/world; volumetric blood flow in the hepatic artery averaged 80-120 ml/min; the average pressure was maintained within 70 mm Hg. Dosing clamps located on the lines provided the opportunity to optimally redistribute the perfusate flows. So in the caudal vena cava, the pressure was maintained within the physiological norm. At the same time, the required level of perfusate for gravitational injection into the portal vein was stabilized in the soft-walled reservoir.

Conclusion: The developed scheme for machine perfusion liver preservation allows stabilizing two blood (arterial and venous) flows of different characteristics during a prolonged experiment. In parallel with the liver, the kidney, which is adequately perfused in the general blood flow, can function as a controller of hemohydrobalance, a stabilizer of acid-base balance, and an eliminator of metabolic wastes.**(TCM-GMJ December 2023; 8(2):P20-P24)**

Keywords: Machine perfusion; Liver preservation; Perfusion scheme;

Introduction

The method of machine perfusion of a liver transplant, which is gaining popularity, justifies the hopes placed on it. First of all, due to the possibility of a longer preservation of the organ, reaching several days (1 ,2, 3, 4, 5, 6, 7). Perfusion-controlled conservation, in a number of cases, makes it possible to improve the condition of an organ rejected for transplantation and thus has a positive effect on the statistics of transplantations in general (8, 9). In addition, in the process of perfusion preservation, the possibility of prolonged physiological, metabolic, and morphological monitoring of the organ arises. This is extremely important for confident prediction of its post-transplant functioning (10, 11). In turn, compared with cold storage of an organ, machine perfusion is an expensive procedure. It involves the creation of a biotechnical complex consisting of a transplant and a perfusion apparatus. They are interconnected according to a certain scheme by artificial blood lines. The existence of a variety of perfusion schemes due to specific experimental or clinical needs (12).

The purpose of this study was the experimental development of a perfusion scheme for long-term preservation of the liver outside the body by its perfusion with native, norothermic, oxygenated blood.

Materials and methods

To create a perfusion apparatus, a roller blood pump console with a control system was used. The device also included a standard membrane, children's oxygenator with a heat exchanger, venous and arterial filters, blood tubes made of polyvinyl chloride and silicone. In addition, a soft-walled venous reservoir and an organ reservoir were used (13, 14). In the composition of the apparatus, we included a converter of constant flow into a pulsed flow (external pulsator) of our own design (15). Its principle of operation consists in electronically con-

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trolled, dosed, external clamping of the arterial line with the frequency of the native pulse (Fig. 1). The internal circulation scheme was developed so that under the conditions of operation of one pump (20) it was possible to form two different blood flows.

The internal circulation scheme was developed so that under the conditions of operation of one pump (20) it was possible to form two different blood flows:

- venous (splanchnic) blood flow (15) from a soft-walled reservoir (25) located above the graft at a certain height, which, together with an external pinch valve, made it possible to control volumetric blood flow and pressure;
- arterial pulsating blood flow with oxygenated blood (16) created in a series circuit of a roller pump (20), an oxygenator (22) and a pulsator (24).

According to the perfusion scheme after the roller pump (20), the blood flow was divided into two lines. The first was sent to the oxygenator (22) with a heat exchanger (21). Then, through a pulsator (24), the flow was fed into the aortic stump (9), from where it entered the hepatic artery (7). The arterial line was dosed shunted (27) with the second, venous (26) line (from the pump to the upper soft -walled reservoir). This mixed flow (with an increased oxygen content compared to venous blood) was sent to the upper soft-walled reservoir (25). From the upper reservoir, simulated splanchnic blood was gravitationally supplied to the portal vein of the liver (6). Sensors for monitoring pressure (30), temperature (31), and volumetric blood flow velocity (29) were placed in different parts of the perfusion scheme (apparatus). In addition, in certain sections of the circuit, external pinch valves (28) metering the flow were strengthened as shown in the diagram (Fig. 2). In a bench test, the perfusion system showed stable operation in a 24-hour experiment with standard and load hemodynamic and temperature parameters. Ease and reliability in control was also recorded when simulating the need for forced movement of the volume of fluid in the circuit of the perfusion circuit of the device (between the upper and lower venous reservoirs). To test the developed scheme "in vivo", 5 pigs weighing up to 20 kg were used as an experimental model. Animals were introduced into the experiment in accordance with the requirements of international organizations on bioethics and the use of laboratory animals in scientific experiments (16, 17). Under general endotracheal anesthesia (premedication - ketamine 15 mg/kg, atropine sulfate 1%-0.05 mg/kg; general anesthesia - isoflurane 1-2%, propofol 1% - 3-5 mg/kg), pressure was measured under laparotomy and volumetric blood flow velocity in the vena cava, portal vein, in the abdominal aorta, in the common hepatic artery and in the renal artery. The data obtained further served as tentative criteria for adequate machine perfusion of these organs "ex vivo". For the same purpose, a control blood test was taken for sugar, hematocrit, pH, and AST. Next, the vessels of the liver, right kidney and their ligamentous apparatus were mobilized and skeletonized. Thus, at the same time, the liver with the right kidney was isolated on a common vascular pedicle. Heparin was injected into the vena cava (2850 IU-0.78 ml - 3000 IU-0.6 ml). The aorta and vena cava were opened at the bifurcation level. The native blood of the animal was forcedly taken by filling the upper and lower venous reservoirs of the perfusion apparatus. The liver was explanted along with the supra- and subhepatic portion of the caudal vena cava and the subphrenic portion of the aorta. The suprahepatic portion of the vena cava and the distal end of the aorta were ligated. The liver was placed in a reservoir for the organ, placing it down the diaphragmatic surface on a special soft, fluctuating air cushion. Injection cannulas were inserted into the aorta and portal vein. A cannula (17) was placed into the distal (subhepatic) end of the vena cava to drain blood into the inferior venous reservoir (19). The right kidney (10) with the feeding artery (11) was taken along with the aorta (9) during liver explantation. The hepatic vein (12) was drained into the venous line. The ureter was also drained, placing the biliary and urinary catheters in graduated test tubes. All cannulas were connected to a perfusion system filled with native blood and normothermic preservation of both organs was started (Fig. 3). In the process of perfusion, the main hemodynamic parameters were taken into account, comparing them with the initial parameters recorded in the body of a conditionally intact animal (under conditions of endotracheal anesthesia before the start of the main stage of organ mobilization). Throughout the experiment, drug correction of vascular tone was not performed. Each experiment was completed for comparison at the end of the 12-hour perfusion, taking into account the currently generally accepted and recognized optimal 8-hour duration of hypo- and normothermic machine perfusion.

Results and discussion

The performance of the roller pump of the perfusion machine did not exceed 500 ml/min. The temperature of the perfusate was maintained within 37°C; volumetric blood flow in the portal vein averaged 350-400 ml/min; the pressure was maintained within 12–24 mm Hg (by the end of the experiment, these figures decreased from the maximum to the minimum); the pulse rate in the arterial line was standardly set within 100 beats/min; volumetric blood flow in the hepatic artery averaged 80-100 ml/min; the mean pressure was 70 mm Hg. These parameters were maintained throughout the experiment. The division of the blood flow in front of the oxygenator into two lines allowed one of them to pump arterial, oxygenated blood into the organs with a pulsating flow, maintaining in them the volumetric blood flow and pressure planned according to the initial indicators. By the other line, supply the perfusate to the soft-walled upper tank, located 50-70 cm above the perfused organ.

The pressure in the portal vein was regulated by vertical movement of this reservoir. Dosing external pinch valves on the lines, on the one hand, ensured the regulation of the volumetric blood flow of the flowing blood, and, on

the other hand, the possibility of optimal redistribution of various flows, while stabilizing the levels in both reservoirs. So, in a soft-walled tank with mixed (splanchnic) blood, the calculated (up to 200 ml) level of perfusate for gravitational supply to the portal vein was standardly maintained. The volumetric flow rate in the portal vein was controlled by the control of an external clamp on the outlet line from the reservoir. In the caudal vena cava, the pressure was maintained within the physiological norm (3-5 mm Hg) to avoid stagnation in the liver. This was achieved by establishing a certain level gradient between the organ reservoir and the lower venous reservoir. The hemodynamic parameters mentioned above were achieved during the first hour of perfusion by a gradual increase from the minimum to the average calculated values.

After relative, reperfusion stabilization of hemodynamics of the liver, the intervals between the moments of the necessary correction of some hemodynamic parameters of the perfusion machine increased, reaching 40-60 minutes. The stabilized blood flow in the biotechnical system did not undergo drastic changes requiring close attention and correction. This allowed the operator to leave to monitor general and biochemical blood parameters.

Conclusions

The results obtained show that:

• The developed scheme for long-term machine preservation of the liver outside the body allows you to stabilize two different blood flows consumed by the liver using only one blood pump;

• The perfusion machine designed according to the proposed scheme simultaneously generates a normothermic, oxygenated, pulsating (arterial) flow for the hepatic artery and a venous (splanchnic) blood flow for the portal vein that is maximally adapted to natural parameters;

• The perfusion machine prototype is easily assembled from reusable and disposable accessories of the cardiopulmonary bypass machine, easy to manage and reliable in operation;

• Each section of the perfusion scheme is hemodynamically maximally adapted to the parameters of native blood flow in these organs;

• The described scheme of liver preservation includes the method of its co-preservation together with the kidney, which is explanted and perfused from similar sources, receiving adequate hemodynamic and biochemical blood flow. Therefore, it can be assumed that the kidney that has restored its function is able to eliminate metabolites and toxins that accumulate in the perfusate plasma and eliminate the need to include a dialyzer in the perfusion scheme of multi-day machine preservation;

• The developed scheme and method of multi-day machine perfusion of the hepato-renal complex on a common vascular pedicle requires careful experimental verification to reliably confirm the identified innovations and evaluate both in terms of bio-medical and economic benefits.

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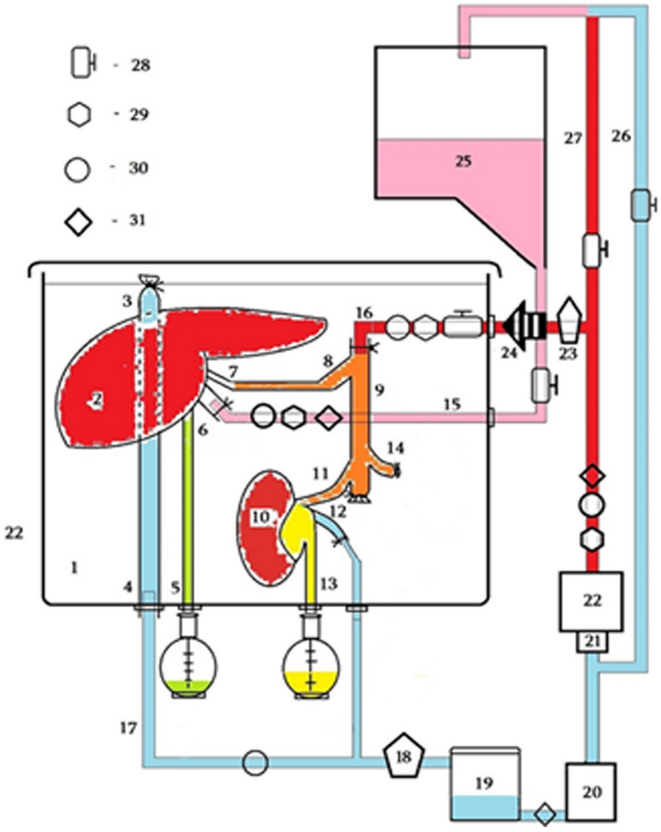
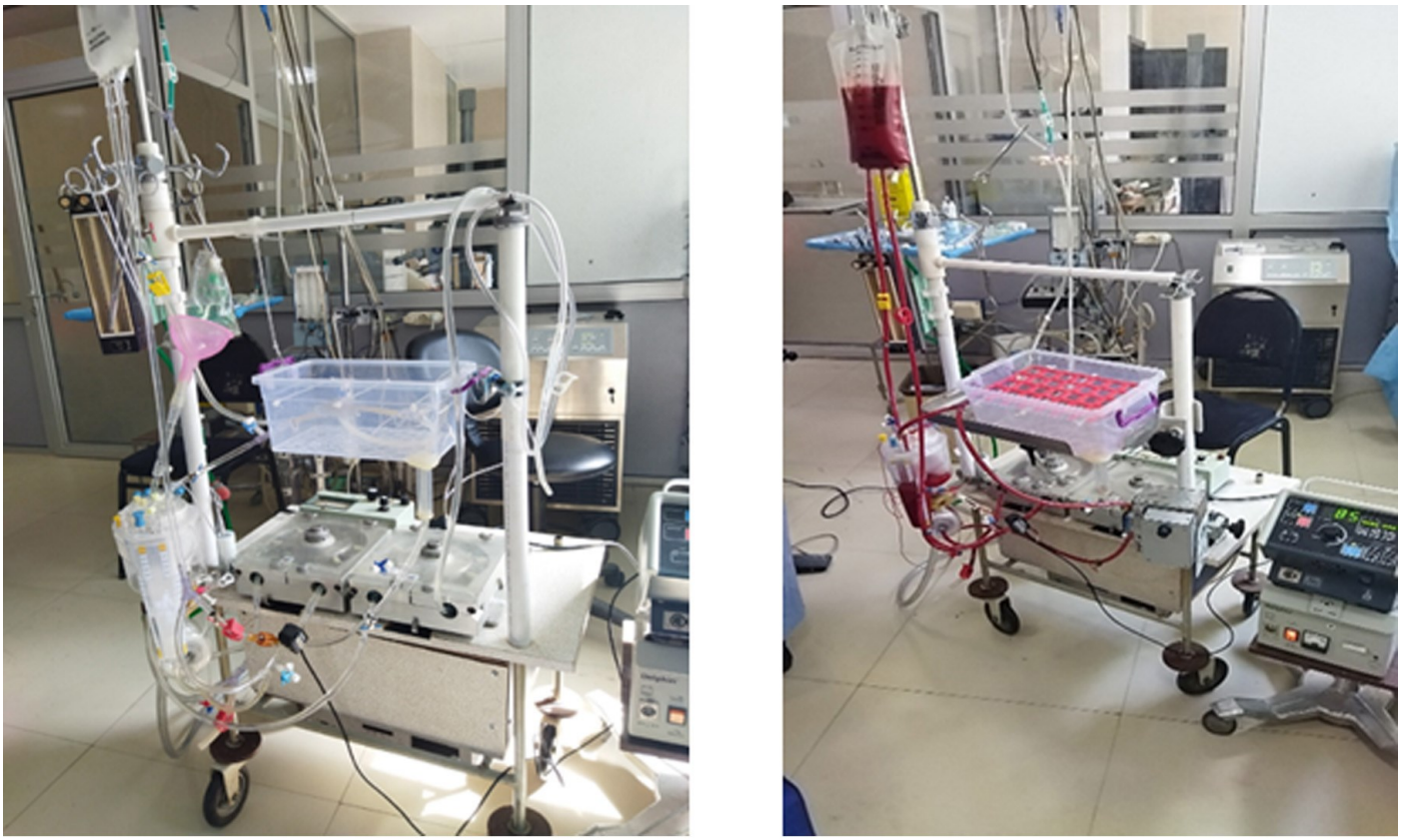
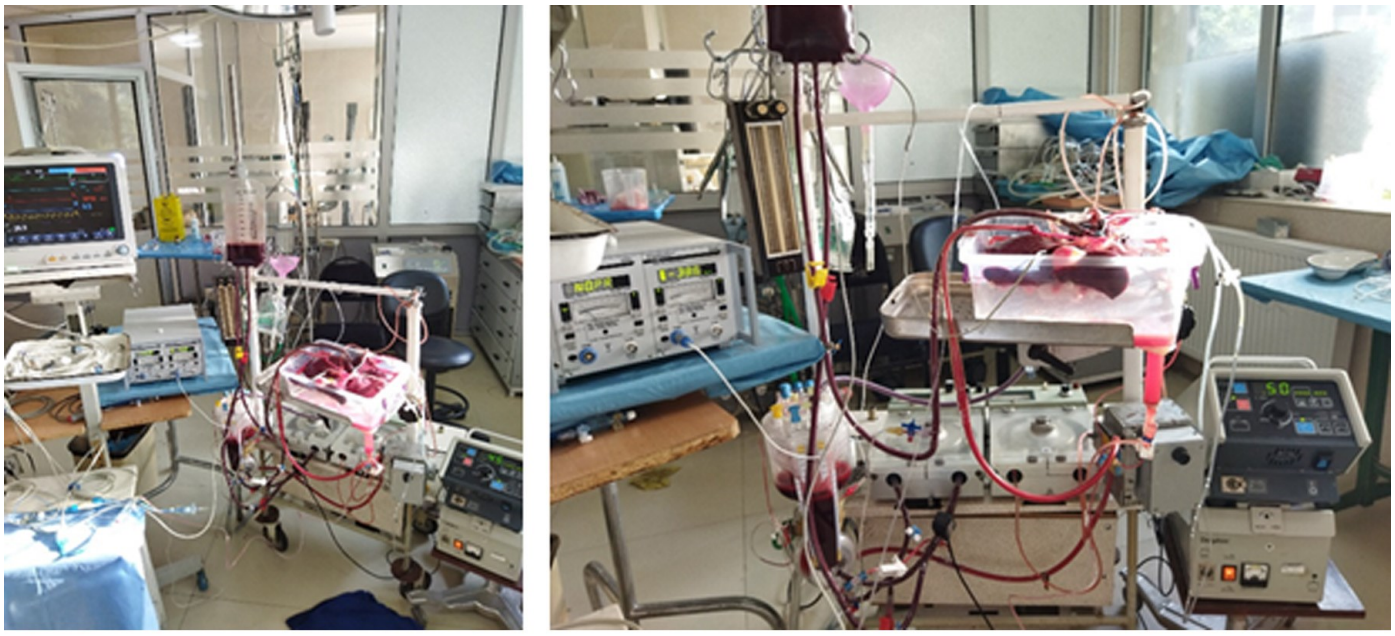


Figure 1: Scheme of biotechnical system: perfusion apparatus and organ complex (liver, kidney on a common vascular pedicle) ;
1. reservoir for an organ; 2. liver; 3. ligated suprahepatic section of the vena cava; 4. cannulated subhepatic section of the vena cava; 5. drained common bile duct; 6. cannulated portal vein; 7. hepatic artery; 8. celiac trunk; 9. aorta; 10. kidney (right); 11. renal artery; 12. renal vein; 13. drained ureter; 14. ligated left renal artery; 15. highway for the blood of the portal vein; 16. aortic cannula; 17. venous return line; 18. venous filter; 19. venous reservoir (lower, hard-walled); 20. blood pump; 21. heat exchanger; 22. oxygenator; 23. arterial filter; 24. pulsator; 25. reservoir of mixed blood (upper, soft-walled); 26. venous blood supply line to the upper reservoir; 27. arterial blood supply line to the upper reservoir; 28. external pinch valve; 29 sensor of blood flow volumetric velocity; 30. blood pressure sensor; 31. temperature sensor



A **B**

Fig. 2. Perfusion apparatus filled with **A** - blood substitute, **B** - native blood in recirculation mode



C **D**

Fig. 3. Long-term preservation of the hepatic-renal complex: **C** - 6th hour, **D** - 10th hour of perfusion

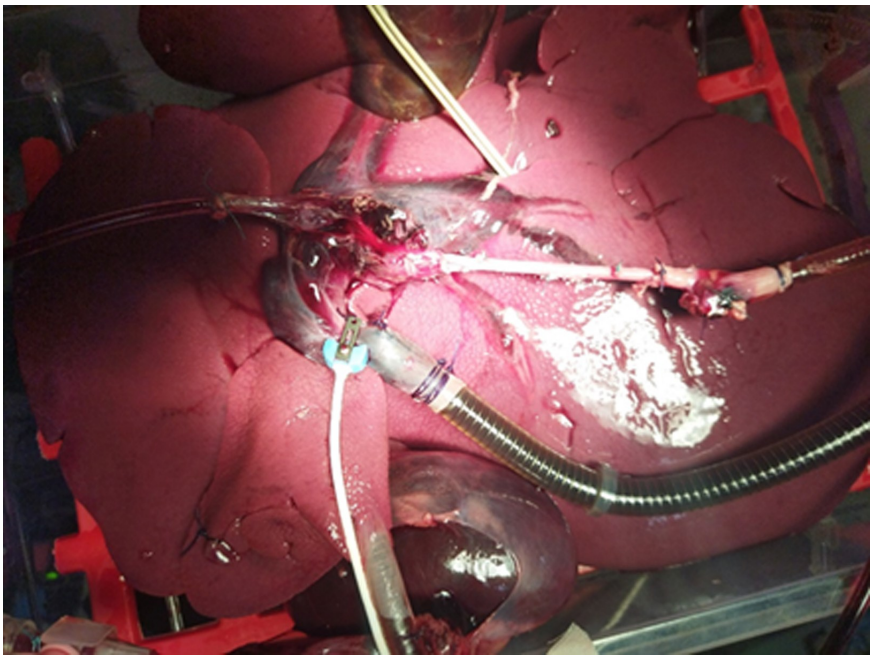


Fig. 4. Perfusion of the hepato-renal complex, 18th hour of conservation

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