Some hemodynamic parameters of the liver during 24-hour perfusion conditioning using a proprietary device

Chkhaidze Z.[1], Khodeli N.[1], Kordzaia D.[1], Pilishvili O.[1]

Abstract

Background: The transplantology is one of the most promising and perspective fields of the modern medicine as the replacement of the organs with unrecoverable disease with the donor organs has no alternative so far.

Aim: Boosting the number of liver pathologies in the world and, on the other hand, successful liver transplantations result in an annual increase in the number of people waiting for transplantation. Therefore, attention was drawn to so-called methods of morpho-functional improvement of organs obtained from "marginal" donors. It was revealed that machine perfusion can improve the condition of the donor organ. Numerous experimental studies show that perfusion of the organ with controlled, normothermic, oxygenated blood is a key factor in liver conditioning and perfusion should be prolonged in order to achieve the desired effect.

Methods: The study was conducted on 5 pigs weighing 20-25 kg. Perfusion of the cannulated liver began one hour after explantation (warm ischemia) with normothermic oxygenated blood. For perfusion, a two-chamber pump of our own design with a hydraulic drive was used, providing pulsating blood flow in the hepatic artery and non-pulsating - in the portal vein. To condition the liver, heparin, insulin, bile preparations, prostacyclin and nutrients in standard doses were introduced into the perfusate. The condition of the liver was assessed by morphological studies, monitoring of hemodynamic parameters in the portal vein and hepatic artery, as well as the amount of bile secreted. Bile acids, cholesterol, bilirubin, glucose, and transaminases were determined in the blood.

Results:In all experiments, perfusion was carried out within physiological hemodynamic parameters (blood pressure in the hepatic artery 82±4.3/58±3.1 mmHg; blood flow in the portal vein 765±36 ml/min. Over 24 hours, the amount of bile released was 114±28 ml). Also, biochemical parameters in the blood iffered slightly from the initial data. Morphological studies showed that less than 3% of cells were suffered by small droplet micro steatosis; mononuclear portal infiltrates were found only in several areas. Mild mixed large droplet micro steatosis and small droplet micro steatosis was found in less than 5 % and 10% of the hepatocytes accordingly on the 16th and 24th hours of perfusion. Similarly the mild venous congestion was present in 1 out of 5 livers after 16-hours perfusion and in 2 out of 5 livers after 24-hours perfusion. The number of necrotic hepatocytes and infiltrated portal triads did not exceed 10% and 15%. However, there were no differences in the degree of biliary damage – cholestasis or ductular proliferation - correlating with the terms of the experiment.

Conclusions: 24-hour liver perfusion conditioning by using of the machine of own design providing the pulsatile blood flow guarantees the satisfactory preservation of liver making it useful for successful transplantation. **(TCM-GMJ August 2025; 10 (2): P17-P21)**

Keywords: Nutrition, Zinc (Zn), Children, Growth, Psychomotor

Introduction

The ever-growing demand for donor organs is forcing transplant surgeons to look for alternative ways to meet the needs of recipients who are in the terminal stages of disease. It is known that not all obtained organs are used for transplantation due to their unfavorable condition [1]. Efforts are being made to improve the quality of rejected organs [2, 3, 4, 5, 6]. The key role in improving of the quality of transplant organs is assigned to the "ex vivo" machine perfusion method. Recently, there has been a tendency to bring the conditions of conservation as close to physiological norms as possible. This gives a rise to the need to create artificial conditions as close as possible to natural ones [7, 8, 9]. It should also be noted here that effective conditioning of a marginal transplant can be achieved by long-term (many hours, many days) perfusion. This was made possible by constant technological improvement of artificial analogues of the heart, lungs, blood vessels, etc. During "ex vivo" machine perfusion for the purpose of long-term preservation of the liver, a number of technical and methodological issues arise [10, 11]. Among them, the management of hemodynamic parameters of perfusion (the nature of the pulse wave, minute volume of blood flow, pressure in the hepatic artery and portal vein) is important. Equally important is the monitoring and management of the biochemical composition of the perfusate/ blood (correction of urea, uric acid, creatinine, proteins, glucose, cholic acid, bile acids, etc.) [12, 13, 14].

In modern perfusion machines, non-pulsating roller and centrifugal pumps provide blood circulation. However,

From the [1]Tbilisi state Medical University , Georgia;
Received March 10, 2025; accepted April 25 , 2025.
Address requests to: Chkhaidze Zurab
E-mail: zurab.chkhaidze@tsu.ge

non-pulsatile flow is characterized by a number of known disadvantages that affect hemodynamics and homeostasis [15, 16]. Therefore, for the maximum physiological hemodynamics of artificial perfusion during liver preservation, the device must provide both venous - non-pulsating, and arterial - pulsating blood flow. Numerous experimental studies show that perfusion of the organ with controlled, normothermic, oxygenated blood is a key factor in liver conditioning and perfusion should be prolonged in order to achieve the desired effect.

The purpose of this study was a preliminary experimental testing of the developed new perfusion apparatus for long-term, 24-hour preservation of the liver, in which two different (arterial and venous) flows are provided for the liver by one pump and an assessment of some hemodynamic parameters and morphological changes in this case.

Methods

The study was conducted on 5 pigs weighing 20-25 kg. All experiments were performed in accordance with the guidelines of Georgia, the European Union and the United States for the protection and use of animals in experiments [17, 18, 19]. Combined intravenous anesthesia and artificial ventilation were used. The femoral artery and vein, also the urinary bladder, were catheterized. The permanent monitoring of body temperature, weight and electrocardiogram of the animal was conducted. The aortic section at the level of the celiac trunk, portal vein, bile duct, and caudal vena cava were cannulated. The liver was explanted together with sections of the aorta and caudal vena cava (Fig. 1). Perfusion was started one hour later (warm ischemia time) after explantation with normothermic oxygenated blood. For perfusion, a two-chamber pump of our own design with a hydraulic drive was used, providing a pulsating blood flow in the hepatic artery and a non-pulsating one in the portal vein. The initial filling volume of the chambers was 130 ml. The perfusate consisted of physiological solution (115 ml), 10% glucose (10 ml), 8.4% sodium bicarbonate (5 ml) and heparin (1000 IU/ml). To condition the liver, insulin, bile preparations, prostacyclin, and nutrients in standard doses were introduced into the perfusate. Artificial circulation was started with increasing blood flow (400 ml/min). The frequency of the pulsating flow was regulated by the "internal rhythm" of the device within 80-100 beats/min. During perfusion preservation with homologous blood, the body temperature was maintained within 38.5-39.0°C. The transfusion volume and the amount of bile were monitored. The levels of hemolysis, pH, BE, Hct, Hb, AST, pO2, pCO2, ALT, AST, K+ and Na+ were determined in the blood. Liver puncture biopsy was performed before the start of perfusion, as well as at 8, 16, and 24 hours of the experiment (using a reusable instrument with Bard Magnum biopsy needles). The device includes a lower venous reservoir with a volume up to 500 ml. It was placed 40-50 cm below the liver. The second reservoir up to 500 ml for "splanchnic" blood was placed 50-60 cm above the liver on a vertically movable holder. In addition to a blood pump of its own design, the perfusion apparatus also included a heat exchanger, venous and arterial filters, an organ reservoir, blood tubes with dosing taps (Fig. 2). The overall control of the apparatus was carried out by a digital control unit, which combines the control of the operation parameters of the pump, heat exchanger, and the flow distribution system within the circulation circuit of the apparatus. The arterial tube after the pump was divided into two branches. One branch was sent to the upper reservoir, and the second branch was sent to the oxygenator. The tube after the oxygenator was again bifurcated. One branch was connected to a tube leading to the upper reservoir, and the second (arterial) branch - to the explanted section of the aorta. (Fig. 3).

Dynamic scheme "ex vivo" of machine preservation. A distinctive detail of this scheme is the usage of only one pump (12), but two different flows for the liver are achieved at the same time. Namely, arterial pulsating blood flow enters the liver (2) from the abdominal aorta (7) through the vascular pedicle (6), and mixed (venous with a high oxygen content) blood from the upper venous reservoir (16) enters the portal vein (5) in a gravitational, non-pulsating flow. All hemocirculation tubes are equipped with dosing taps (22), which makes it possible to create the required blood flow in their lumen and regulate both pressure and volumetric blood flow. Thus, a stable blood level is maintained in both reservoirs and the required oxygen saturation of the venous blood in the upper reservoir (16) is maintained as well. On the perfusion scheme, sensors for temperature (19), pressure (20) and volumetric blood flow (21) are located in different areas.

The condition of the liver was assessed using morphological studies, monitoring of hemodynamic parameters in the portal vein and hepatic artery, as well as the amount of bile secreted.

Results and discussion

In all experiments, the preservation time reached 24 hours, and perfusion was carried out within the limits of physiological hemodynamic parameters (Tab. 1).

The perfusion apparatus pump capacity did not exceed 500 ml/min. The specified parameters were maintained throughout the experiment. The division of the blood flow before the oxygenator into two lines allowed one of them to pump arterial, oxygenated blood into the liver with a pulsating flow, maintaining the planned volumetric blood flow and pressure according to the initial parameters. The perfusate was supplied through the other line to a soft-walled upper reservoir located 50-70 cm above the perfused organ. The pressure in the portal vein was regulated by vertical movement of this reservoir. The dosing taps on the lines ensured, on the one hand, regulation of the volumetric blood flow of the incoming blood, and on the other hand, the possibility of optimal redistribution of various flows, stabilizing the levels in both reservoirs. Thus, in a soft-walled reservoir with mixed (splanchnic) blood, the calculated (up to 200 ml) level of perfusate for gravitational entry into the portal vein was maintained as standard. In the caudal vena cava, the pressure was maintained within the physiological norm (3-5 mm Hg) to exclude congestion in the liver. This was achieved by establishing a certain level gradient between the organ reservoir and the lower venous reservoir. The above hemodynamic parame-

ters were achieved by gradually increasing from minimum to average calculated values. The stabilized blood flow in the biotechnical system did not undergo abrupt changes requiring close attention and correction. This allowed the operator to leave to monitor general and biochemical blood parameters.

Histopathological evaluation of donor liver tissue is an important tool for predicting organ function after transplantation, especially in the case of marginal liver [9]. Steatosis is one of the risk factors for early graft dysfunction, which develops in 10-50% of patients with liver transplantation. Steatosis has been shown to be associated with an increased risk of ischemia and, accordingly, with worse clinical outcomes. An increase in the volume of hepatocytes due to fat accumulation is associated with deterioration of liver microcirculation. The latter causes a decrease in ATP production and an increase in lipid peroxidation [13]. However, different centers use different protocols for histological evaluation of donor liver for steatosis and other pathologies.

In five experiments that lasted successfully for 24 hours, liver damage was minimal and did not exceed the criteria for their suitability for transplantation (Fig. 4). No changes in glomerular structures were observed after 24 hours of "ex vivo" machine perfusion ($p<0.05$). However, the dilatation of the wall thickness of several arteries were found ($p<0.05$).

No bile ductular proliferation, cholestasis and venous congestion were revealed ($p<0,05$). Mild mixed ld-MaS and sd-MaS were found in less than 10% of the hepatocytes after 24 hours of "ex vivo" machine perfusion. At the same time, the number of necrotic hepatocytes, as well as portal triads infiltrated with mononuclear cells, did not exceed 3%. These data are higher than at the perfusion starting ($p<0.05$) but they continue to remain in the frame of standards suggested for the donor organs.

Based on all of the above, the results obtained indicate that changes occur in the liver preserved as a result of 24-hour machine perfusion that are quite compatible with successful transplantation and satisfactory functioning of the transplanted graft in the recipient's body.

Conclusion

- It is enough to use only one pumping equipment and two venous (upper and lower) reservoirs in accordance with the perfusion circuit developed and tested by us to achieve two different hemodynamic flows with different blood composition and content.
- The first performances (5) of experiments on animals in which a stable 24-hour duration was achieved with confirmed physiological activity of the isolated liver are indicative of the possibility of increasing the duration of multi-day perfusion.
- The perfusion device connected to isolated organ for long-term perfusion is the most challenging biotechnical complex. An analysis of regulation of such complex considers the two-way communication with the computerization of processes occurring both in the organ (monitoring, data analysis, decision making and performance), and in the device (pump capacity, oxygenation regulation, blood gas composition, pressure).
- The results of the experiments performed with a stable 24-hour period of ex vivo perfusion of the isolated liver indicate the possibility of a further increase in the duration of perfusion with the improvement of both the perfusion machine and the proposed technique.

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Table 1.

Hemodynamic parameters and blood condition during 24-hour liver preservation

Time / Parameters	Initial	8th h	16th h	24th h
Frequency (beats/min)	118±15	92±9	98±9	105±11
Total blood flow of the perfusion machine (ml/min)	-	600±60	600±50	600±50
Mean pressure in aorta (mmHg)	90,4±6,6	83,7±5,7	80,0±4,3	82,0±4,0
Pressure in portal vein(mmHg)	36±4	38±3	36±5	34±6
Pressure in caudal vena cava (mmHg)	5±0,5	5±0,5	4±0,5	3±0,5
Blood Flow in aorta (ml/min)	100±5	95±7	100±5	109±7
Blood Flow in portal vein (ml/min)	450±90	400±140	380±110	380±120
Blood flow in caudal vena cava (ml/min)	110±20	500±45	500±45	490±60
Amount of bile (ml)	-	48±14	82±28	114±45
pH	6,8±0,1	6,7±0,2	6,9±0,1	6,7±0,2
PO2 (mmHg)	78,6±3,2	68,4±4,0	70,3±4,8	62,0±6,5
pCO2 (mmHg)	27,3±2,2	29,9±3,4	29,0±4,5	24,2±6,0
Ht (%)	≤25±2,0	≤25±2,0	≤25±2,0	≤25±2,0
AST (unit per liter)	131±15	260±60	360±75	248±70
ALT (unit per liter)	32±10	31±14	32±21	59±33
Glucose (mg/dl)	110±8	122±8	240±10	156±11
ACT (min)	5±1	8±1	10±3	12±2
Bile acids (mmol/l)	35,0±5,4	38,0±5,0	38,0±6,1	39,0±6,6
Cholesterol (mmol/l)	2,6±0,4	2,5±0,6	3,1±1,0	3,6±0,7
Bilirubin (mmol/l according to Van Den Berg method)	0,34±0,11	0,44±0,15	0,33±0,2	0,34±0,22
Na+ (mmol/l)	165±21	1690±25	185±28	174±30
K+ (mmol/l)	5±0,5	5±0,5	7±1,2	6±0,9

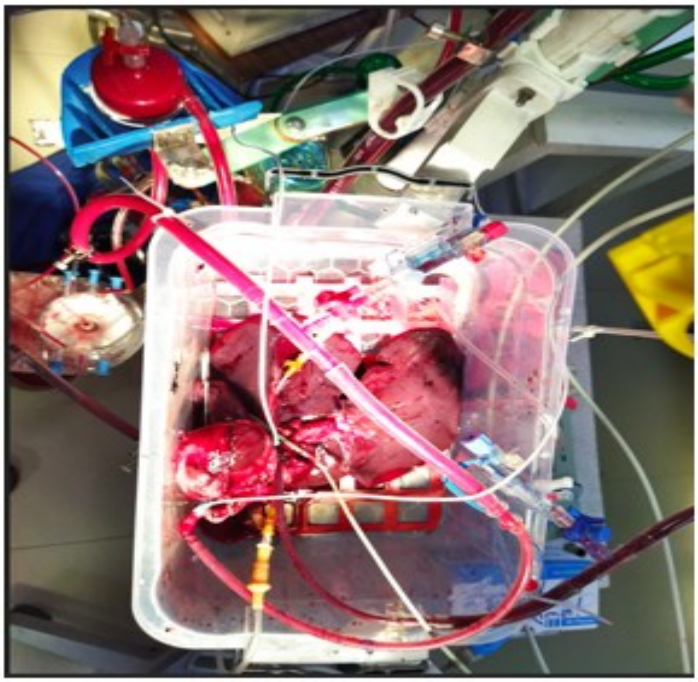


Fig 1

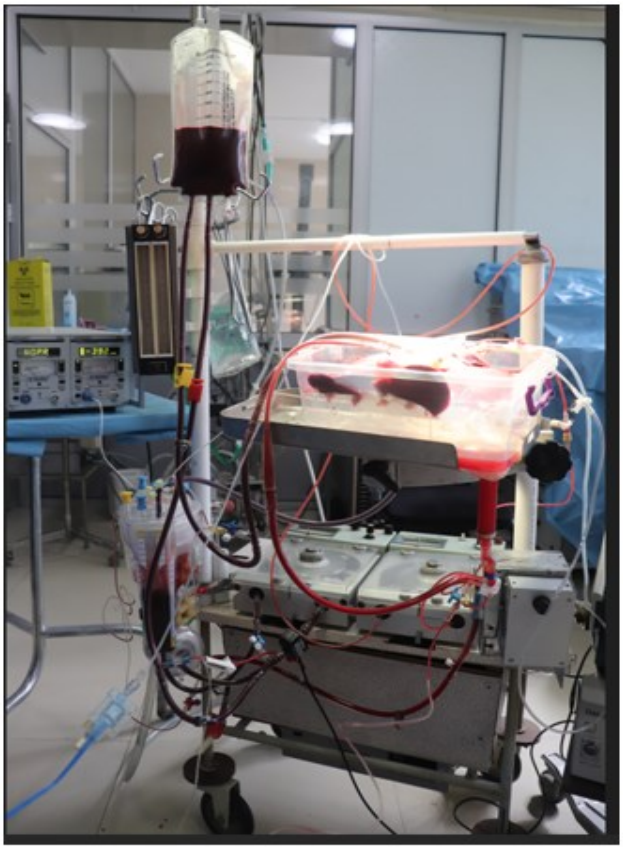


Fig 2

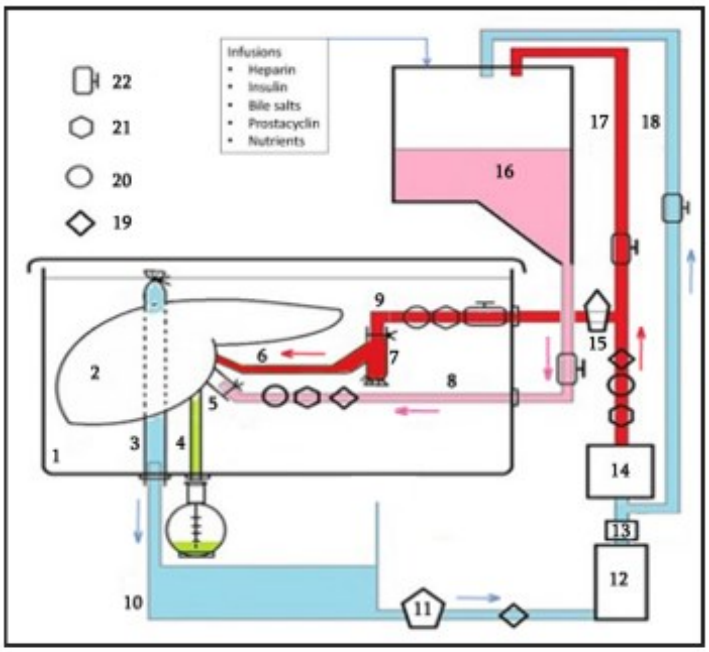
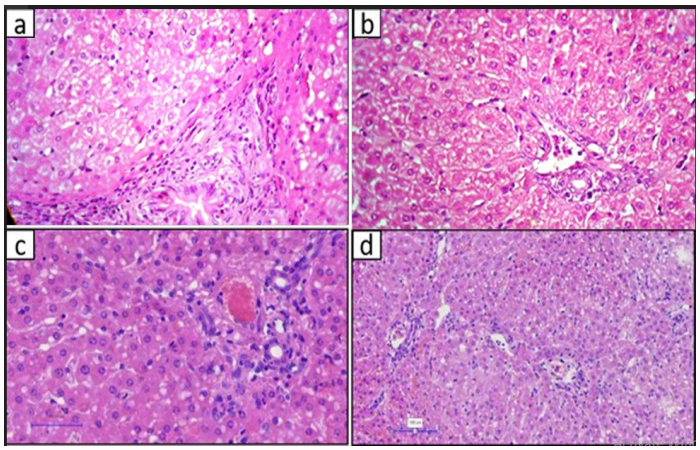


Fig. 3



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