Challenges and perspectives of surgical treatment of liver failure.

Current status and last achievements in Georgia

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Liver transplantation is considered to be the last hope of treatment for irreversible liver failure caused by different diffuse and/or space-occupying lesions of this organ. The strict limitation of the donor organs stipulates for development of alternative approaches for the solving this problem.
The presented review of literature and our experience aims to discuss the modern aspects of management of different hepatic pathologies causing liver failure with the view of creation of the auxiliary, bioengineer-based functional tissues and/or organs and innovative surgical interventions allowing to conduct the operations in cases, which were up to date considered as inoperable.
There are highlighted the last achievements of the experimental and translational studies performed in four University research centers of Georgia, which, on the one hand, provoke the specific professional interest, and on the other hand, require the international cooperation and collaboration for further progress and advances in this field of surgery.

KEY WORDS: Artificial liver, Bio-Artificial organs, Liver failure, Innovative surgery, Tissue engineering

Introduction

Liver transplantation is considered to be the last hope of treatment for irreversible liver failure caused by diffuse and/or space-occupying lesions of this organ. At the same time, the acute shortage of donor organs [1], as well as the complications, accompanying the rejecting reactions of the transplanted organ and/or prolonged immunosuppression [2], lead to the intensive search for alternative treatment methods.

This search develops into two strategic directions:
Bioengineering - as an organ replacement therapy, based on creation of auxiliary bioartificial functional tissues and/or organs in vivo and/or in vitro, which can be transplanted orthotopically or heterotopically [3-9].
Innovative surgery, allowing liver resections in cases which were previously considered as inoperable. The following techniques obtained the particular interest:
– Extensive and atypical extracorporeal hepatic resection with the further replantation of the remained part of the organ [10,11]; the method has successfully passed the clinical testing and currently the experience for its standartisation has been being gained;
– Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS); this approach implies ligation of the portal vein branch supplying the large volume of pathological liver tissue with its in situ separation (splitting) from normal tissue to induce rapid regeneration of future liver remnant (FLR); on the second stage the "regenerated" FLR allows the safe excision of

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the large abnormal hepatic mass without development of liver failure [12-15]. ALPPS methodology has been developed both in humans and in various experimental models, including small rodents [16-19], which is a good prerequisite for planning and development of new researches in this direction.
The presented paper aims to review some of above-mentioned approaches for the treatment of hepatic pathologies causing liver failure. In the review there are highlighted the last achievements of the experimental and translational studies performed in the Universities/ Research Centers of Georgia, which, on the one side, cause the wide professional interest, and on the other side, require the extended collaboration for further development of obtained results.

Methods

We used the Pub Med database for our search. From 2007 to 2018, approximately 150 articles were searched with the following complexes of keywords in the title and/or abstract: 1. "liver failure" and "surgical intervention " or "surgery" or "operation"; 2. "liver Bioengineering" or "bio-artificial liver" and "liver failure". Later, we have selected articles, published in the journals indexed in WOS and having impact factor (IF) or in Scopus. The obtained database was supplemented with those papers of Georgian researchers as well as their abstracts presented at international conferences - considered to be relevant to the aim of the given review.

Challenges and Perspectives of Liver Failure Treatment

Currently more than 350 million people are suffering from chronic HBV [20] and about 130-170 million people are infected with chronic HCV [21] throughout the world. Besides, a significant number of patients do not know that they have the viral hepatitis or its complications [22]. WHO estimates that 1.4 million people die each year due to such complications of above-mentioned pathology as acute hepatic failure, cirrhosis or liver cancer [23].
The autotransplantation of bone marrow derived stem cells (BMDSC) which is widely studied in the experiments and gradually replaced into clinical practice is considered to be one of the prospective method(s) for the treatment of cirrhosis or acute-on-chronic liver failure [24-30]. However, in the patients with decompensated liver cirrhosis this method is ineffective and the mortality rate ranges from 48% to 67% [31,32]. From this point of view liver transplantation is proved to be the last hope for their survival [33]. The same should be told in case of Wilson's disease when severe acute liver failure (ALF) with grade I or II encephalopathy is presented [34-36], as well as in Budd-Chiari Syndrome [37] or chronic hepatic failure, e.g. in long-term antitubercular treatment [38,39].
It is established, that the outcome of acute liver failure is lethal in the 50-90% of cases [40]. Liver Transplantation for Acute or Chronic Liver Failure is Based on EASL-CLIF Diagnostic Criteria. Living donor liver transplantation (LDLT) has excellent outcomes in patients with EASL-CLIF grade 1 and 2 ALF. Without transplantation, ALF patients have a very poor prognosis [41]. Generally, acute-on-chronic liver failure predicts adverse outcomes after orthotopic liver transplantation. Given the dismal prognosis without transplantation, the ALF patients can be transplanted with comparably good outcomes, in particular, the patients who improve under conservative therapeutic measures [42].
The deficit of donor organs and lack of timeliness prevents the application of the liver transplantation as an effective method of treatment [43,44].
In spite of the fact that many countries have established the deceased donor programs, it is the remarkable deficit of transplantable livers, that continues to make the thousands of patients hopeless [45].
In the developing countries, where the deceased donor programs are not established, only the half liver transplantation from living donors is remained as a real approach. The same situation is in Georgia where half liver transplantation has been performed only 31 times during 2014 – 2017 years [46]. All the above mentioned stipulate the attempts for seeking and development of alternative approaches for the treatment of liver failure.

Bioengineering-Based Interventions

The latest research confirms that a "liver bud" derived from human endogenous epithelial, endothelial and mesenchymal stem cells in vitro in three-layer culture (iPSC-LBs) can be successfully vascularized after its in vivo transplantation. The functional and structural development of the transplanted bud is similar to the liver development in embryogenesis [47]: in 48 hours the vascular network of the transplant becomes connected to the blood vessels of the host body and begins functioning and stimulating the further maturation of the transplanted liver bud. It begins synthesizing the liver-specific proteins - alpha-feto protein, retinol-tightening protein 4, transitrate and albumin [48]. The similar results confirming the metabolic activity of Scaffold-free 3D bioprinted human liver tissue were obtained by Kizawa H et al. (2017) [49]. These results raise the hope that growth of the bio-artificial liver tissue in the 3D bio-matrixes (bio-scarfolds), including the bio-degradative ones, should be even more effective [50,51].
The transplantation of in-vitro recellularized liver matrix as well as in vivo recellularization of the decellulaized single liver lobe have been studied through the experiments [52-54]. The results of the investigations of such

chimerial livers are quite promising, as their morphological and biochemical features reveal similarities to the native liver [55]. It appeared that histologically it is possible to identify the vascular and biliary structures as well as labyrinth of liver cells. In addition, the cells of these chimerical bio-artificial livers produce hepatocyte proteins and bile components and express the cytokeratins of progenitor cells and/or hepatocytes [56]. The functional activity of the in-vivo recellularized liver lobule was observed during 6 hours [57]. This finding provides the new stimuli for further studying of long-term functioning of recellularazed liver or its single lobe.

It is considered that liver cells optimally realize their synthetic and metabolic functions in a typical environment [58]. In this regard, interesting results are promised from the repopulation of the fully decellularized human hepatic scaffold by liver stellate cells, as well as hepatocellular and hepatoblastic cancer cells, which are distinguished by high viability and proliferation. The experiments showed that the cells successfully managed not only to maintain their viability, but also started the remodeling of the matrix.This observation represents a real breakthrough on the path towards development of bioartificial liver [55,59-61].

Special interest is raised by decellularized scaffolds that are obtained from non-autologous entire organs [62] or is created by means of artificial printing with the analogy of native liver matrix. The effectiveness of repopulation of cells in such scarfolds is largely determined by the ability of adequate vascularization [63]. This, in turn depends on how much the "printed" scaffold structure repeats the structure of the matrix of the native organ [64] and/or to which level it can activate (ensure) the membrane potential of the cells embedded in it [65].

Today, the scaffolds printed with the various polymers mainly focus on the peculiarities of microcirculation, particularly, the distance between the beds of the vascular structures should not exceed 1 mm. Nevertheless the preference is given to the reciept of the entire liver decellularized scaffolds, in which not only the matrix of microcirculatory network is preserved but also the large vessels (liver artery, portal and liver veins) which could be recellularized (endothelized) by the application of endotelicocytes of the umbilical vein. It should be noted that the complete successful decellularization of liver matrix was carried out with controlled machine perfusion during several days, with increasing pressure (the outflow of 350 ml the injection solution) [66].

Such perfusion can be achieved in the conditions of high-tensile strength of the above-mentioned vascular beds, which in addition to the firmness of their connective tissue structures should be also determined by sites of merging of connective tissue sheaths of portal triads and hepatic veins (intrahepatic porta-caval fibrous connections - IHPCFC) described by us previously [67,68] (the study was performed at Tbilisi State Medical University). It was shown IHPCFC provides especial strength and firmness to liver matrix stipulates the remaining of wholeness of the organ in condition of different impact; Later we found IHPCFC in dogs and rats that enabled us to introduce the hypothesis about the universality of these IHPCFC in all mammals and suppose the importance of taking into account their existence while printing of liver scaffolds [69] (the study was performed at Institute of Morphology, Tbilisi State University).

The combining of tissue bioengineering with surgical approaches showed the effectiveness of the pancreatic islets concluded into biocontainers for treatment the type 1 diabetes. The isolated and demucosated segments of the small intestine with maintained circulation [70], as well as auto vein segments were used as the biocontainers [71]. In the experiments conducted at Tbilisi State Medical University and at Uppsala University, pancreatic islets transplantation into the biocontainers have successfully replaced the pancreas transplantation for the treatment of insulin deficiency in case of type 1 diabetes. The same approaches were realized for the auxiliary liver [72]. The research hypothesis that the demucosated segment of small intenstine with intactive blood supply could host the transplanted liver micro fragments was tested in mice and rats [71,73,74]. The results of the studies (performed at Tbilisi State Medical University) confirmed that the liver tissue transplanted into intestinal segment (so call "sausage") was remodeled with the formation of new blood vessels and expression of different genes needed for angiogenesis. Transplantation of liver fragments into isolated segment of small intestine of genetically determined Dpp4-deficient rats and Non Albumin Rat (NAR) showed satisfactory metabolism, ability of synthesis and exocrine functioning, including the release of liver proteins. Similar results were obtained by Hata T, et al. [75]. The experimental studies showed that transplantation of liver mass (artificial liver) in the amount of 20-30% of host liver mass ensures the survival of the animals. So,it was concluded that, the liver generated in the intestinal biocontainer may be an effective alternative to the treatment of the liver failure and play the role of a "bridge" (temporary rescuer) between the therapy and transplantation.

Creation of applicable organs for transplantation by the means of scaffolds represents one of the most urgent problem(s) of modern transplantology [76]. The decellularized tissues of different organs were proposed for this purpose, however, finding the healthy donor tissue remains very serious problem [49,60].

Taking the above mentioned into the consideration, Choi et al. proposed the creation of the model of "auxiliary liver" from the human placenta [77]. Some authors believe that the use of human placenta as a scaffold is the best way in tissue engineering for the creation of a new bioinengineered organ, since the placenta is rich in well-developed arterial and venous vascular network, it is volumetric for seeding the transplanting cells and tis-

sues, its extracellular matrix contains numerous cytokines, chemokines and growth factors, chemotactic triggers (for instance, GCP, SDF-1) as well as triggers for angiogenesis and vasculogenesis (i.e. VEGF, HGF, EGF, FGF, PDGF, TGF-beta) [73,78]. It should be noted that some of these factors (VEGF, HGF, FGF, EGF, IGF-1, IGF-BP and etc.) are also featured by the hepatotrophic effect. In addition, as far as the placenta is the "organ that is being thrown away", it is easily accessible.

Z. Kakabadze and co-authors [79] suggested the implantation of the liver fragments (instead of cells) in the placental scaffold, as far as these fragments contain all types of liver cells. The investigators transplanted in the sheep the "hepatized placenta" prepared in accordance with this principle. The decellularized placenta with implanted autologous multiple liver fragments of 1-2 mm^3 size was introduced subcutaneously, in the ilio-inguinal areas of 7 Turkish female sheep, weighing 15-20 kg, that have developed acute liver failure due to the previously performed 85% hepatectomy. Seven female sheep of the same weight, which had also undergone 85% partial hepatectomy, were used as a control group. All seven animals of the control group died due to the liver failure within 3 days following the surgery, while 5 animals in the experimental group (71,4%) survived (two animals died due to vascular thrombosis and transplantant necrosis). Moreover, if a healthy sheep liver weighed 877 ± 216 g (n = 3), in the animals of control group (at the time of their death) the liver weighted 114 ± 55 g (n = 7), while in "hepatized placenta" transplanted animals, at the moment of their withdrawal from the experiment (after 20 days from the surgery) the liver weighed 370 ± 78 g (n = 5); Thus, the "hepatized placenta" implanted in the animals assist the survival of the animals following the massive hepatectomies as well as the initiation of the regeneration of remained liver and may be propose as the "auxiliary liver" in treatment of ALF (experiments were conducted at Tbilisi State Medical University) [79]. The authors conclude that the human decellularized placenta has a suitable anatomic structure for creating the different bioinengineered organs: its mechanically strong beds of blood vessels provide a good perfusion of tissues. Histological, X-ray (with contrast medium), radioactive (with the technetium (99mTc) mebrofenin and radiological (dopplerography and CT) studies have shown that the blood flow is satisfactory preserved after hepatization of the placental cotyledons.

Taking into the consideration that the in-growth of the implanted tissue into the extracellular matrix of the placental scaffold is accompanied by the migration of native endothelial and stromal cells promoting the revascularization of transplanted liver fragments, the authors suppose that the factors necessary for cells migration are maintained in the extracellular matrix of placenta [77].

Generaly after transplantation of recellularized organs the thrombosis is developed quite often due to aggregation of the thrombocytes in the blood vessels. This makes the additional restriction for the application of the decellularized organs for the transplantation, since limits the viability of the implanted tissue by several hours or days [78]. However, as far as placenta is characterized by higher mechanical resistance of the blood vessels and "binding ability" of arterial and vein defects, its transplantation should be accompanied by less complications due to the circulatory disorders [80].

Innovative surgical approaches

In the 70s of the last century, the hypotheses on the effectiveness of temporary transplantation of auxiliary liver from the deceased newborns to adult recipients in the cases of the ALF was developed.

The methods of transplantation of the still borns' livers to adults intraperitoneally (under the own liver, for long-term support) or extraperitoneally (in the right ileac fossa, for temporary support) were developed on the cadavers and later implemented in dogs (the study was conducted in Tbilisi Institute of Surgery) [81]. In the control group ALF was modeled with formation of "side-by-side" porto-caval shunt followed by one-hour total ischemia of liver with further restoration of the blood supply to the organ (Misra and Diaz model) [82-84]. After the implementation of the described model in adult dogs, the heterotopic transplantation of the livers obtained from the puppies aged 6-60 days was performed.

All animals of the control group died in 18,5 +/-0.95 hours after the surgery because of the hepatic coma. Morphologically, the massive necrosis of liver tissue was revealed. However, some hepatocytes remained viable (potentially reversible lesion).

The animals, which underwent auxiliary liver transplantation showed the perfect bile excretion and the rapid growth of the transplant, which was significantly higher than in the normal puppies of the same age. This indicated that the newborn's liver that was transplanted into the adult recipients went through the full functional and structural adaptation to the increased load. The hepatocintiography using Bengali pink I-131 has shown the normal functioning of the transplant in 48 hours after surgery and the maximal restoration of the recipient's liver in 3-5 days following the surgery. The normalization of the most physiological and biochemical indicators of the blood were detected. The morphological study confirmed a significant restoration of the liver's microstructure. Eight animals lived for 3-7 days after the auxiliary liver transplantation; 1 recipient who had undergone transplantatectomy on the third days after transplantation, lived for seven months. It was concluded that the functional capability of liver of the newborns is sufficient for restoration of the liver functioning and maintain the homeostasis of adult recipients in case of ALF caused by severe but still potentially reversible lesion [85,86].

The advanced and atypical extracorporeal liver resections with the further replantation of the organ is more and more attractive for the surgeons because the method allows the resection of advanced tumors, that were previously considered to be inoperable and the only method of their treatment was the total hepatectomy and orthotopic liver transplantation [10,11].

The universal "Machine for Artificial Blood Circulation" (Geopatent # U 1888) constructed by the scientists of the Alexandre Natishvili Institute of Morphology, Ivane Javakhishvili Tbilisi State University should facilitate the further development of this elite surgical method and confirmation of its efficiency. The pump of this machine is fundamentally different from the pumps, existing on the market. The device provides perfusion with the non-pulsative (laminar) as well as pulsatile blood flow. The universality of the device is determined by the fact that it is equipped with the pulsator that can work synchronously with the heart. The device is designated for the full heart-lung bypass, for ensuring continuous in-vivo and/or ex-vivo blood supply of the organs of brain- or heart dead donors.

Such isolated perfused ex vivo liver models have been used to study drug toxicity, liver failure, organ transplantation and hepatic ablation and combine advantages of both previous models [87], for the perfusional preservation of isolated organs and also for regional perfusion in the body of recipient [43,88]. However, the above-described device was successfully used also in experimental liver replantation on the various animals (rabbits, dogs, sheep).

On the one hand it allowed veno-venous bypass for the management of the ahepatic phase circuit (in order to minimize its negative effects), while on the other hand it provided continuous hemodynamic protection of transplant (replant) with native blood physiological flows throughout the whole surgery from the moment of explantation up to the end of replantation [89,90], thus excluding the reperfusial lesion of the replanted organ. Its blood vessels were switched to the venous and arterial lines of the perfusion system step by step. The splanchnic blood of the recipient was supplied to the portal vein with the manageable flow while the liver artery was supplied with the blood from the caudal vena cava, additionally provided by artificial pulsating and oxygenation [43,91].

Recently the interest of the scientists in this field has increased when the the same device was used for liver preservation by extracorporeal perfusion [92] (the study was performed at Tbilisi State University).

The investigation was performed on 6 sheep with simulated cardiac arrest and undergone 8-hours extracorporeal circulation. The device was connected to the body through the femoral artery and vein with special cannulas. The biopsy of the liver was performed before the starting of perfusion, and on 4 and 8 hours of the experiment. The histological slices were stained by H&E and were assessed by standard criteria: the degrees of steatosis (large-droplet macrovesicular steatosis [ld-MaS] and/or small-droplet macrovesicular steatosis [sd-MaS]), mononuclear portal inflammatory cell infiltration, bile ductular proliferation, cholestasis, venous congestion and hepatocellular necrosis.

Before the perfusion, no venous congestion, hepatocellular necrosis or ld-MaS were observed; less than 3% of cells were suffered by sd-MaS; mononuclear portal inflammatory cell infiltrates were found only in several areas. Mild mixed ld-MaS and sd-MaS was found in less than 5 % and 10% of the cells accordingly on the 4 and 8 hours after in vivo Machine perfusion. Similiarly the mild venous congestion was present in 1 out of 6 livers after 4-hours perfusion and in 2 out of 6 livers after 8-hours Perfusion. The number of necrotic hepatocytes and portal triads infiltrated with mononuclear cells did not exceed 10% and 15% accordingly. However, there were no differences in the degree of biliary damage – cholestasis or ductular proliferation - correlating with the terms of the experiment.

Taking into the account all internationally accepted criteria of donor liver histological assessment, 8-hour in vivo perfusion of the liver in Cardiac Death Donors by using of the machine providing the pulsatile blood flow guarantees the satisfactory preservation of liver making it useful for successful transplantation.

Conclusion

Bioengineering based interventions as well as innovative surgical approaches continue to be important methods alternative to-or supplemented the liver transplantation for treatment of acute/chronic liver failure.

We believe that the studies, that have been conducted at the universities/research centers of Georgia, should contribute to the determination of the future prospectives in this direction. These investigations, on the one hand, provoke the specific professional interest, and on the other hand, require the international cooperation and collaboration for further progress and advances in this field.

Riassunto

Il trapianto di fegato è considerato l'ultima speranza di trattamento per l'insufficienza epatica irreversibile causata da diverse lesioni diffuse e/o occupanti spazio di questo organo. La rigorosa limitazione degli organi donatori prevede lo sviluppo di approcci alternativi per la risoluzione di questo problema.

La revisione della letteratura presentata e la nostra esperienza hanno lo scopo di discutere gli aspetti moderni della gestione di diverse patologie epatiche che causano insufficienza epatica al fine di creare tessuti e / o organi

funzionali ausiliari, basati sul bioingegnere e innovativi interventi chirurgici che consentano di condurre le operazioni in casi che erano stati considerati inoperabili.
Nella rassegna vengono in particolare evidenziati i risultati degli studi sperimentali e traslazionali condotti in quattro centri di ricerca universitari della Georgia, che, da un lato, suscitano l'interesse professionale specifico e dall'altro richiedono la cooperazione e la collaborazione internazionale per ulteriori progressi e progressi in questo campo della chirurgia.

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