Role of the oxidative stress and arterial blood supply in the function of the transplanted liver graft

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Introduction

Every year hundreds of thousands of people die of hepatic failure all over the world. In Hungary this number is about 7000. The majority of patients waiting for transplantation are suffering from chronic hepatic disease, but in 15% of all cases transplantation is indicated because of fulminant hepatic failure, which has a high mortality of over 80%. LTX is probably the most effective therapy for acute and chronic hepatic failure because of its radicality and its prolonging patients’ survival. The results of LTX are today very good with a 65-80% 3-year Kaplan-Meier survival. In Hungary more than 150 LTX have already been done in the past few years with a survival rate of 67% without reference to the indications generating need of transplantation. Postoperative prognosis is influenced not only by the indicating diseases but also by the general conditions of patient and the perioperative management.

Although LTX is part of the routine surgical interventions in our country, there are many separate factors during the operations. The efficiency depends not only on the basic disease and on the surgical techniques but on immunological and oxidative courses as well. The early and late graft function hangs on the preservative and reperfusion injuries and the blood supply. Technically the most difficult moment is to provide good arterial blood supply. The arterial circulatory failure of the liver-graft causes serious hypoxia and therefore increases the preexisting high-grade oxidative stress. The above-mentioned factors are leading to acute and chronic graft lesions and usually the patients can be saved only by retransplantation. So there is growing expectation to eliminate all the factors that may cause the worsening of survival probability of the patients, who are in a bad condition anyway. One of these agents is the absence of the “ideal” arterial blood supply. Arterial thrombosis after LTX is the most common complication. Its frequency is above 10% and it is more often by pediatric recipients.

Risk factors of the hepatic artery thrombosis are the following: small size of the arteries, wide range of anatomical variations and usage of donor arterial conduits.

It often happens in significant portal hypertension that the common hepatic artery is thin and the arterial tension is less than the average. The significantly developed splenic artery “steals” the blood because of the hyperkinetic splenic circulation. In these cases aneurysms of the splenic artery are more common (13%) and their incidental rupture after transplantation has a tragical outcome. As LTX techniques are progressing, the variations of arterial perfusion difficulties grow and there is an increasing need for finding new alternatives. Among the factors influencing graft function, uncontrolled free oxid chain reactions have destructive effects on all cells. The main particle of the known mediators and enzymes in the antioxidative system is myeloperoxidase enzyme (MPO) synthetised by neutrophiles.
Aimes

The main purpose of my essay was to evaluate the role of oxidative stress, which determines the prognosis of patients after LTX and to find new alternatives for solving difficulties in connection with arterial blood supply of the graft.

My aim was to measure the rate of the oxidative stress before and after the operation.

I wanted to analyse the change of MPO in the mirror of the postoperative complications, with special regard to the early arterial circulatory failure. In connection with the arterial supply of the liver-graft I wanted to measure up the risk factors, that are most common in the view of the complications. I analyzed the condition of arterys from the aspect of portal hypertension, the relation of the liver and the splenic artery, and the danger of aneurysms in the splenic artery. Besides working out the procedure how to generate arterial anastomoses, I processed my experimental results of the “ideal” arterial graft.

In my script I wanted to find solution to the following issues:

1. How does the level of the MPO change before and after the LTX?
2. What kind of connection is there between the rate of the MPO and the complications, prognosis?
3. How much do arterial thrombosis and complications influence the rate of the oxidative stress and graft function?
4. Can we use MPO for monitoring the rate of the oxidative stress after LTX?
5. Which are the most common risk factors in the development of hepatic artery thrombosis in LTX patients?
6. What are the features of arterial blood supply in portal hypertension?
7. What could be the ideal solution for a vascular graft in LTX?
8. What can we do for reducing the oxidative stress in case of LTX and how can we prevent the complications in the arteries?

Summary: my aim was to examine the oxidative stress status in the recipient patients in the mirror of the clinical complications and achievements, with special aspect to arterial blood supply difficulties.

Material and Methods

I used clinical and experimental methods in order to examine the blood supply of arteries in implanted liver and to understand the role of free oxides. I analysed the LTX patients data in a prospective and retrospective way and the possibilities of the “ideal” arterial graft through animal experiments.
Patient’s data

Patients group for the MPO: prospective clinical examination was based on 32 liver transplanted patients. Patients group for splenic artery aneurysm: 337 Dutch and 150 Hungarian LTX patients data have been analyzed through retrospective and prospective examination.

Methods

Patients demographic and clinical data were compared in the course of following liver transplantations, with particular attention to the acute complications.

We performed the MPO enzyme monitoring deliberately in the peri- and postoperative period. We took MPO-EIA ”sandwich” ELISA technology (Bioxytech MPO-EIA, catalogue number 21013, Oxis Research). We measured polyclonal anti-MPO reaction in pursuance of the examination (aL 405 nm).

In the course of examining the splenic artery aneurysm we analyzed the angiographs made earlier-, and one year after (as a control) the operation. We analyzed the rate of the liver and splenic artery besides the splenic artery aneurysm.

We operated dogs for the serial vascular graft experiments and used posterior rectus sheath lined with peritoneum as a tubular autologous graft (RS-graft).

We sewed in the graft as an external iliac artery interpositum and it was followed for a 6 month period.

After the first experiment the RS-graft was examined for 1 month without immunosuppression. We used von Willebrand factor (vWF) expression technology (immunohistochemistry) and hematoxylin-eosin fluid for represent the endothelial cells. The point of the experiment was to examine the RS-graft for a longer period by using immunosuppression. During the examination a wider histological elaboration was lead for adjudging the vitality and the morphology of the graft. We extended the labor examinations for having a better view of the tissue oxygenisation.

We used immunohistochemistry methods (von Willebrand expression, CD 34) to analyze the endothelial cells. To prove the neoangiogenesis we followed the change of the micro-vessel density in the RS wall. We controlled the viability of the finer structures by electronmicroscopical examinations (Philips, 2000*-150000) and made efforts to describe the cell layer covering the wall of the lumen. We set cell cultures from the newly removed preparations.
Results and theses

The early and late function of implanted liver is influenced not only by the used preservation solutions and reperfusion injuries but also by the adequate blood supply of the graft. Most of the complications are related to lack of this basic requirement. Failure of arterial patency may occur the existing systemic oxidative stress becomes worse. Based or on studies the following conclusions can be made.

1. The function of donor liver after implantation depends on preservation, reperfusion as well as on arterial blood supply. After reperfusion the body is exposed too a considerable oxidative stress which could get even worth with subsequent hypoxia and necrosis if occlusion of hepatic artery occurs. The incidence of arterial trombosis is about 10% and it is higher in pediatric recipients.

2. Prospective clinical studies give the evidence that monitoring MPO in blood can be used to estimate oxidative stress after liver transplantation.

3. The most commonly reported indication was hep c infection (43%). In 32 cases 34 operations were performed. Two of these were retransplantations. The operations were successful in 24 cases (75%), 8 patients died of different complications. In two cases the indication were acute hepatic failure which proved to be unmanageable by transplantation. In other 2 cases PNF occurred after the operation. In the remaining 4 cases late complications were blamed to patients mortality.

4. In 4 cases of 32 (11%) early hepatic artery trombosis occurred but thanks to the immediate interaction (trombectomy use of aorta conduit or retransplantation) the complication became under control.

5. Before the operation we found an abnormally high MPO level of 65 ng/ml in peripheral blood of the recipients. 48 hours after the transplantation there was an even higher level of MPO (123 ng/ml) which was a significant difference compared to the initial preoperative state (p < 0.0001). By the end of the third week the MPO concentration fell back near to the initial level (65 ng/ml). In some cases of acute hepatic failure and arterial trombosis we could detect an extreme high MPO level of more than 300 ng/ml. This significant change in the level of MPO was characteristic for acute cases after transplantation. The only exception is rejection when there is no significant change.

6. Patients with hepatic cirrhosis waiting for LTX have a special splenic circulation. In portal hypertension because of hypercinetic perfusion of the spleen the splenic artery improves a stronger blood flow. As a result hepatic perfusion declines considerably since adequate intraluminar pressure is at least as important as good patency of the artery for sufficient blood flow. In cirrhotic patients the higher blood pressure in splenic artery can lead to aneurysms in the arterial wall, which easily ends up in a sudden rupture and subsequent bleeding.

7. Out of 150 Hungarian recipients 13 patient developed splenic artery aneurysm (3 of them were soliter, 10 multiplex dilatations). Only cirrhotic patients had such complications, fortunately without any bleeding. 2/3 of them were women, which let us suppose a female predominance. There were no aneurysm at all in hungarian pediatric patients.
8.45 recipients of 337 Dutch patients had similar complications. Adult recipients had more frequently aneurysms than children. The 2:1 female-male proportion showed female predominance again. Aneurysms were multiplex in 87% and most of them (another 87%) were located to the distal part of splenic artery. The splenic artery was dominant to hepatic artery in size and flow in all cases. The proportion between the diameters were 1.34 with solitary and 1.23 with multiplex aneurysms in splenic artery wall. When patients had no cirrhotic disease they failed to develop splenic artery aneurysm neither. On the contrary cirrhotic patients with parenchymal liver disease had more often this vascular abnormality: 17% (p<0.001). Two patients died early after the operation due to fatal bleeding caused by splenic artery aneurysm rupture. Controll examinations showed no further change in number or size of developed aneurysms.

9. Hepatic artery thrombosis is a major cause of early graft failure. In many cases the only solution possible is retransplantation with a relative high mortality. Considering this fact it is easy to understand why it is so important to provide an ideal arterial blood supply for liver. During the transplantation procedure fresh or cryopreserved arterial segments from cadaver are widely used, however there are many problems with them. Their quality is often questionable and the amount and size of them are not always appropriate. These grafts could serve as a possible solution for anatomical hepatic artery variations, especially in case of segment liver transplantation (split, living donor). If the recipient has a weak hepatic artery the vessels from donor could be used as aortic conduit. Preservation, storage, and postoperative rejection could damage these arteries and cause a long term sclerosis of graft wall within months or years.

10. Our experimental study was triggered by arterial complications after liver transplantation. We tried to create a vascular graft from autologous material. According to the results of 1 and 6 months studies we can say that grafts made of posterior rectus fascia sheath lined with peritoneum on the inside can be used as vascular replacement. Grafts were all viable after 6 months (8 cases) and no thrombosis occurred. Doppler US found normal flow everytime. Histological examinations showed a special structure similar to that of an arterial wall. There were fibroblasts, smooth muscle cells and a great deal of elastin as a product of these cell types in the graft wall. Small capillars as sign of neoangiogenesis were also seen. The intarluminar surface of the graft was lined totally with endothelial cells but there were no mesothelial cells at all. EM showed intact mitochondria in smooth muscle cells which was another evidence of viable structure. The postoperative immunosuppression did not harm the graft wall. Tissue oxygenisation in the examined limb was sufficient. There were normal MPO level and blood gas parameters in all cases. The low thrombogenicity of the graft could be explained by the preexisting mesothelial monolayer of the graft which was exchanged gradually by endothelial cells. Further investigations are required to get more information of the graft and its clinical behaviour.

Conclusions and practical advice

Based on our clinical and experimental studies we can conclude that function of implanted liver depends on presence of preservation and reperfusion injuries and on adequate arterial blood supply. After reperfusion the liver and the whole body is exposed to a considerable oxidative stress, which easily get worse if there is any
arterial complications. Monitoring the level of MPO before the operation it is clear that recipients are in a higher oxidative stress status and this becomes more serious after the transplantation especially in case of acute liver failure and arterial thrombosis. Calcineurin inhibitors are well-known antioxidants and so do selen and antioxidant vitamins. In order to reduce reperfusion injuries the time of cold and warm ischemia should be shortened (piggy-back) and surgical techniques should be performed properly. Sudden changes in temperature are to avoid and good arterial blood supply should be maintained. Although different types of arterial variations can mean a real challenge for operating surgeons the construction of ideal arterial anastomoses and appropriate blood supply is extremely important. It is also very important to find out before the operation whether there is any aneurysm of splenic artery (in portal hypertension it is a very common complication: 8-13%). Solitary aneurysms can be resected or the splenic artery can be ligated in case of multiplex disorders. This way the hepatic artery circulation could be better and the splenic artery itself could be used when creating the anastomosis. All in all we have to make efforts to avoid reperfusion injuries and so oxidative stress and to provide good blood supply to all implanted liver grafts.
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