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Impact of Ig-Therasorb® Immunoapheresis on Stability of Xenogeneic *Ex Vivo* Porcine Liver Perfusion – Value of Aminotransferases and Flow Rates for the Assessment of Metabolic Graft Viability

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Due to growing shortage of donor organs, the concept of extracorporeal pig liver perfusion in the treatment of acute liver failure has been rediscovered. Immunomodulation, such as immunoapheresis or inhibition of complement, results in long-term perfusion without exact knowledge of the remaining metabolic graft viability. This study was aimed at the comparison of conventional parameters of graft stability such as perfusion rates and release of aminotransferases with parameters of metabolic graft function. Ig-Therasorb® immunoapheresis (IA) of the xenogeneic perfusate was performed to protect the discordant pig livers from hyperacute rejection, mediated by preformed naturally occurring human xenogeneic antibodies. The application of IA created stable autologous graft reperfusion after a short time of xenoperfusion, but it was not able to prevent the livers from severe synthetic and functional damage. In the future, improvement of xenogeneic graft function, rather than pure prolongation of perfusion, must be the principal aim.

Key words: Extracorporeal pig liver perfusion; Immunoapheresis; Metabolic function.

Abbreviations: ALF, acute liver failure; ECLP, extracorporeal pig liver perfusion; GE, galactose-elimination-rate; GLDH, glutamate dehydrogenase; HXR, hyperacute xenograft rejection; IA, Ig-Therasorb® immunoapheresis; MEGX, monoethylglycinexilidide-test; THP, total hepatic perfusion; XNAb, xenoreactive naturally preformed antibodies.

Introduction

The dramatic shortage of donor livers has led to a high mortality of patients awaiting urgent liver grafts. In this context, extracorporeal pig liver perfusion (ECLP) has experienced a revival in the 1980's and 1990's (1, 2). As a discordant organ, the porcine liver undergoes hyperacute xenograft rejection (HXR) mediated by preformed, naturally occurring, human anti-pig xenogeneic antibodies (XNAb) and complement (3, 4). Immunomodulation of the xenogeneic perfusate results in a significant prolongation of perfusion periods but without any indication of the remaining metabolic graft viability and associated potential benefit for the patient with acute liver failure (ALF) (5–7). Conventional parameters for determination of xenogeneic liver function are the release of aminotransferases and development of perfusion flow rates (1, 2). However the value of these parameters for evaluation of the livers' metabolic capability for detoxification and thus for the clinical use remains speculative. The aim of this study was the comparison of conventional parameters of graft stability with the capability for detoxification and synthesis of a xenogeneic pig liver using autologous reperfusion. Ig-Therasorb® immunoapheresis (IA) of XNAb was performed to delay HXR. The results are expected to help identifying beneficial graft function and thus to avoid harmful periods of perfusion for the patient with ALF.

Materials and Methods

Animals ex vivo perfusion

For ECLP, livers of 18 Landrace pigs (body weight: 25–30 kg) were carefully explanted after *in situ* flush with 2000 ml University of Wisconsin solution (0 °C) and collection of autologous blood. The grafts were normothermic *ex vivo* perfused with 1500 ml of fresh human blood (8, 9). Flow rates and vascular pressure were continuously measured online (Labtech, Spectra Computersystems GmbH, Karlsruhe, Germany). Cannulation of the Ductus choledochus permitted determination of bile production.

Ig-Therasorb® immunoapheresis

To deplete XNAb we used a pyrogen-free Ig-Therasorb® glass column containing polyclonal sheep anti-human IgG antibodies (heavy and light chain-specificity) conjugated to cyanogen bromide-activated Sepharose beads (Plasmaselect, Teltow, Germany). They are able to bind all four subclasses of IgG antibodies as well as IgM and IgA antibodies. By enzyme-linked immunosorbent assay (ELISA) and nephelometry we could measure a reduction of IgG, IgM and IgA of 92%, 75% and 78% respectively, comparable with results already published (7, 8).

Analytical studies

Graft stability was assessed by online measurement of total hepatic blood flow (THP, sum of portal and arterial flow rates). The release of the mitochondrial glutamate dehydrogenase (GLDH) served as a parameter for severe hepatocellular damage (Hitachi 917 Rack, Roche Diagnostics, Mannheim, Germany). Synthetic graft function was analyzed by continuous measurement of bile production. Metabolic capability for detoxification was determined by monoethylglycinexylidide-test (MEGX) and galactose-elimination-rate (GE). MEGX production was measured before and 30 minutes (180 min of

reperfusion) after injection of lidocaine (1 mg/kg liver weight) by fluorescence-polarisation-immunoassay (TDx/TDxFLx-System, Abbott Diagnostics, Chicago, USA) (10–13). GE was analyzed after 120 min of autologous reperfusion by spectrophotometric evaluation of concentration (NaF-plasma7D-galactose DH UV-test, Roche Diagnostics, Mannheim, Germany, spectrophotometer DU 7500, Beckmann Instruments Inc., Fullerton, CA, USA). The concentration of D-galactose 5 min after adding to perfusate the dose of 0.5 g/kg liver weight was set as 100% value. The following concentrations (15, 30, 60 min) were calculated as retention in percent (8).

Experimental groups

Xenogeneic perfusion was stopped after 15 min, when hepatic flow-rates deteriorated due to HXR. In group 1 (G1) porcine livers were perfused with their own blood (autologous, control group). Pig livers of group 2 (G2) were perfused with human blood without pre-treatment (xenogeneic). In group 3 (G3), Ig-Therasorb® immunoapheresis was used for depletion of XNAb (apheretic). After perfusion, all organs were cold reflushed. Reperfusion of the organs was performed with autologous blood for 5 hours. Values were expressed as means ± SD. Statistical differences were analyzed by two-way repeated analysis of variance and by Student's t-test. They were considered significant at $p < 0.05$.

Results

Hemodynamics

In the first 30 min of autologous reperfusion, THP was low in all groups. Subsequently, perfusion improved in

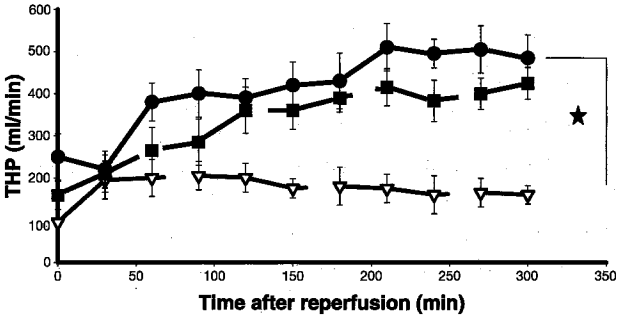

Fig. 1 Total hepatic perfusion (THP) in the studied groups. At the beginning of autologous reperfusion, THP was low in all groups. In G1 (autologous; ●) and G3 (apheretic; ■) perfusion recovered subsequently but not in G2 (xenogeneic; ▽) (*p<0.05).

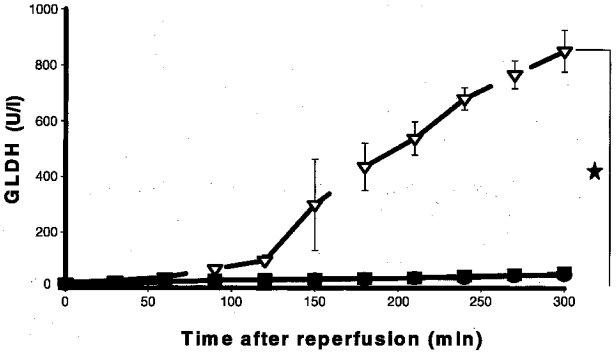

Fig. 2 Glutamate dehydrogenase (GLDH) activity in the studied groups. GLDH levels increased significantly in G2 (xenogeneic; ▽) (*p<0.05). GLDH levels in G1 (control; ●) and G3 (apheretic; ■) were comparable.

Tab. 1 Bile production during autologous reperfusion.

Bile production (µl/g liver/h)	Time after reperfusion (min)	Group 1 (autologous)	Group 2 (xenogeneic)	Group 3 (apheretic)
	60	6.3 ± 1.8*	0.2 ± 0.1	0.6 ± 0.2
	120	12.8 ± 2.8*	0.9 ± 0.2	1.4 ± 0.2
	180	9.8 ± 1.8*	1.0 ± 0.2	1.9 ± 0.3
	240	6.5 ± 1.7*	0.8 ± 0.1	1.2 ± 0.3
	300	6.8 ± 1.7*	0.4 ± 0.1	1.0 ± 0.2

Mean ± SD are given; * $p < 0.05$ G1 *vs.* G2 and G3

Tab. 2 MEGX-production and galactose elimination rate during autologous reperfusion.

Galactose elimination (Retention, %)	Time after reperfusion (min)	Group 1 (autologous)	Group 2 (xenogeneic)	Group 3 (apheretic)
Start	120	–	–	–
	125	100	100	100
	135	56.5 ± 4.2*	82.3 ± 5.6	76.5 ± 5.5
	150	18.4 ± 4.8*	68.8 ± 4.6	64.6 ± 4.9
	180	16.3 ± 3.8*	59.6 ± 3.9	55.9 ± 4.1
MEGX-production (µg/l)	210	141 ± 18.5**	41.5 ± 8.9	84.7 ± 9.8***

Mean ± SE are given; * $p < 0.05$ G1 *vs.* G2 and G3, **$p < 0.05$ G1 *vs.* G2 and G3, ***$p < 0.05$ G3 *vs.* G2

the control group (G1) and the apheretic group (G3). Autologous reperfusion did not lead to recovery of THP in the xenogeneic group (*p<0.05) (Figure 1).

Hepatocellular damage

There was a significant increase of the mitochondrial GLDH level in G2, despite autologous reperfusion. GLDH levels were not significantly different between G1 (control) and G3 (apheretic) (Figure 2).

Liver function

Bile production of xenogeneic (G2) and apheretic (G3) liver grafts was significantly lower during autologous reperfusion (Table 1).

While MEGX-production in G3 was still higher than in G2, galactose elimination rate did not differ significantly from xenogeneic grafts (Table 2).

Discussion

The increasing knowledge about the value of XNAb and complement has led to several strategies to prolong experimental and clinical discordant xenogeneic ECLP (3, 8). Terajima *et al.* reported xenogeneic pig liver perfusion of 9 hours after administration of prostaglandin E1 to the perfusate (5, 14). Immunoapheresis has been demonstrated to delay HXR and thus to improve perfusion (6). Just recently, Levy *et al*. reported two patients with liver failure that were bridged to transplantation by ECLP with transgeneic organs (15). In all these cases, however, it still remains speculative whether long-term ECLP is beneficial or even harmful for the patient. We assumed the clinical benefit to be very much dependent on the remaining xenogeneic grafts' metabolic capability during ECLP. The results of our study demonstrate the harmful effect of a short-term xenogeneic pig liver perfusion despite autologous reperfusion. The application of IA resulted in stable reperfusion but not in appropriate graft function. In the clinical situation, sufficient metabolic support and capability of detoxification is the main intention of ECLP. The ability to produce bile is one of the most valid parameters of liver function (14, 16). In addition, MEGX formation has been shown to provide useful prognostic values regarding experimental and clinical liver graft survival (9, 13). MEGX formation is mainly mediated by the microsomal cytochrome P-450 system by de-ethylation of lidocaine. Decrease in the activity of this system is known to be associated with hypoxic liver damage, which could be also a result of endothelial disruption due to xenoreactive organ damage (14, 16). Indeed, galactose elimination rate is a very non-specific parameter of function; however, in our study it confirmed the metabolic insufficiency of xenogeneic perfused grafts.

For the clinical application of ECLP, measurement of perfusion rates and release of aminotransferases are not parameters with sufficient power to provide assessment of the therapeutic course. In the future we must focus on improving xenogeneic organ function, rather than on pure prolongation of xenogeneic graft perfusion.

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