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Expression of human decay accelerating factor (hDAF) in transgenic pigs regulates complement activation during ex vivo liver perfusion – immunopathological findings

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Abstract Ex vivo perfusions of human decay accelerating factor-expressing transgenic ($n = 3$), and nontransgenic ($n = 6$) porcine livers with human blood revealed a higher degree of organ damage in nontransgenic pig livers. Transgenic livers were protected from immunohistologically detectable complement deposition, despite corresponding IgM and IgG deposits in both groups. Complement activation and consumption of C3 and C4 turned out to be lower in transgenic pig livers. In contrast to livers of normal landrace pigs, livers from genetically manipulated pigs showed no morphological alterations after perfusion.

Key words Decay accelerating factor · Transgenic pig · Ex vivo perfusion · Immunohistology · Complement

Introduction

Extracorporeal liver perfusion for elimination of toxic metabolic products causing hepatic encephalopathy after fulminant hepatic failure has regained importance in the last few years. Due to progress in immunosuppression and, for example, antibody depletion techniques, some obstacles for its clinical use could be cleared. The current case reports [4, 6] about discordant heterotopical hepatic transplantation and extracorporeal perfusion, however, show that there are still severe problems ahead, influencing the viability of the perfused organ and the clinical outcome of patients as well. Xenogeneic ex vivo perfusion of isolated pig livers is closely related to the idea of therapeutic extracorporeal perfusion, providing the possibility of simulating benefits and, even more important, risks of extracorporeal liver perfusion prior to clinical application. A new aspect is the use of genetically manipulated porcine livers expressing the membrane-bound human decay accelerating factor (hDAF) [5, 9, 11]. The purpose of this study was to compare the degree of complement activation during perfusion of transgenic, hDAF-expressing and nontransgenic pig livers, as well as to examine differences in the deposition of complement components on their endothelial cells.

Materials and methods

After hypothermic perfusion via the portal vein and the hepatic artery with 4 °C University of Wisconsin (UW) solution, three transgenic pigs were hepatectomised in deep anaesthesia within a multiorgan explantation. Cold storage and preparation time were 90 min in total. The median liver weight (LW) was 535 ± 42 g. Liv-

ers of six nontransgenic pigs (LW = 689 ± 29 g) were treated equally and served as the control group. The livers were wrapped in a waterproof plastic bag and suspended in a waterfilled perfusion chamber. Heparinised human blood (10 IU/ml) of two donors with identical blood group (BG 0, A or B) and Rh factor was diluted to a haematocrit of 30 % for perfusion. The volume of diluted blood amounted to approximately 1500 ml. After the first passage through the liver, a blood volume which corresponded to about 1 ml/g organ weight was removed from the circuit, to avoid a wash- in of UW solution, potassium, etc. into the circuit. Perfusion was carried out for 3 h. During perfusion, pressure, temperature, pH and oxygenation were controlled, and blood samples taken at several time points. Tissue specimens taken before and after perfusion were prepared for routine staining, detection of neutrophils and platelets, immunohistology and fine-structural analysis. An indirect immunoperoxidase technique was used for immunohistology. An avidin-biotin complex (ABC) kit (Dianova-Immunotech, Hamburg, Germany) was combined with an avidin-biotin blocking kit (Vector Laboratories, Calif., USA) and aminoethylcarbazole (AEC, Sigma-Aldrich, Deisenhofen, Germany) as chromophore. The antibodies chosen for detailed analysis of complement and preformed natural antibody deposition included monoclonal mouse anti-human antibodies against C3c, C3d, C4c, C4d, SC5b-9 neoantigen (MAC), factor P (Quidel, San Diego, Calif., USA), IgM and IgG (Dako, Hamburg, Germany). Sections from at least ten corresponding loci of each organ were stained for these antigens. In addition, biochemical parameters such as electrolytes, transaminases and other enzymes, as well as bile flow, increase of organ weight and vascular resistance, indicating liver cell damage and functional restrictions of the organs, were determined (data not shown). CH50 and AP50, as well as serum levels of C3 and C4 as markers for complement activation and consumption were analyzed.

Results

In the control group ($n = 6$), organs were positive for all complement factors in six to nine sections of, in average, 14 treated sections per single factor and organ (43–65 %). Livers of transgenic pigs ($n = 3$) were C3d-, C4c- and MAC-negative. C3c staining occurred in one single case. C4d and properdin were detectable though less extensive than in nontransgenic livers. The main effects concentrated on branches of the interlobular arteries. Venous staining occurred less often and, in this case, affected portal venous vessels. In both, control and transgenic livers, there was no complement and antibody deposition on hepatocytes, sinusoidal lining cells, central veins, epithelia of bile tracts and on hepatic veins. Hence, the immunoreactive product was predominantly detectable on endothelial cells and, to a minor degree, on the myothel and perithel of arteries. No difference occurred in the deposition of the preformed natural antibodies (PNAB), IgM and IgG, between transgenic and nontransgenic organs. Concerning morphological alterations, predominantly midzonal, but sometimes also pericentral areas of necrosis were present in nontransgenic livers, accompanied by hyperaemia, sometimes even haemorrhagia, whereas specimens from transgenic livers showed no signs of necrosis. Correspondingly, the fine structural analysis of transgenic livers revealed no alterations of morphology. In both groups, however, disseminated infiltration and sequestration of granulocytes appeared in the sinusoids, accompanied by leucocyte casts in vessels. Furthermore, there was evidence of thrombocyte aggregation in sinusoids of livers in each group. Moreover, thrombosis, especially in portal venous vessels, occurred in transgenic and nontransgenic pig livers. Biochemical analysis of the blood samples supported the histological results. In particular, transaminases, glutamate dehydrogenase and potassium reached higher levels in control livers (data not shown). Plasma levels of C3 and C4 showed a decrease during perfusion of both transgenic and nontransgenic livers. In human DAF-expressing livers, a steady state was reached at about 85 ± 5 % (C3) and 84 ± 2 % (C4) of original values after 60 min. In normal pig livers, no steady state was reached and levels decreased to 60–70 %. CH50 and AP50 determination by total haemolytic complement assay confirmed a higher degree of complement activation in the control livers. Classical and alternative pathways of the complement cascade were activated similarly in the control group.

Discussion

The results of ex vivo liver perfusions of hDAF-expressing organs evidenced the effectiveness of this strategy to overcome the activation of the human complement cascade following the binding of human preformed natural anti-pig antibodies to the porcine liver endothelium [3]. Though it was reported previously that the targets of these antibodies, gal α(1,3)gal epitopes, were more or less omnipresent in pig livers [7], binding of IgG and IgM as well as complement factors concentrated on arterial and, less distinctly, on portal venous vessels in nontransgenic livers. Despite the corresponding antibody deposition in transgenic livers, only trace deposits of C4d and properdin could be observed in transgenic livers. The plasma complement levels concurrently remained higher during perfusion of transgenic livers. Morphological changes in the nontransgenic livers and the lack of alterations in transgenic organs confirmed a higher extent of organ damage. These findings suggest that genetic manipulation of domestic pigs in the sense of expressing human complement regulatory proteins [1] is an advance towards the reliable clinical application of pig liver perfusion either for transient therapeutic purposes till the original liver has regenerated or as a bridge to allotransplantation [2]. In combination with an improved perfusion protocol, including optimal circulation in the portal venous low pressure system by floating suspension and pneumosynchronous pressure changes [8, 10], a prolonged viability and function of the livers can be reached, reducing the need for organs during one perfusion procedure.

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