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Transplantation of genetically modified hepatocytes after liver preconditioning in Watanabe heritable hyperlipidemic rabbit

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ABSTRACT

Background: Hepatocyte transplantation is a potentially less invasive alternative to liver transplantation for treating inherited metabolic liver diseases. We developed an autotransplantation protocol of *ex vivo* genetically modified hepatocytes combining lentiviral transduction and transplantation after liver preconditioning by partial portal vein embolization. We investigated the metabolic efficiency of this approach in Watanabe rabbits, animal model of familial hypercholesterolemia.

Methods: Our autotransplantation experimental protocol was used in two groups of rabbits ($n = 10$), experimental and sham, receiving transduced and control hepatocytes, respectively. Isolated hepatocytes from left liver lobes were transduced using recombinant lentiviruses. Median lobe portal branches were embolized under fluoroscopic control. Functional measurement of low-density lipoprotein (LDL) receptor expression was assessed by LDL internalization assays. Cholesterol level evolution was monitored. Rabbits were killed 20 wk after the procedure.

Results: Three rabbits of each group died several hours after hepatocyte transplantation; autopsy revealed portal vein thrombosis in two rabbits from each group. The protocol was therefore modified with hepatocytes being transplanted through splenic injection. Lentiviral hepatocyte transduction efficacy was 64.5%. Fluorescence microscopy revealed Dil-LDL internalization of transduced hepatocytes. Seven rabbits in each group were considered for lipid analysis. Four weeks after autotransplantation, median total cholesterol level decreased in the experimental group, without reaching statistical significance (8.9 [8.0-9.8] g/L *versus* 6.3 [0.5-8.3]; $P = 0.171$). In the experimental group, enzyme-linked immunosorbent assay detected significant antibody expression against human low-density lipoprotein receptor.

Conclusions: Autotransplantation protocol allowed a nonstatistically significant improvement of the lipid profile in Watanabe rabbits. Further experiments involving a larger number of animals are necessary to confirm or refute our findings.

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Introduction

Hepatocyte transplantation is an innovative and potentially less invasive alternative to orthotopic liver transplantation for the treatment of life-threatening inherited metabolic liver diseases.[1] Autotransplantation of *ex vivo* genetically modified hepatocytes in comparison to allogenic hepatocyte transplantation could allow to overcome problems associated with shortage of donor organs, quality of hepatocyte sources, and immunosuppression.[2-4] However, this approach requires high efficacy of hepatocyte transduction since a limited number of hepatocytes are isolated from each resected liver lobe and subsequently transplanted. In addition, transplanted cells have a low capacity of spontaneous liver engraftment and necessitate the development of preconditioning techniques which would be transposable to clinical practice and promote significant liver repopulation.

Our team has previously developed strategies to overstep the limits of hepatocyte autotransplantation for the treatment of inherited metabolic liver diseases.[1,5-10] Partial portal vein embolization (PVE), a minimally invasive preconditioning approach, has allowed significant improvement of hepatocytes' engraftment.[7,9] Additionally, transduction in suspension of isolated hepatocytes with a lentiviral vector and immediate transplantation (SLIT) has obviated the need for primary culture and allowed important transduction efficacy (up to 90%) resulting in long-term transgene expression.[11] The combination of SLIT and PVE in nonhuman primates resulted in the engraftment of 6% of lentivirally transduced hepatocytes.[9]

Familial hypercholesterolemia (FH) is an inherited autosomal disease due to a mutation in the gene encoding for the low-density lipoprotein receptor (LDLR), leading to severe hypercholesterolemia and premature coronary heart disease.[12,13] The current treatment methods for homozygous FH are generally inefficient (diet, bile acid binding resins, statins) or laborious (low-density lipoprotein [LDL] apheresis).[13] Currently, orthotopic liver transplantation represents the only curative option for these patients but is limited by the shortage of donor livers, the specific morbidity of the procedure and life-long immunosuppression.[13,14] The Watanabe heritable hyperlipidemic (WHHL) rabbit is the only existing large animal model of FH.[15] Like FH patients, WHHL rabbits spontaneously develop hypercholesterolemia and atherosclerosis due to genetic and functional LDLR deficiencies.[16]

In the continuity of our previous experimental studies and before proceeding to clinical experimentation, we aimed to assess the metabolic efficiency of our preclinical model of autotransplantation of *ex vivo* genetically modified hepatocytes in the WHHL rabbit model of inherited metabolic liver disease.

Materials and methods

Animals

WHHL male and female rabbits aged between 12 and 16 wk and weighing between 1.8 and 3 kg were purchased from Centre de Production Animale (Olivet, France). All animals were housed at the animal facility of Ecole Nationale Vétérinaire de Nantes (Nantes, France) and checked daily. They were maintained in separate cages on a standard diet and kept in 12-h light–dark cycles. All animals were treated in accordance with the European Community laws for animal care. The study was approved by the local ethics committee and by the French Ministry of Research (ARC 02008.02). Study end points were defined to avoid unnecessary suffering of animals.

Experimental protocol

The complete experimental protocol was established in a first series of New Zealand White rabbits as previously reported.[10] This preliminary study allowed to assess feasibility of the procedure in rabbits; the efficacy of the SLIT + PVE approach in terms of hepatocyte transduction, engraftment, and long-term transgene expression; and the *in vitro* correction of the WHHL hepatocyte phenotype by transduction with a lentiviral vector encoding human LDLR. The aim of the present experimentation was to assess the metabolic efficacy of our approach. Two groups of WHHL rabbits were included in this study, both receiving the complete experimental protocol of autotransplantation. The experimental group was transplanted with transduced hepatocytes, whereas the sham group was transplanted with nontransduced hepatocytes.

Left lobe resection and permanent partial PVE

Surgery was performed as previously described.[10] Briefly, rabbits were sedated by an intravenous injection of alfaxalone and maintained under isoflurane. A left lobectomy, corresponding to approximately 25% of the liver volume, was carried out through midline laparotomy. A 12-G catheter was positioned in the hepatic vein of the left lobe for hepatocyte isolation. Before surgery, rabbits received prophylactic antibiotherapy by cephalexin (IV), while intravenous morphine was administered, when necessary, for perioperative analgesia.

Permanent PVE was performed by injecting a mixture of cyanoacrylate (Histoacryl, B. Braun Medical, Diegem, Belgium) and lipiodol, as it is frequently the case in clinical practice for future liver remnant hypertrophy in order to prevent postoperative liver failure after liver resection.[17] An initial portogram using a 3-F introducer placed in the superior mesenteric vein was taken to map the portal branches before embolization. Portal branches of the median lobe, corresponding to approximately 45% of the liver volume, were selectively embolized using a microcatheter (Terumo Progreat, Guyancourt, France) that was positioned accordingly under fluoroscopic control. The microcatheter was then replaced by a 3-F venous catheter connected to a perfusion chamber (Access Technologies, Skokie, IL), which was placed subcutaneously in the left anterior thoracic region to provide chronic access to the portal vein. Transdermal fentanyl was used as postoperative analgesic.

Generation of lentiviral vectors

The self-inactivating lentiviral vector, APOAII.LDLR.WPRE (woodchuck hepatitis virus posttranscriptional regulatory