Abstract

The treatment of acute liver failure, a condition with high mortality, comprises optimal clinical care, and in severe cases liver transplantation. However, there are limitations in availability of organ donors. Hepatocyte transplantation is a promising alternative that could fill the medical need, in particular as the bridge to liver transplantation. Encapsulated porcine hepatocytes represent an unlimited source that could function as a bioreactor requiring minimal immunosuppression. Besides patients with acute liver failure, patients with alcoholic hepatitis who are unresponsive to a short course of corticosteroids are a target for hepatocyte transplantation. In this review we present an overview of the innate immune barriers in hepatocyte xenotransplantation, including the role of complement and natural antibodies; the role of phagocytic cells and ligands like CD47 in the regulation of phagocytic cells; and the role of Natural Killer cells. We present also some illustrations of physiological species incompatibilities in hepatocyte xenotransplantation, such as incompatibilities in the coagulation system. An overview of [...]
Review

Current status of hepatocyte xenotransplantation

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HIGHLIGHTS

- An overview is presented of the innate immune barriers in hepatocyte xenotransplantation.
- Some illustrations are presented of physiological species incompatibilities in hepatocyte xenotransplantation.
- An overview of methodology in cell microencapsulation is presented.
- An overview of proof-of-concept studies using microencapsulated porcine hepatocyte xenotransplantation are presented.
- An outline of a provisional clinical trial is presented.

ABSTRACT

The treatment of acute liver failure, a condition with high mortality, comprises optimal clinical care, and in severe cases liver transplantation. However, there are limitations in availability of organ donors. Hepatocyte transplantation is a promising alternative that could fill the medical need, in particular as the bridge to liver transplantation. Encapsulated porcine hepatocytes represent an unlimited source that could function as a bioreactor requiring minimal immunosuppression. Besides patients with acute liver failure, patients with alcoholic hepatitis who are unresponsive to a short course of corticosteroids are a target for hepatocyte transplantation.

In this review we present an overview of the innate immune barriers in hepatocyte xenotransplantation, including the role of complement and natural antibodies; the role of phagocytic cells and ligands like CD47 in the regulation of phagocytic cells; and the role of Natural Killer cells. We present also some illustrations of physiological species incompatibilities in hepatocyte xenotransplantation, such as incompatibilities in the coagulation system.

An overview of the methodology for cell microencapsulation is presented, followed by proof-of-concept studies in rodent and nonhuman primate models of fulminant liver failure: these studies document the efficacy of microencapsulated porcine hepatocytes which warrants progress towards clinical application. Lastly, we present an outline of a provisional clinical trial, that upon completion of preclinical work could start within the upcoming 2–3 years.

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1. Introduction

The treatment of acute liver failure includes the removal of toxic components, optimal supportive medical care, and, in the most severe cases, liver transplantation. The latter option represents the only treatment available in case of end-stage liver failure. However, the shortage of organ donors limits its application. Thus, the development of alternative therapies is crucial to meet the medical need.

Hepatocyte xenotransplantation is a promising alternative [1–5] with several advantages. The procedure is moderately invasive, allows the use of encapsulated and/or genetically modified cells, has the advantage of a potentially unlimited supply, and has the perspective of low morbidity and high safety. Moreover, repeated administrations can be considered, the costs may be inferior to those of liver transplantation, and single cells can undergo genetic modification and cryopreservation. Considering species compatibility, it is emphasized that pig hepatocytes have metabolic similarities to humans [6–8]; however, important physiological incompatibilities remain and may represent a barrier to xenotransplantation. Importantly, there is no risk of acute vascular rejection when compared to whole organ xenotransplantation, as the vascular endothelium of the liver is not transplanted [9,10]. Moreover, there is some evidence that hepatocytes are resistant to complement-mediated injury [11]. Remarkably, genetically modified hepatocytes could provide protection against human humoral and cellular immune responses [12]. A relevant proof-of-principle was reported in a study documenting up to 100% reconstitution of the liver of immunotolerant albumin-urokinase transgenic mice with rat liver cells [13].

Various routes can be exploited to administer hepatocytes: intraportal, directly into the hepatic parenchyma, intrasplenic, or in the peritoneal cavity. Autologous, allogeneic and xenogeneic hepatocytes have shown therapeutic efficacy in fulminant liver failure models and/or engraftment abilities in rodents [14,15] and nonhuman primates [16–18]. In rodents with fulminant liver failure, hepatocyte transplantation resulted in improvement in bilirubin and serum ammonia, a decrease in hepatic encephalopathy and an increase in survival rate of over 60% compared to control. In view of these encouraging results, programs to develop clinical trials have been initiated [19].

Exploratory trials showed the clinical utility of hepatocyte transplantation as a bridge-to-transplantation in patients with fulminant hepatitis. An improvement in neurological status and in biochemical parameters has also been observed, but no significant increase in survival has been reported. Allotransplantation or genetically modified autologous hepatocytes are other options for the treatment of inherited metabolic diseases [20].

2. The humoral and cellular innate immunity barriers in hepatocyte xenotransplantation

Hepatocyte xenotransplantation is still limited by several immunological and non-immunological barriers (Fig. 1). With the creation of transgenic pigs, a potential reality is getting closer for certain organs. For instance, for organs from α1,3-galactosyltransferase gene-knockout (GT-KO) homozygous pigs prolongation of survival upon transplantation in nonhuman primates has been documented, and the organs studied include the liver [21,22]. These genetically-engineered pigs could also serve as donors of cellular xenografts. For instance, pig hepatocytes have the benefit of an immediate restoration of liver function. There are issues such as the barriers imposed by the innate immune system and the physiological incompatibilities between species, which will be discussed below with a special emphasis on hepatocyte xenotransplantation.

2.1. The innate immune system

The innate immune system is the first line of defense against foreign organisms. It includes phagocytic cells and complement activation representing two important effector mechanisms, which also contribute to xenograft rejection.

2.1.1. Complement and natural antibodies

One of the major obstacles in pig-to-human organ xenotransplantation is hyperacute rejection initiated by the binding of naturally-occurring antibodies against carbohydrate epitopes such as galactose-α1,3-galactose (Gal) on the pig vascular endothelium. This antibody binding in turn activates the complement cascade resulting in congestion and thrombosis of the graft’s vasculature, which in minutes leads to an irreversible damage and loss of the graft. However, cellular xenografts generally do not appear to be sensitive to hyperacute rejection despite the expression of Gal [9,10,23,24]. Isolated primary porcine hepatocytes show cell surface expression of Gal, in an age-dependent manner with less expression in the livers from pigs at 100 days of gestation and 3-day-old newborn pigs, and higher expression in pigs at one month of age and older [25].

Despite this higher Gal expression, adult primary hepatocytes are preferred in potential treatment: these cells appear to be resistant to complement-mediated lysis when compared to
endothelial cells which are known to be extremely susceptible to destruction by complement [11]. This decreased susceptibility was originally ascribed to decreased binding of complement-fixing antibodies, resulting in a decreased complement activation; but it was shown that this was rather related to an intrinsic resistance to complement-mediated lysis [11]. Thus, hepatocyte xenotransplantation represents a potential therapy for liver failure as shown in small animal models [26–29].

2.1.2. Phagocytic cells and ligand recognition

Due to the substantial species disparities, the activation of the recipient’s immune system is not only due to the immunogenicity of the graft, but also due to the lack of ligands that deliver host inhibitory signals when binding to its receptors. One of such ligands is CD47, an ubiquitously expressed molecule from the immunoglobulin superfamily that binds to its receptor, signal-regulatory protein (SIRPα), leading to tyrosine phosphorylation of intracellular immune receptor tyrosine-based inhibitory motifs (ITIMs) and producing a “don’t eat me signal” [30]. CD47-positive cells from hematopoietic xenografts, especially from porcine origin, are unable to interact with mouse or human SIRPα leading to phagocytosis of the xenogeneic cells by host macrophages; this emphasizes the interspecies incompatibility of the CD47-SIRPα system in the xenogeneic setting.

Recently this system has been shown to play an important role in hepatocyte xenograft rejection. In a syngeneic murine model of liver repopulation, CD47-KO hepatocytes were transplanted to resemble xenografts for the CD47 incompatibility; intrasplenic transplantation induced a strong activation of myeloid-derived cells when compared to wild-type hepatocytes, but CD47-KO hepatocytes were unable to repopulate the liver in the long-term. This was due to the destruction of grafted cells during the first two weeks after transplantation [31].

The importance of CD47-SIRPα incompatibility in hepatocyte xenotransplantation was also demonstrated in a different approach: upon ectopic expression of murine CD47 via lentiviral transduction in primary human hepatocytes or HepG2 cells, HepG2 cells were less susceptible to phagocytosis in vitro by RAW264.7 macrophages than non-transduced HepG2 cells [32]. Subsequently, human hepatocytes expressing murine CD47 transplanted in BALB-ΔRAG1γc-μPA mice showed a better engraftment compared to non-transduced hepatocytes [32]. These results possibly explain the better engraftment of human hepatocytes in murine models when innate immune cells such as macrophages are depleted using clodronate [33]. This is also the case for the human immune system, as demonstrated by a more stable and long-term engraftment (6–7 months) of human hepatocytes in a murine model comprising stably co-engrafted human hemato-lymphoid system [34]. While the CD47-SIRPα system is not the only one, the evidence suggests that it represents an important barrier to hepatocyte xenotransplantation that needs to be taken into account when considering this form of therapy.

2.1.3. Natural Killer (NK) cells

NK cells are a special subset of lymphocytes with primary contributions to antiviral and antitumor immune responses. The GaIT-
KO modification of pigs overcomes acute humoral rejection, but provides no advantage in preventing graft destruction by NK cells (either by direct or by indirect mechanisms). This presents an impeding barrier to organ and cellular xenotransplantation, because the recognition of pig cells by NK cells is not dependent on the expression of Gal [35]. Being non-vascularized grafts, direct mechanisms are involved in the destruction of xenogeneic hepatocytes.

In humanized transgenic mouse models based on the severe combined immune deficiency (SCID) or SCID-beige background, depletion of NK cells resulted in a better repopulation of xenogeneic hepatocytes [34,36]. In addition, a superior engraftment has been shown for human hepatocytes in Rag2−/−gama c−/−/Alb−uPA mice, animals with severe impairments in T and B-lymphocyte development and function together with a complete absence of NK cells; this indicates the importance of NK cells in mediating xenograft rejection [36].

2.2. Physiological incompatibilities as a barrier to hepatocyte xenotransplantation

Physiological species incompatibilities are considered a severe barrier in organ xenotransplantation, but not much is known about these in cellular xenografts. Severe coagulation dysregulations are known for liver transplantation across species, the liver being the major organ involved in the production of coagulation proteins. This indicates the importance of physiological incompatibilities besides the immunological barriers. Hepatocytes are involved in coagulation, drug metabolism and detoxification. There are only limited data on species compatibilities in these important functions, but there is some evidence suggesting a species-specific regulation of certain important proteins involved in the coagulation system such as fibrinogen [37].

In the clinical transplant setting, porcine hepatocytes would in theory be regulated by the human environment and would have to respond to human factors and proteins. In lieu of this, porcine hepatocytes upon stimulation with human tumor necrosis factor α (TNFα) increase their fibrinogen production and form stable and widely branched fibrin polymers: this contrasts to the response by human hepatocytes that would normally decrease fibrinogen synthesis without fibrin generation in this condition [37].

Similarly, substantial differences between porcine and human hepatocytes have been observed in the expression of different isoforms of the cytochrome P450 [38], as well as in the production of albumin and C-reactive protein [39], when exposed to human acute phase proteins such as TNFα and interleukin-6.

These incompatibilities are of clear relevance when considering hepatocyte xenotransplantation for acute liver failure. For instance, the inflammatory environment associated with liver failure could stimulate xenogeneic hepatocytes to fibrinogen production which in turn contributes to the already hypercoaguable state [40]. Also, the effect of acute phase proteins on the function of porcine hepatocytes in drug metabolism is a critical issue. While these studies have only been done in vitro, in vivo data would add value and shed further light on the importance of these processes related to physiologic species incompatibilities.

3. Xenogeneic hepatocyte microencapsulation

Despite the progress in producing genetically-modified pigs, the rejection by the innate immune system and the destruction of xenogeneic cells by an elicited immune response remain a major obstacle to the success of cell transplantation. Thus, microencapsulation in biocompatible polymers has been developed to protect the cells from the immune system [41–43] (Fig. 2). Capsules or microspheres are designed to protect cells against circulating antibodies (~150 kDa) and immune and inflammatory cells while allowing nutrients such as oxygen (16 Da) and glucose (180 Da) to diffuse inside the microsphere (Fig. 1): hence, immunosuppression with its intrinsic adverse effects can be reduced or eliminated. The passage of cytokines and inflammatory mediators is dependent on the size of these proteins and the permeability of the microspheres: for instance, for microspheres based on alginate and poly(ethylene glycol) of different molar weight and prepared using different ratios, the permeability can be adjusted between 70 and 150 kDa [44].

Hydrogel microspheres are composed of biocompatible macromolecules that are linked together by ionotropic interactions or covalent crosslinking. Alginate-based hydrogels like sodium alginate and alginate-poly(l-lysine)-alginate (Fig. 2) are most frequently used in cell microencapsulation. Sodium alginate has a great biological acceptance and permeability upon ionotropic gelation with divalent cations [45,46], but it has the disadvantage of relatively low physical resistance and stability [47]. Variants of sodium alginate have been tested including barium alginate microbeads [48], ornithine alginate [49], carrageenan alginate [50], synthetic methacrylate-based polymers [51], poly-l-lysine [52], agarose [53], sodium cellulose sulfate [54] or poly(ethylene glycol) [55]. Poly-l-lysine allows the design of microspheres with a layer that create a "real" capsule around cells rather than beads of sodium alginate that are solid compounds where cells are included. Because of potential toxicity, poly(l-lysine) has also been replaced by poly(L-ornithine) [56] or chitosan [57,58].

Based on the advantages of both alginate (biocompatibility) and poly(ethylene glycol) (mechanical resistance), novel types of hydrogel microspheres have been designed [44,59,60]. These combine ionotropic interaction of alginate molecules with chemical crosslinking of vinyl sulfone-terminated multi-arm poly(ethylene glycol) molecules. These hydrogels allow adaptation of physical properties in a controllable manner, like permeability and swelling, and also offer excellent biocompatibility, mechanical resistance, and stability [44]. Of note, xenografted cells maintained their viability for up to six months in these microspheres [60]. More recently, conformal coating of cells was shown to be very efficient in providing immune protection while giving optimal diffusion of nutrients and oxygen [61].

3.1. Preclinical studies with encapsulated xenogeneic hepatocytes

Encapsulated hepatocytes have been investigated in several
animal models of liver failure. Guinea pig hepatocytes macro-
encapsulated in acrylonitrile-sodium methallyl-sulfonate co-
polymers survived and conjugated bilirubin for over two months
after intraperitoneal transplantation in Gunn rats, a model for
inherited deficiency of bilirubin glucuronidation, representing
the clinical disease of Crigler-Najjar syndrome [62]. In another study,
encapsulated guinea pig hepatocytes transplanted in the perito-
neum reduced mortality from about 80% to 36% in rats with
fulminant liver failure induced by 95% liver resection [63].

Microencapsulated primary or immortalized human and rat
hepatocytes transplanted intraperitoneally into mice with
fulminant liver failure could sustain liver metabolic functions
resulting in increased survival [28,29]. In this study cells were
microencapsulated in alginate-poly(l-lysine)-alginate micro-
pheres. Porcine hepatocytes microencapsulated in alginate-
poly(l-lysine)-alginate microspheres manifested in vitro produc-
tion of albumin and urea, and degraded ammonium, diazepam
and lidocaine; in vivo a 55% increase in survival of mice with
fulminant liver failure was shown [29,64]. Recently, porcine he-
patocytes microencapsulated in alginate-poly(l-lysine)-alginate microspheres (Fig. 2) showed efficacy in baboons with fulminant
liver failure induced by 75% hepatectomy and 60 min warm
ischemia: upon transplantation a substantial improvement in
survival and biochemical parameters was achieved (Machaidze
et al., submitted for publication). This study is the first in a large
animal model of fulminant liver failure, and is considered relevant
in progressing towards clinical explorations.

3.2 Provisional clinical trials with a porcine hepatocyte product

Thus far, there have been no clinical trials testing porcine he-
patocyte transplantation. Regarding human hepatocytes, based on
the promising results in preclinical models, a team from King's
College in London [4] has proposed clinical trials testing micro-
capsulated human hepatocytes in a child with acute liver failure
[20], with success. Recently, the group reported optimized pro-
tocols to produce GMP-grade alginate-encapsulated human hepa-
tocytes that are suitable for clinical transplantation [65].

A provisional clinical trial with microencapsulated porcine he-
patocytes could be as follows. First, patient selection will be made
according to the criteria defining acute liver failure, namely, coag-
ulation abnormalities (i.e., the coagulation parameter International
Normalized Ratio (INR) ≥ 1.5) and encephalopathy without pre-
existing cirrhosis, and an illness course of less than 26 weeks
[66]. Conditions related to acute liver failure will typically include
acetaminophen overdose, idiosyncratic drug-induced liver injury,
viral hepatitis, mushroom intoxication, vascular disease, alcoholic
hepatitis or autoimmune disease. Patients for whom there is no
liver transplant possible (because of organ shortage or because of
disease-related contra-indications) will be included in a phase I
trial, testing microencapsulated hepatocytes together with best
supportive care. Another target population comprises patients with
biopsy-proven alcoholic hepatitis who present with symptoms like
fatigue or undetermined abdominal discomfort, or manifest jaun-
dice, ascites, variceal bleeding or hepatic encephalopathy, and who
are unresponsive to a short-term treatment with corticosteroids.
These patients have a poor prognosis; i.e., the 6-months mortality is
about 70%. Both target populations are in clinical trials testing
extracorporeal liver assist devices for temporary liver support
[67,68].

Porcine hepatocytes will be isolated from pathogen-free pigs
fulfilling the criteria regarding the absence of infectious agents [69],
and then encapsulated in alginate-based microspheres, and trans-
planted intraperitoneally. The numbers of hepatocytes to be
transplanted are based on liver volume and existence of remaining
liver function (coagulation Factor V assessment), i.e., maximal ~50% of
the initial liver volume, ~500–700 ml packed cells: the final
volume of microspheres will be three times the hepatocyte volume,
~1.5 L. For patients that are eligible for a liver transplant, the
primary efficacy endpoint is a clinical condition enabling the suc-
cessful entry in a transplant program within 2–3 weeks after he-
patocyte transplantation. The following parameters will be
recorded on a regular basis: porcine and human albumin, aspartate
aminotransferase, alanine aminotransferase, total bilirubin, pro-
thrombin time, factor V and platelets. If an organ becomes avail-
able, the patient will undergo a liver transplantation; of note,
intraperitoneal microspheres will then be removed and will not
compromise surgery. Patients with alcoholic hepatitis are generally
not eligible for a liver transplant: hence, in these patients the pri-
mary efficacy endpoint is an improvement in liver function
accessed following scores like the MELD (Model for End-Stage Liver
Disease) score.

4. Conclusion and perspective

Porcine hepatocyte transplantation is foreseen at first for pa-

tients with acute liver failure that are eligible for a liver transplant
and require metabolic support during the waiting time for a
transplant, or during the time needed for their own liver to
regenerate. Liver transplantation is an efficient and safe treatment
modality: however, organ donor shortage and the life-long
immunosuppression are limitations to its application. To meet the
medical need, hepatocyte transplantation can provide the function
as bioreactor in temporary liver support, representing a novel and
innovative approach. The proof-of-principle regarding efficacy has
been achieved for porcine (microencapsulated) hepatocytes in ro-
dent and nonhuman primate models of induced fulminant liver
failure, with substantial increase in survival. A potential limitation
includes the safety issue, for instance the transmission of porcine
endogenous retroviruses. The preclinical efficacy data are in line
with clinical data on hepatocyte transplantation in patients with
metabolic liver diseases [2,3,20]. The development of microspheres
with adjustable physical properties and optimal biocompatibility
and mechanical resistance will increase the chance of success. Upon
completion of advanced preclinical studies, the phase transition to
clinical trials could be foreseen in the coming two-three years.

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Conflict of interest

The authors declare that there is no conflict of interest.

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