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Research Article

No PERV transmission during a clinical trial of pig islet cell transplantation

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ABSTRACT

Xenotransplantation of pig islet cells is a promising alternative for the treatment of diabetes with insulin and may help to prevent numerous late complications such as blindness and amputation. First encouraging results using porcine islets have been reported in preclinical animal models as well in the first clinical trial in New Zealand. The goal of this manuscript is to examine the biological safety of a second trial performed in Argentina, specifically in regards to the transmission of porcine endogenous retroviruses (PERVs) using improved detection methods As in the first trial encapsulated islet cells from the well-characterised Auckland Island pigs were used. The animals were not genetically modified. The islet cells were transplanted in eight human recipients using a modified clinical protocol. Sera taken at different time points after transplantation (up to 55 weeks) were screened for the presence of antibodies against PERV proteins by Western blot analysis using viral antigens from highly purified virus particles. Positive sera obtained by immunization with recombinant PERV proteins were used as control sera. In none of the patients antibodies against PERV were detected, indicating the absence of infection. In parallel at different time points (up to 113 weeks) white blood cells (WBC) have been tested for PERV DNA, and WBC and plasma for PERV RNA by real-time RT-PCR. All tests were negative. In addition, using primers detecting pig mitochondrial cytochrome oxidase (COX) gene, patients were screened for microchimerism. In summary, the data are further evidence for the safety of pig islet cell transplantation.

1. Introduction

Type 1a diabetes is an autoimmune disease and diabetes type 1b is an idiopathic disease, both caused by the destruction of the insulin-producing beta cells in the pancreas. The prevalence of type 1 diabetes in Western Europe and North America is about 0.5% of the population, now affecting approximately 2 million subjects (Reichart et al., 2015). In the United States type 1 diabetes affects approximately 1.25 million children and adults (National Diabetes Statistics Report, 2014). Replacement of the destroyed beta cells by transplantation of either a complete pancreas organ or isolated human beta cells is the only effective way to cure the disease. Since there is a shortage of human donors, an alternative approach is to use xenotransplantation, i.e. transplantation of an organ from another species (van der Windt et al., 2012). For several reasons (size, physiological similarity, ability to be genetically modified and to produce large numbers of progeny) pigs are the preferred donor animals. Pig insulin differs from its human counterpart at only a single amino acid and had been used successfully in the treatment of diabetic patients for decades. In addition, a similarity between human and pig glycose homeostasis was described, e.g. the normal

Abbreviations: AEC, Animal Ethics Committee; CA, capsid; COX, cytochrome oxidase; DOL, Diatranz Otsuka Ltd; ECL, enhanced chemiluminescence; GMP, good manufacturing practice; FeLV, feline leukaemia virus; HIV, human immunodeficiency virus; HRP, horseradish peroxidase; HTLV, human T - cell leukaemia virus; IEQ, islet equivalents; IgG, immunoglobulin G; kDa, kilodalton; LCT, living cell technologies; MTF CT, MTF, mammalian tissue free collagenase thermolysin; NHP, non-human primates; NZ, New Zealand; PERV, porcine endogenous retroviruses; RT-PCR, reverse transcriptase polymerase chain reaction; SU, surface envelope; TM, transmembrane envelope; WBC, white blood cells; TEM, transmission electron microscopy.

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blood glucose level of healthy adult male is 70–100 mg/dL, that of pigs 66 to 116 mg/dL.

The insulin therapy cannot reproduce the complex pattern of physiologically controlled insulin secretion, and therefore patients are at risk of hypoglycaemia and many patients develop severe vascular complications such as myocardial infarct or stroke. Such late adverse effects as well as blindness and amputation, which represent the highest financial burden when treating with insulin alone, may be prevented performing pig islet cell transplantation.

Encouraging results in pig islet xenotransplantation have been obtained over the last years in pre-clinical NHP models (for review see Hering and Walawalkar, 2009; Ellis and Korbutt, 2015). For example, in 2006, two groups published survival of functional porcine islet cells in diabetic NHP for longer than 3–6 months with immunosuppression (Cardona et al., 2006; Hering et al., 2006). Subsequent studies with transgenic porcine islets have shown islet survival for more than one year (van der Windt et al., 2009). Encapsulation of islets also showed effectiveness in NHP (Dufrane et al., 2006). For example, pig islet cells in a subcutaneous macrodevice corrected diabetes in primates up to six months without immunosuppression (Dufrane et al., 2010). There is also progress in clinical trials. In 1994 the transplantation of foetal porcine islet-like cells clusters in diabetic kidney transplant patients was reported and porcine C-peptide was detected in urine up to 400 days (Groth et al., 1994). In 2009, the New Zealand based company Living Cell Technologies (LCT) performed a clinical trial in New Zealand (n = 14, applying 5000–20,000 islet equivalents, IEQ) (Matsumoto et al., 2014). Auckland island pigs had been used as source of the islet cells and the animals as well as the islets had been characterised in great detail beforehand showing absence of potentially zoonotic pig microorganisms other than PERV (Garkavenko et al., 2008a,b). The patients enrolled in the New Zealand study were analysed using the previous generation of detection methods and no transmission of PERV and other microorganisms was observed (Wynyard et al., 2014). Here another study is analysed, performed by the same company in Argentina in 2011 (Cooper et al., 2016). Eight patients were tested using methods of the next generation to determine if they were infected with PERV. Blood taken from each patient was investigated at different time points after islet cell transplantation. Immunological methods for the detection of antibodies against PERV as indirect evidence for transmission and PCR-based methods as direct evidence were used. In comparison to the New Zealand study the clinical protocol was different and the virus detection methods were improved. The testing was performed according the recommendations of the International Xenotransplantation Association (Denner et al., 2016).

This report describes the PERV results for blood samples taken from 1-week post-transplant up to week 113 post-transplant in some cases. Altogether 484 results were obtained, 432 for the PCR (216 for PERV and 216 for COX) and 52 serological results.

2. Materials and methods

2.1. Ethics statement

Patients were recipients of DIABECELL® [immunoprotected (alginate-encapsulated) porcine islets] as part of a phase I/IIa open-label investigation of the safety and effectiveness of DIABECELL® in patients with type 1 diabetes mellitus. The study protocol (ClinicalTrials.gov Identifier NCT01739829) was granted ethics approval in NZ by the Health Research Council's Gene Technology Assessment Committee and Medsafe. Approval was also obtained in Argentina from the Ministry of Health, Buenos Aires Province. All enrolled patients provided written informed consent.

2.2. Animal ethics approval

LCT adheres to codes of ethical conduct in strict accordance with the Animal Welfare Act 1999 which protects the welfare of animals within New Zealand. All animal studies are approved by an Animal Ethics Committee (AEC) appointed by LCT. The AEC is comprised of LCT employed scientific staff, an LCT employed veterinarian, an independent veterinary consultant, animal welfare representative from the Royal New Zealand Society for the Prevention of Cruelty of Animals (RNZSPCA) and laypersons nominated by local or regional council without affiliation to LCT. All animal surgery was performed under isoflurane anaesthesia.

2.3. Islet isolation

Islets were isolated using the Ricordi Isolator (Biorep Technologies, Miami, FL, USA), which provides an automated system for the isolation of islets from pancreatic tissue. The pancreas was perfused within the Ricordi chamber using a digestion solution containing Liberase MTF CT GMP grade (Roche Applied Science, Auckland, New Zealand), warmed to 37 °C and recirculated for 10–20 min. The chamber shaking speed and displacement were preset. The digestion was stopped by cooling and diluting with RPMI 1640 (Life Technologies, Auckland, New Zealand) containing 2% porcine serum. The digestion time is adjusted as required based on an in-process microscopic check to determine the percentage of free islets released. DIABECELL products are packaged for transport in accordance with section 47 of the NZ Medicines Act 1981 (22 Sept 2011). All procedures are validated and quality controlled and compliant to GMP standard. Each batch is transported in temperature monitored Credo Cubes series 4, 1296 (Minnesota Thermal Science). Transport is validated for cold transport at 2 °C–10 °C and room temperature 18 °C–25 °C.

2.4. Patients

Encapsulated neonatal porcine islets were transplanted into the peritoneal cavity via a laparoscope at Hospital Interzonal General de Agudos Eva Peron, San Martin, Provincia de Buenos Aires. Two groups consisting of patients who received twice either 5000 islet equivalent (IEQ)/body weight kg (group 1, n = 4) or 10,000 IEQ/kg (group 2, n = 4). The second transplantation was conducted at three months after the first transplantation. According to the study protocol blood samples were scheduled to be taken at 1 week pre-transplant and weeks 1, 4, 8, 12, 24, 52, 104 and 260 post-transplant and then 5-yearly for life. However, it should be noted that patients were not always available for sampling at these time points so there is some deviation (up to 8 weeks) from the scheduled collection dates. Sample collection dates for each patient and testing are specified in Tables 1 and 2.

2.5. Virus production and purification

To isolate PERV for the Western blot analysis, supernatant from 293 T cells infected with and producing PERV-A/C recombinant virus (Karlas et al., 2010) was harvested on the third day after cell splitting. Totally 500 ml of supernatant were collected and processed. All procedures of virus isolation were performed at 4 °C. The supernatant was centrifuged at 1200*g* for 10 min and at 4000*g* for 10 min to eliminate cells and cellular debris. Then it was filtered through 0.45 mm filters (Millipore) followed by centrifugation at 34000 rpm (rotor SW41Ti, Beckman, Ireland) for 1 h 40 min. The pellet was resuspended in PBS and centrifuged through a 20% sucrose cushion at 36000 rpm (rotor SW 50.1Ti, Beckman) for 2 h. The pellet was resuspended again in PBS (to obtain a 1000 and 2000 fold virus concentrate) and was kept frozen at −80 °C until use. The