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# Xenotransplantation – Clinical Activities and Regulatory Development

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## Clinical xeno-organ transplantation

The gap between the number of patients waiting for various organ transplantations and the number of available grafts increases steadily. Xenotransplantation – the use of animal organs and tissues for transplantation to man – may provide a solution to this problem. Small series of xenotransplantations were performed already in the 1960's. In most cases, non-human primates such as chimpanzees or baboons, were used as source animals. In one case, a chimpanzee kidney transplant functioned for nine months in a young woman. The result is remarkable, considering the primitive immunosuppressive therapy available in those days. In most cases, however, organs from non-human primates were rejected within days (10). In a few cases, transplantations of organs from pig or sheep were attempted, but the function of these organs ceased already during the operations. It became obvious that the immunological response was difficult to control, and that the greater the phylogenetic disparity between source animal and recipient, the more aggressive was the rejection response. When the donation of human organs from cadaveric donors became established, the interest for xenotransplantation temporarily ceased.

The results with human organ transplantation have gradually improved since, and more and more patients are opting for a transplant. Waiting times increase and patients waiting for life-supporting organs such as livers, hearts and lungs die on the waiting list. It has always been especially difficult to find grafts for small children in need for size-matched thoracic transplants. In 1984, an American group therefore attempted to transplant a baboon heart to a newborn infant dying from congenital heart malformation (1). The transplantation was technically successful, and for the first days the graft functioned well. However, graft failure occurred after 20 days, probably due to rejection. The so-called "Baby Fae case" was given great public attention, and the ethics behind performing such a highly experimental procedure on a dying child was intensively discussed.

Besides increasing the number of available grafts, xenotransplants may also provide an advantage by being resistant to certain diseases that tend to recur and destroy a human graft. One such example is, that baboons are resistant to human hepatitis B, a disease that inevitably recurs after human liver transplantation. In 1992-1993, two cases of baboon-to-man liver transplantation were performed in Pittsburgh, USA (8). Both patients suffered from Hepatitis B and were in those days not considered eligible for human liver transplantation. The first patient had initially a very promising course. He was in hepatic coma at the time of transplantation. After 24 hours he could be taken off the ventilator, and after five days he was back at the

ward, eating and moving around. As could be expected, several biochemical parameters changed to a baboon profile, but this did not seem to cause any problems, at least in the short run. However, infectious complications developed and the patient died at day 70 in a cerebral bleeding caused by an *Aspergillus* infection, most probably related to the intense immunosuppression. The second patient survived for 26 days, but never regained consciousness or renal function. In both cases, there was little histopathological evidence of rejection in the transplanted livers.

Obviously, non-human primates offer the best physiological compatibility, and the disease immunological barrier also seems more manageable. However, due to infectious risk considerations, ethical concerns and the limited supply of non-human primates, most groups today consider the pig to be the most suitable species for clinical xenotransplantation. One major problem is then that vascularized organs transplanted from pig to man undergo hyperacute rejection, a rapid immunological process that destroys the transplanted organ within a few hours. All humans have preformed antibodies directed against pig-specific antigens mainly the Gal-epitope. After revascularization of the graft, these antibodies bind to the pig endothelium and complement is activated. The process leads to vascular damage, thrombosis and bleeding and more or less immediate organ failure. Prevention of hyperacute rejection requires very intense pre-treatment of the recipient with antibody removal or complement inhibition. Such pre-treatment was considered far too risky for routine clinical use.

In the beginning of the 1990's, a British group produced transgenic pigs specifically designed as source animals to man (7). These pigs carry a human complement regulatory factor (e.g. hDAF) and hyperacute rejection can thus be avoided. Organs from such pigs have now been transplanted to non-human primates, a model that should mimic the immunological barrier between pig and man. Transgenic pig hearts and kidneys have provided life-supporting function for up to a few months, but the later stages of the rejection process are still difficult to control. So far, livers from similar transgenic pigs have in a few cases been used for extracorporeal perfusion in patients with acute liver failure. The livers were then temporarily connected to the patients circulation and seem to have provided some detoxifying effects. Whether they provided any immunological advantage compared to wild type pig livers has been hard to judge in this model, and so far, transgenic pig organs have not been transplanted as permanent grafts to man. During 2002 the development of Gal knock-out pigs was reported. The hypothesis is that these organs will cause a less intense rejection response, but so far no data on primate studies are available.

**Clinical xeno-cell transplantation**

The majority of xenotransplantations performed during the 1990's have been cellular transplantations. In the beginning of 1990's, ten patients at Huddinge University Hospital, Stockholm, Sweden underwent transplantation with porcine foetal pancreatic islets (5). The study was performed in collaboration with the Biomedical Centre in Uppsala, Sweden, and proceeded with experimental studies showing maturation and function of the foetal beta-cells in adult recipients. The sows and the foetal tissue also underwent an extensive microbiological screening. In eight patients, the tissue was injected to the liver via the portal vein. In four of these patients, function of the pancreatic tissue was observed for a maximum of 450 days as measured by excretion of porcine C-peptide in urine. In the last two patients the porcine grafts were injected under the capsule of a simultane-

ously transplanted human kidney. Surviving pig cells were then found in a kidney graft biopsy taken three weeks after transplantation. The finding was remarkable as this was the first time pig tissue was shown to survive in a human being. In no case, however, could the patients stop their insulin injections. The postoperative courses were uneventful, and there were no signs of infectious complications.

In the United States, a biotech company has performed transplantations of foetal pig neurons to treat patients with Parkinson's disease, Huntington's disease, and in a few cases, epilepsy or sequel after stroke (4). In the pilot study on Parkinson's disease, several patients reported remarkable improvements. The treatment has since then been analysed in a randomised placebo-controlled trial. Survival of the cellular graft occurred in several patients. However, no clear therapeutic effect was noted in the controlled trial.

Bovine adrenal cells have also been evaluated as treatment for patients with morphine-resistant cancer pain (3). The cells were placed in a semi-permeable membrane, and put into the spinal canal. No immunosuppressives were given in this study. The cells were protected by the membrane, and by being placed behind the blood-brain barrier where the immunological response is weaker than in the rest of the body. Secretion of therapeutic substances, and survival of cells for more than six months, were observed, but the therapeutic effect was questionable. In Switzerland, xenotransplantation has been attempted as a treatment for amyotrophic lateral sclerosis (ALS) (11). In this study, baby hamster cells were genetically modified to secrete a human neurotrophic factor. Encapsulated cells were placed in the spinal canal, and again secretion of the therapeutic protein was observed, and cells survived for several months even without immunosuppression.

Xenotransplantation is usually defined as any contact between a human patient's body fluids and animal organs or tissue. Using this definition several hundreds of patients in both Europe and the United States have been "xenotransplanted" when treated with a bioartificial liver. Most patients suffered from acute liver failure, and the treatment was an attempt to keep the patients alive until a human graft for transplantation became available. Their plasma was passed through a "liver dialysis machine" containing encapsulated pig cells (6). Improvements in hepatic encephalopathy and certain biochemical parameters have been observed.

**Regulatory development**

The scientific development of xenotransplantation during the 1990's led to several national and international initiatives to regulate the clinical use of the procedure. The main concern was the risk for infectious complications, affecting not only the patient, but also potentially introducing new pathogens into the general population. Two land-mark reports were published in the United Kingdom. The first was produced by the Nuffield Council on Bioethics (13) and was within a year followed by a governmental report "Animal tissue into humans – a report from the advisory group on the ethics on xenotransplantation" (14). The ethical basis for these reports is the traditional weighing of benefits against risks. They conclude that the scientific basis at that time was insufficient for entering clinical trials. The term source animal is recommended instead of donor as the animals obviously do not donate their organs. Pig is considered acceptable as source animals while non-human primates are not considered acceptable for the reasons given earlier in this article. The production of transgenic pigs is also considered acceptable as long as the pigs "remain pigs", and are not caused to suffer from the genetic modification. The potential infec-

tious risk for the public is emphasised and considered as a major area for further investigations. The importance of education of and extensive information to future xenograft recipients and their families to make it possible to obtain a truly informed consent is underlined. The reports led to the foundation of the United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA). The UKXIRA has produced a number of reports within the field, which can be found on their web site. The UKXIRA has set high standards for approving a clinical xenotransplantation trial, and so far, they have not received any applications.

The regulatory development in the rest of Europe varies (9). Some countries have decided on, or are considering a moratorium for clinical xenotransplantation e.g. Switzerland and The Netherlands. Others, such as the UK and Spain, have issued reports suggesting a regulatory framework within which applications for xenotransplantation trials could be considered.

Among the Nordic countries, Norway and Denmark at present have moratoriums for clinical xenotransplantation trials, while Finland and Iceland have no official stand on the subject. In Sweden, a governmental report has suggested a legal framework making it possible to perform limited trials, but the report has not yet been presented to the Parliament.

The United States authorities have taken a more liberal standpoint to clinical xenotransplantation trials. Several studies on cellular xenotransplantation have been approved. They are performed within a strict regulatory framework and extensive microbiological testing and follow-up are required (2). Samples from source animals and recipients are to be stored for 50 years. A central registry of xenotransplant recipients and a central archive for the safety samples is being developed. Applications are handled by the Food and Drug Administration, and a number of reports and recommendations on clinical xenotransplantation trials can be found on the FDA web site. As in the UK, primates are not considered acceptable as source animals.

The infectious risks connected with xenotransplantation were highlighted a few years ago, but more data are now available, and most experts in the field today agree that the risk to the public is minimal provided that strict safety precautions are followed. Obviously, microbes do not respect geographical borders, and countries having a moratorium on clinical xenotransplantation, may still have to face the effects of trials performed elsewhere. With this background, several international organisations, including the WHO (12) and the OECD have organised workshops and published reports in the field. The European Council has also appointed a working group producing a state of the art report and draft guidelines for clinical xenotransplantation trials. The international perspective is also important to prevent so-called xenotourism. This term refers both to patients travelling to countries were the treatment is offered, but lacking proper follow-up at home, and to companies or researchers having applications turned down in their own country and will then perform xenotransplantations in countries lacking regulatory framework and the resources to control the trials. The international regulatory initiatives will hopefully provide all countries with baseline recommendations for clinical xenografting and promote rapid exchange of experiences across borders.

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