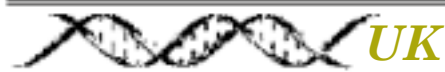


ANIMAL ORGANS FOR HUMANS:

The Science and Ethics of Xenotransplantation

GeneWatch



Briefing Number 19
June 2002

Xenotransplantation is the term used to describe the transfer of organs, cells or tissues between species and from animals to humans. Since there is a shortfall in the number of human organs available for transplantation and patients in need, it has been proposed that organs from animals could be used instead. Because of problems with the rejection of animal organs by the human immune system, attempts are being made to genetically modify animals (mainly pigs) to make them more suitable as organ donors. This briefing examines the science, ethics and safety issues involved.

The organ 'gap'

Organ transplantation has progressed since the 1960s through increased understanding of the immunology of organ rejection; the development of immunosuppressive drugs; and improved methods of tissue matching, organ storage and transport^{1,2}. Another important factor in the success of heart and other transplants was the acceptance of criteria to demonstrate brain stem death which allowed the use of so-called 'heart-beating' donors³.

In 2000, there were 1,487 kidney transplants and 217 heart transplants in the UK. However, there were 6,284 people on the kidney transplant waiting list and 178 waiting for hearts⁴. This disparity in numbers between those in need of organ transplants and organs available is known as the 'organ gap'. Improvements in road safety leading to fewer deaths and thus fewer organs for transplantation has been blamed, in part, for this shortfall. It is against this background of an organ gap that new technologies are being researched and promoted, one of which is xenotransplantation.

Xenotransplantation – its history and the application of genetic technologies

Pig heart valves are routinely used as replacements in cases of human heart disease, but the valves are not living as the tissue has been fixed and preserved and infectious organisms killed with the use of a chemical, glutaraldehyde. However, animal-to-human organ transplantation is far from routine and, to be successful, organs will have to be living and functional as the heart has to beat and pump blood. Attempts to use animals as kidney and heart donors for humans date back to the early 1900s when primates such as chimpanzees and baboons were used⁵. Survival times were very low - often patients did not survive for more than a day. Even with high doses of immunosuppressive drugs, maximum survival times were about two months. The most famous experiment was the transfer of a baboon heart into a newborn baby - Baby Fae - in 1984, who died 20 days later.

It is therefore clear that many practical obstacles have to be overcome if xenotransplantation is ever to be successful. The main barrier is thought to be organ rejection because the transplanted organ is detected as 'foreign' by the human immune system and attacked. This immunological reaction to a xenotransplant has three stages⁶:

- **hyperacute rejection** – occurring very soon after transplantation, involving an antibody response which then triggers the activity of a molecule called 'complement' and a series of damaging reactions;
- **delayed rejection** – this involves blood vessel cells in a rejection response;
- **cell-mediated rejection** – where immune system cells attack the transplanted organ.

Research typically involves experiments with mouse-to-rat transplants and then pig-to-primate transplants

These reactions are thought to be triggered because certain molecules on the surface of cells differ from species to species. The immune system detects these differences in the transplanted organ and a whole cascade of reactions begins as the body tries to kill what it sees as a foreign invader.

To try to overcome this, scientists are genetically modifying animals in one of two ways:

- to remove the molecule that marks other species as foreign to the human immune system – in the case of pigs, this is known as α -gal⁷;
- to include a gene for a human protein - either CD55 (or DAF - decay-activating factor) or CD59 - which inhibits the complement system^{8,9,10}.

Genetic modification is also being used to inhibit other parts of the rejection response and boost protective mechanisms. Research typically involves experiments with mouse-to-rat transplants, and then - to test xenotransplantation techniques further for their suitability for humans - pig-to-primate transplants. Pigs have been selected as the species of choice as organ donors for humans because their organs are about the right size (miniature breeds of pig are often used as other breeds may become too large), they are relatively cheap and are thought not to pose the same ethical concerns as primates. Importantly, using pigs rather than primates should also reduce the chance of disease-causing viruses being transferred along with the organ (but see below).

As well as whole organs, xenotransplantation of pig nervous tissue to treat Parkinson's and Huntington's disease and pig pancreatic islet cells (the cells which produce insulin) to treat diabetes are also under investigation¹¹.

However, if xenotransplantation technology is to be economically viable, it has to be able to supply genetically modified pigs on demand. Because genetic modification of embryos is technically difficult, the nuclear transfer technique (cloning) is being used to produce GM pigs from GM cells. The cloned GM animals will then be bred naturally to produce a herd of GM organ donor pigs.

Success rates

Progress has not been as rapid or smooth as the proponents of xenotransplantation had promised

There has been much hype about the promise of xenotransplantation. In 1995, a leading xenotransplantation company, Imutran, claimed that the technology was "*ready for testing in humans*" because monkeys receiving GM pig hearts survived for 60 days rather than the usual one hour¹¹. However, this was when the monkey's own heart was still in place to pump blood and survival was only for 5-9 days when the transplanted heart had to pump blood. Progress has therefore not been as rapid or smooth as the proponents of xenotransplantation had promised. The UK's regulatory authority, UKXIRA (UK Xenotransplantation Interim Regulatory Authority), was established in 1997 to oversee xenotransplantation in the UK and its 1999/2000 Annual Report concluded that:

*"In summary, the evidence of efficacy has not advanced at the rate predicted when the UKXIRA was established some three years ago. Clinical trials involving whole organs are clearly still some way off."*¹²

Originally, single gene changes, altering one key surface marker molecule (α -gal which is present in pigs but not humans), or expressing the human protein which suppresses the complement reaction were expected to overcome the problems of hyperacute rejection and allow progress. But despite some

success, it is evident that the later stages of rejection pose more serious problems than anticipated and are triggered by many diverse factors, not α -gal and complement alone⁶. These problems have not yet been overcome either through further genetic modification or immunosuppressive regimes. Therefore, it is evident that much more complex genetic modifications will be needed than originally predicted or other strategies adopted.

Approaches which are being investigated include attempts to 'educate' the body to accept pig cells. For example, by infusing the patient's bone marrow cells into a pig foetus it is hoped that both the pig and the human cells would come to consider each other as compatible. The pig/human hybrid bone marrow would then be infused into the patient before organ transplantation. Infusing pig bone marrow cells into the patient sometime before organ transplantation and using anti-rejection drugs whilst the body adapts to the pig cells has also been proposed¹³.

All these approaches are highly speculative and the prospects for animal to human transplants remain extremely remote. However, much hype continues. In March 2000, when PPL Therapeutics announced that it had successfully cloned pigs at its laboratories in the USA, claims were made that human experiments could start in six years¹⁴. In January 2002, PPL announced the birth of cloned piglets with the α -gal gene 'knocked out'. The press release went on to claim that: "*the promise of xenotransplantation is now a reality*"¹⁵. However, only one of the piglets' two α -gal genes are knocked out so all of the piglets still produce α -gal¹⁶ and will now have to be bred naturally with other GM knockout pigs to breed a pig that has both α -gal genes knocked out. Even when pigs are produced with both α -gal genes knocked out, there are many other causes of acute rejection so the approach is likely to fail¹⁷. The announcement was widely interpreted as having been made for commercial reasons in order to boost the PPL share price.

The later stages of rejection pose more serious problems than anticipated and are triggered by many diverse factors

The ethics and risks of xenotransplantation

Despite the poor performance of xenotransplantation trials, there is still considerable investment in research. For example, from January 2001, Novartis has committed \$10 million per year for three years to the xenotransplantation company, Immerge BioTherapeutics, a joint venture with BioTransplant. However, as well as the practical question of whether a human body will ever accept a different species' organ, there are other serious risks and ethical concerns:

- 1. Transfer of disease-causing organisms.** – One of the most serious risks of xenotransplantation is that a disease-causing organism could be transferred with the organ. The dangers of cross infection are greater the more closely species are related, and because primates are so closely related to humans they have been rejected as donors on these grounds. Although pigs were considered safer in this respect, it was shown in 1997 that they can carry certain viruses (porcine endogenous retroviruses – PERVs) that can infect human cells in laboratory tests¹⁸. These have been found in a variety of pig tissues including pig pancreatic islet cells which have been proposed to treat diabetes¹⁹.

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Retroviruses become part of the host's genetic material and so are still found in animals kept in conditions which usually exclude most disease-

causing organisms. These viruses do not usually cause disease in the natural host but may cause disease if they spread to another species. Whilst many retroviruses remain harmless, some can:

- cause tumours;
- combine with other retroviruses to produce novel viruses with unexpected properties;
- alter gene expression²⁰.

Because transplant patients have their immune system suppressed with drugs, they may be especially vulnerable to the effects of retroviruses and any infection could then spread in the population. Such cross-species transfers have caused widespread disease outbreaks in the past. For example, Ebola and Marburg monkey viruses have caused outbreaks of disease in humans; HIV may have originated from monkey retroviruses; and in the 1950s, millions of people were infected with Simian Virus 40, a monkey virus which contaminated vaccines made in monkey cell lines¹¹. A review of 159 patients who had been in contact with pig cells in experimental treatments for liver, spleen and kidney failure (their blood was passed through pig organs outside the patients' bodies); burns (pig skin grafts); or islet cell transplants for diabetes showed no sign of having acquired pig retroviruses²¹. However, the majority of exposure times were low (hours rather than days) with only one case of islet cell transplant extending to 460 days.

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The risk of PERV transfer is likely to remain unquantifiable and may only be determined via direct observation of the outcomes of animal-to-human transplants. Therefore, whether it is ethically justifiable to allow such risks to the whole population to save one life has been questioned²². In 2000, the Roslin Institute pulled out of xenotransplantation research because of the risks from retroviruses, focusing instead on tissue regeneration from stem cells through its alliance with the US biotech company, Geron²³.

- 2. Incompatible physiology.** – Even if an animal's organ is not rejected and it carries no infectious agents, it may simply not work properly in a different species because, for example, the physiology of a pig is not identical to a human's. This is particularly important for kidneys and livers, which carry out complex biochemical functions in the body. For example, there are small but important differences in the structure of the hormone, vasopressin, which controls urine production, and whether a pig's kidney will respond to human vasopressin is unclear. How well the hormones produced by the pig kidney (renin to control blood pressure and erythropoietin to stimulate red blood cell formation) will work in humans is also not known. Therefore, animal organs may not be able to support life in humans. Similar problems may arise with pancreatic islet cell transplants if the pig insulin produced acts differently than human insulin. Insulin for the treatment of diabetes used to be isolated from pig or cattle pancreas, but has largely been replaced by artificial insulin made by genetically modified organisms in contained facilities. Human insulin was considered an advance which avoided side effects caused by bovine or porcine insulin.
- 3. Threats to animal welfare.** - Thousands of animals have been used in xenotransplantation research ranging from mice to chimpanzees. For example, kidneys have been transferred between sheep, tiger, pig, cat, lion, wolf, fox and dingo to dog; dog to wolf; cat, hare and pig to rabbit; rabbit to cat; pig to dog, baboon, monkey, goat and rabbit; sheep and pig to goat;

and guinea pig and mouse to rat²⁴. Many of the recipients will not only have endured surgery but will also have suffered the effects of organ failure and the side effects of immunosuppressive drug regimes. Because genetic modification techniques are variable in their effectiveness, many other animal 'failures' will have been destroyed. The cloning process is also inefficient, with many offspring dying around the time of birth. Whether the prospects for xenotransplantation justify the scale of animal suffering seems questionable to say the least. Using pigs as organ donors would also change our relationship with them, further treating them as commodities for human use. Whether pigs deserve less moral attention than primates is also questionable²⁵.

Because genetic modification techniques are variable in their effectiveness, many animal 'failures' will have been destroyed

Who's involved in xenotransplantation?

Table 1: Companies involved in xenotransplantation research

COMPANY	LOCATION	ORGANS	COMMENTS
Advanced Cell Technology	Worcester, MA	Kidney, heart	Using cloning and GM techniques.
Alexion Pharmaceuticals	New Haven, CT	Nerve cell based therapies	Focusing on Parkinson's Disease and spinal cord damage using GM pigs.
Algenix	Shoreview, MN	Liver	Developing bio-artificial livers using pig cells for external use.
Circe Biomedical	Waltham, MA	Liver, pancreas	Developing bio-artificial livers using pig cells.
Diacrin	Charlestown, MA	Nerve, liver and retina cell based therapies	In partnership with Genzyme Corp using tissue from GM pigs for treatment of neurological disorders.
Immerge BioTherapeutics	Charlestown, MA	Kidney, heart	Joint venture between Novartis and BioTransplant Inc. Agreement with Infigen (an animal cloning company) to collaborate on the production of GM miniature pigs for xenotransplantation.
Nextran/Baxter	Princeton, NJ/Deerfield, IL	Liver	Uses GM pigs and has tested pig liver as an external support for liver failure.
PPL Therapeutics	Edinburgh, Scotland	Kidney, heart	Combining cloning and genetic modification technologies on pigs.
ReNeuron	England	Nerve cell therapies for stroke victims	Developing mouse stem cell lines.
Ximerex	Omaha, NE	Liver	Formed by a scientist from the University of Nebraska Medical Center. Uses GM pigs to produce human/pig hybrid liver by introducing human cells into foetal pigs.

Whether the prospects for xenotransplantation justify the scale of animal suffering seems questionable to say the least

Supplying organs or replacement tissues is seen as a lucrative market and has led to considerable commercial investment

Supplying organs or replacement tissues is seen as a lucrative market and has led to considerable commercial investment in the technology. In 1998, the xenotransplantation market was predicted to be worth up to \$6 billion in 2010¹¹. Several companies - all except two of which are located in the USA - are developing xenotransplantation techniques to use for a variety of organs and tissues (see Table 1). PPL Therapeutics is the only company in the UK involved in whole organ xenotransplantation research. ReNeuron, another UK company, is developing mouse stem cell lines to produce nerve tissue to treat stroke patients. In 2000, following revelations about the suffering of animals in their xenotransplantation research, Novartis closed its UK division of Imutran, which has now been incorporated into Immerge BioTherapeutics. In 1992, at its UK research base in Cambridgeshire, Imutran had been the first to produce a genetically modified pig (called 'Astrid') which was designed to reduce rejection by expressing a human complement inhibiting protein, CD55.

The companies involved in xenotransplantation are trying to develop either whole organ transplantation; tissues for use in nervous system disease or damage; or bio-artificial machines outside the body which use animal cells to support liver or kidney function as the patient's blood is passed through them. Many of the companies have research collaborations with universities and hospitals in the US and GM pigs are the most commonly used donor animal. All approaches for organ transplantation envisage using immunosuppressive drugs in partnership with xenotransplantation because the problems of rejection are not considered to be completely resolvable – even patients with human organ transplants require lifelong immunosuppression drugs.

Alternatives to xenotransplantation

An important question when considering whether xenotransplantation should be pursued is whether there are other options for improving the availability of organs for transplantation. Alternatives that could be used to address the organ gap include:

- **Prevention** – to address the root causes that lead to the need for organ transplantation. These include life-style improvements to reduce heart disease and early diagnosis of diabetes (which is an important cause of kidney failure).
- **Better transplantation services** – The British Medical Association and others have called for a range of measures to improve services, including better coordination and increased provision of intensive care beds²⁶. In Spain, such measures - together with new ways of increasing organ donation - led to 33.6 organs per million of the population being transplanted in 1999 compared to 13 per million in the UK²⁷.
- **Increasing organ donation rates** – An opt-out scheme has been proposed where it would be assumed that a person would be willing to donate their organs after death unless they specifically registered that they did not wish this to happen²⁸. Whilst this approach raises important questions of the moral acceptability of such presumed consent²⁹, other options include mandated choice³⁰ (where a person's willingness to donate cannot be overridden by their relatives' wishes) and increased use of altruistic donation by living donors in the case of kidney transplants (people have two kidneys but can survive with one).
- **Biomechanical devices** – Improvements in artificial heart technology^{31,32}, in dialysis machines and artificial livers may also lead to more effective ways of treating organ failure. Miniaturisation of artificial livers and kidneys could

There are other options for improving the availability of organs for transplantation

lead to people being able to move around while they are using them and living a more normal life.

- **Stem cell technologies** – Attempts are being made to regenerate tissues from stem cells, a type of cell that retains the ability to develop into different cell types³³. Stem cells would be ‘reprogrammed’ to develop into the tissues required. To avoid the problems of rejection, the stem cells could either be genetically modified or the nucleus from a cell of the patient could be used with an empty egg to produce a compatible organ. This later approach is called ‘therapeutic cloning’ to distinguish it from ‘reproductive cloning’ where an individual would be created. Stem cells can be isolated from embryos or adults. Embryo research raises particular ethical concerns about the creation of embryos for use by another person. All such research is a long way from producing whole organs but the production of heart or liver tissue to support failing organs, nerve cells to treat neurological disease and islet cells to treat diabetes is more realistic in the medium term.
- **Improving transplant tolerance** – Ways of promoting tolerance so that cross-matching and anti-rejection drugs are no longer required are being investigated in experimental animals. This includes injection of donor cells into the recipient and modifying the transplanted organ using targeted gene therapy so that it produces proteins which interfere with the rejection response³⁴.

Conclusions

As the population ages and technological advances allow us to keep people alive for longer, the demand for new organs is likely to keep on increasing. Filling the organ gap through the production and sale of genetically modified animal organs, rather than through unpaid donations, is an attractive prospect for the biotechnology industry. However, the prospects for xenotransplantation are poor and research involves a vast number of animals in painful experimentation each year. It may be impossible to remove the risks of transfer of diseases which could threaten not only the patient but also the wider population. Incompatible physiological differences may also obstruct development. There are alternatives, some of which could address need immediately, such as improvements to the provision of NHS services and encouraging donation. Other areas of science, such as the regeneration of tissues from stem cells also offer solutions for the future. Therefore, GeneWatch UK believes that the risks to human health and the suffering of animals involved in xenotransplantation research cannot be justified.

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The prospects for xenotransplantation are poor and research involves a vast number of animals in painful experimentation each year

This briefing has been adapted from a more detailed 100-page GeneWatch report: **“Genetically Modified and Cloned Animals. All in a Good Cause?”** (£5 for individuals - £20 for businesses and organisations.)

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