Xenotransplantation: an overview of microbiological risks and potentials for risk management

D.E. Onions (1) & C.J. Witt (2)

(1) Q-One Biotech Ltd, University of Glasgow, Todd Campus, West of Scotland Science Park, Glasgow G20 OXA, United Kingdom
(2) Scientist, Department of Communicable Diseases Surveillance and Response, World Health Organization, Avenue Appia, CH-1211 Geneva 27, Switzerland

Summary

Xenotransplantation is the use of animal organs, tissues or cells for transplantation into humans to treat a variety of medical conditions. If proven efficacious, the technique could be used as one means of alleviating the disparity between the growing demand for transplantable organs, tissues and cells, and the availability of human-origin transplants world-wide. Just as the practicality and efficacy of the technology need to be investigated, so too does the potential for associated infectious disease risk. While much remains to be learned about the microbiological risk associated with xenotransplantation, the elements to be incorporated into xenotransplantation risk management schemes can be considered, using what is currently known about the infectious agents potentially relevant to the xenotransplantation setting.

Keywords


Introduction

Organ, tissue and cell allotransplantation (the transplantation of living organs, tissues and cells from a human donor into a human recipient) has become a widely-used practice for treating patients with a variety of health conditions. However, donation has not kept pace with demand, and this has led medical researchers to search for alternative measures to overcome transplant shortages (27). One alternative under investigation is the use of xenotransplantation (from the Greek term xenos, meaning strange or foreign), a technology which uses animals as the sources of cells, tissues and organs for transplantation into humans. Xenotransplantation is also being investigated as a treatment method for diseases with no other effective therapeutic interventions, such as refractory Parkinson's disease or Huntington's disease, or as an additional therapeutic approach to conditions such as diabetes mellitus (49). In the future, if any of the proposed uses of xenotransplantation are proven practical and efficacious, xenotransplantation could contribute to the alleviation of suffering and improvement of human health world-wide.

However, the adoption and use of xenotransplantation requires careful consideration. Xenotransplantation can present a risk of xenozoonosis, infectious disease spread from xenotransplant-source animals to human xenotransplant cell, tissue or organ recipients, and potentially the wider human population. This risk is currently unquantifiable, but should be recognised as expanding the traditional scope of zoonoses (infections transmitted from animals to humans under natural conditions) to include those agents which acquire communicable or pathogenic characteristics when exposed to a xenotransplant environment in human transplant recipients. Therefore, the risk of xenozoonosis should be weighed carefully against the potential benefits this technology could offer.

In an effort to promote informed consideration of the relative public health risks and benefits of xenotransplantation, this paper discusses the potential microbiological risks associated with xenotransplantation. In addition, suggestions are offered on the applicability of xenotransplantation source-animal health assurance and clinical investigation programmes to help manage this risk.
General microbiological risk associated with transplantation

In any transplantation practice, a risk of transmission of infection from donor to recipient exists. Viral, bacterial, fungal and parasitic transmission has occurred in allotransplantation, and has been a major cause of post-procedure morbidity and mortality in transplant recipients. Infection and resultant disease can be attributed to both the level of immunosuppression required in the transplant recipient and to the use of organs or tissues contaminated with microbial agents (24). Usually these infections are caused by the use of human donors with asymptomatic and previously undiagnosed infections. However, in some instances donors with diagnosed transmissible diseases have been used in allotransplantation because no other ‘infection-free’ donors were available (37).

Microbiological risks associated with xenotransplantation

Domestic pigs are considered the most likely sources for transplantable organs, tissues and cells for xenotransplantation. This is largely due to the relative suitability of the size and anatomy of their organs for use in humans, the current understanding of and ability to provide for the husbandry and welfare requirements of this species, and the expectation that scientific research will lead to methods of reducing the immunological disparity between transplants of porcine origin and human xenotransplant recipients (7). However, domestic pigs do carry a variety of infectious agents which can be risk factors for the practice of xenotransplantation. These infectious agents include bacteria, fungi, parasites, viruses and potentially new or previously unrecognised agents (17).

Bacterial, fungal and parasitic agents

In general, bacterial and fungal agents can present a microbiological risk for xenotransplantation. Factors influencing that risk include the following:

- the systemic presence of these agents in a xenotransplant source-animal at the time of transplant harvesting
- the latent sequestration of these agents in a xenotransplanted organ, tissue or cell (i.e. micro abscesses with viable Salmonella bacilli in transplanted livers or Mycobacterium fortuitum in porcine heart valves) (7)
- agent contamination of a xenotransplant during harvesting procedures.

If one of these conditions occurs, the xenotransplant recipient could become infected. The risk of productive disease in the recipient will be influenced by the nature of the agent, the immune-suppression of the recipient, and other factors, alone or in combination. The disease produced may or may not be identical to that produced by a naturally transmitted infection, but the mechanism of disease production is expected to be similar to that produced by a contaminated allograft (37).

Parasites, both protozoa and helminths, can also be microbiological risks to xenotransplant recipients. The exact level of risk will depend on the tissue tropism of the parasite and the presence of the parasite in the xenotransplant. Agents like Toxoplasma gondii or Trichinella spiralis, which can be ubiquitous in source animals, probably present the greatest risk. Other agents which are naturally confined to specific organs not harvested for xenotransplantation would not present a risk unless the xenotransplant becomes contaminated during harvesting.

The ability to eliminate a parasite from potential xenotransplant source animals also influences the level of risk associated with parasitic infections. For example, Cryptosporidium parvum is a parasite commonly found in pigs. When the lack of success in eliminating this agent from immune-compromised humans is coupled with the potential for contamination during xenotransplant harvesting, C. parvum could be considered a significant microbiological risk factor for xenotransplantation (28).

Viral agents

Viruses are the infectious agents thought to present the highest microbiological risk associated with xenotransplantation. The associated risk of infection and subsequent disease will vary with the individual characteristics of each virus and will be influenced by the ability of the virus to survive and propagate in the new host, the ability to be transferred between hosts, and virulence of the virus in the new host. A virus which has (or gains) the potential to cause severe morbidity or mortality in the xenotransplant recipient should be designated as presenting a high level of microbiological risk. Viruses which can create persistent, but latent infections in the xenotransplant recipient and which have the capacity for person-to-person transmission, should also be considered as possessing a high level of risk. Some of the considerations necessary to determine the level of risk potentially attributable to different groups of viruses are described below.

Categories of viruses of concern

The viruses of concern to xenotransplantation can be divided into several functional groups, as follows:

a) zoonotic viruses
b) viruses that replicate in human cells in vitro (although not regarded as zoonotic)
c) viruses that could be transmitted from porcine to human cells by syncytial spread
d) viruses that might undergo abortive replication in human cells but could be oncogenic

e) viruses that have not been shown to replicate in human cells but belong to groups that have shown host range changes

f) porcine viruses that could damage the transplant under conditions of reduced immuno-surveillance

g) human pathogens that could use the products of human transgenes to infect the transplanted pig organ and may develop new properties

h) virus infections that indicate that the biosecurity of the facility housing the animals has been breached.

These functional groups, but not the individual viruses, are described below. Recommendations on individual viruses that should be excluded from donor pig herds have recently been submitted for publication (46).

Zoonotic viruses

These are viruses known to be transmitted from pigs to humans. The influenza A viruses form the most dramatic example of this group. The pig is susceptible to both avian and human strains of influenza A and evidence exists to demonstrate that the pig forms a reservoir in which new genetic reassortments between human and avian strains occur (11). The influenza pandemic of 1918-1919 is believed to have caused the deaths of between 20 and 40 million people and retrospective serological studies indicated that the porcine influenza strain causing disease in pigs was antigenically very similar to the human virus responsible for this catastrophic event. This classical H1N1 swine influenza virus is still endemic in the pig population and has been isolated from humans (in which the virus can cause severe and occasionally fatal disease). Currently, classical H1N1 swine influenza virus is present in both Europe and the United States of America. However, in Europe the virus has largely been replaced by H1N1 strains of avian origin (10). In addition, 'human-like' H3N2 viruses are present in the pig population in Europe, and it is these viruses that have undergone genetic reassortment with avian-like H1N1 viruses. Several features of the biology of influenza A viruses favour interspecies transmission. The receptor for viral binding is sialic acid, a moiety which is ubiquitous on avian and mammalian cell membranes, and the segmented genome of the viruses results in the rapid generation of new variants through reassortment of these strands. Furthermore, as in other ribonucleic acid (RNA) viruses, the error rate of the virion RNA polymerase is to the order of 1 in 10^3 nucleotides per replication cycle; therefore, mutations rapidly accumulate. The avian influenza epidemic of 1983 in Pennsylvania was caused by the introduction of a wild bird H5N2 strain to domesticated chickens; within a few months, the acquisition of seven mutations in the haemagglutinin gene resulted in an increase in mortality from 10% to 80%, accompanied by an increase in the transmissibility of the virus (59).

Although these viruses are important in human and veterinary medicine, xenotransplantation does not increase the risk of infection for the general public. Moreover, control of these viruses should be possible. In the case of influenza virus, control should be feasible by ensuring that the housing is bird-proof and that the staff are vaccinated.

Porcine viruses that replicate in human cells \textit{in vitro}

A number of porcine viruses replicate in human cells \textit{in vitro} whilst evidence of zoonotic potential is weak or absent. For instance, while pseudorabies (Aujeszky's disease) virus (PrV), an alpha-herpesvirus, can infect human cells in vitro, only anecdotal evidence exists to suggest a role as a zoonotic virus. Nevertheless, the virus causes fatal encephalitis in other species, such as cattle, cats and dogs. Two broad classes of activity may affect the capacity of PrV to induce disease in non-porcine hosts, namely, the ability of the virus to infect and be released from cells at the mucosal barrier, and the extent to which the virus produces latent or lytic infection in neuronal cells. While PrV, like other alpha-herpesviruses, binds to glycosaminoglycans, entry is dependent on interaction with other receptors and requires the activity of at least four glycoproteins (gB, gD, gH and gL). The second receptor for the human alpha-herpesvirus, herpes simplex virus, has recently been identified as a novel member of the tumour necrosis factor (TNF) family, which is likely to show a degree of species specificity (40). Exit of the alpha-herpesviruses is also dependent on viral glycoproteins, particularly gE, and the critical role of the rate of exit of viruses from cells is indicated by the attenuating effect of gE deletion on wild type virus. Xenotransplantation may overcome the natural barriers to infection, particularly the mucosal barrier, and once replication is initiated in human cells, further selection for human adapted strains is likely to occur under the conditions of reduced immuno-surveillance.

Some barriers to infection may not be so easily bypassed; many complex deoxyribonucleic acid (DNA) viruses (adenoviruses, herpesviruses and poxviruses) carry genes, the products of which modulate the host immune response. These products are often homologues of cytokines, cytokine receptors or proteins that downregulate the expression of class I major histocompatibility complex (MHC) genes. These products are often species-specific, therefore in heterologous hosts, the immune response is unimpaired and effectively eliminates the virus. For instance, the structural genes, and many non-structural genes, of canine adenovirus have clearly identifiable homologues in the human adenoviruses (41). However, the E3 region genes of the human adenoviruses (B, C, D and E subgroups), which contain several genes that inhibit TNF-α induced apoptosis (21), or that sequester MHC class I in the endoplasmic reticulum (3), have no obvious homologues in the canine virus (41). Nevertheless, complex viruses have shown species 'jumps'. An example is adenovirus 76, an endemic virus in commercial chickens which originated as an adventitious duck virus in live vaccines.
Viruses that may be able to infect human cells by syncytial spread from cell to cell

Some enveloped viruses may be able to spread from the xenotransplant to human tissue through the formation of syncytia, even if cell-free infectious virions are not produced. Enveloped viruses, such as those of the families Paramyxoviridae and Herpesviridae, are particularly adept at this mode of spread. The gD glycoprotein of several alpha-herpesviruses is essential for the infectivity of virions. However, when genomes lacking this glycoprotein are transfected into permissive cells, spread of the infection can occur through cell fusion. In the Paramyxoviridae, viral tropism within a host and between hosts can be affected by the tissue-specific proteases required to cleave the fusion glycoprotein.

Viruses that infect human cells, fail to undergo productive replication but may initiate disease

Experimental transmission of certain members of the herpesvirus, adenovirus and polyomavirus families can result in tumour formation outside the natural host. For instance, human adenovirus 12 is oncogenic in hamsters, as is equine herpesvirus 1. These events are associated with abortive replication and with integration of part of the viral genome into chromosomal DNA. Abortive replication of animal adenoviruses in human cells has been observed for several viruses, including canine and bovine adenovirus, features which make these viruses potentially interesting as vectors, but which may also indicate that wild type virus could be harmful.

Other serious, non-neoplastic diseases have been associated with natural transmission of herpesviruses from one host to another. For example, sheep carry a gamma-herpesvirus, ovine herpesvirus 2 (OHV-2), which has not been associated with disease in this species. Natural transmission to cattle results in the production of a fatal lymphoproliferative condition, malignant catarrhal fever, but further cattle to cattle transmission does not occur. Partial DNA sequences of two porcine gamma-herpesviruses have recently been described (16) and caution will be required in determining the disease potential of these viruses in humans.

Porcine viruses that are not known to replicate in human cells but which belong to groups that have shown significant changes in tropism or host range

One of the most problematic viruses in pig herds is the porcine parvovirus. This small, unenveloped, single-stranded DNA virus is one of the most resistant viruses in the environment and this, coupled with the ability to cross the placenta, makes eradication very difficult. Relatively minor mutational changes in the genomes of the autonomous paroviruses have been associated with dramatic host range changes or changes of virulence and tropism. In 1978, a new pandemic virus infection of dogs which resulted in high morbidity and mortality spread world-wide. The origin of this virus (canine parovirus [CPV] type 2) is almost certainly a related parovirus of carnivores, most probably the feline parovirus (FPV). Experimental mutation of the FPV genome indicated that two critical amino acid changes within the surface loops of the capsid protein were sufficient to confer significant changes to FPV, namely, acquisition of the canine host range, a CPV-specific neutralising epitope and haemagglutination characteristics of CPV (13). The restriction of FPV replication in canine cells is post-entry as both CPV and FPV bind to and enter canine cells at the same rate (26). Following the original identification of CPV type 2, the virus has become further adapted to replication in dogs, and two variants, CPV 2a and 2b, are now endemic in the canine population. Moreover, recent infections of cats with parovirus have also involved these viruses (C. Parish, personal communication).

Four broad pathogenic types of pig parovirus (PPV) have been distinguished, as follows:

- non-pathogenic strains (e.g. National Animal Disease Laboratory 2 [NADL-2])
- strains pathogenic to non-immunocompetent foetuses
- highly pathogenic strains causing death in immunocompetent foetuses (Kresse strain) and dermatitis in adults
- enteric strains.

A high degree of identity exists between prototype, non-pathogenic strains, and highly virulent strains; only five amino acids in the VP2 (viral protein 2) capsid protein distinguish the Kresse strain from NADL-2 (6). The possibility of host range changes in this virus should not be discounted, particularly as PPV is able to replicate in non-porcine cells in vitro: a property determined by both the capsid and non-structural (NS) genes (57).

The porcine coronaviruses have also shown dramatic changes in tissue tropism. An example is the transmissible gastroenteritis virus (TGEV) which causes severe enteric disease. In the 1980s, a new porcine respiratory coronavirus (PRCV) spread rapidly through the pig population in Europe and was determined to be a mutant of TGEV containing a large deletion in the spike protein (9). In North America, PRCV strains appear to have evolved independently and are more virulent than counterparts in Europe. Both TGEV and PRCV belong to the closely-related antigenic group 1 coronaviruses which include the human respiratory coronavirus and the feline and canine coronaviruses. Genetic interchange may occur within this group of viruses; evidence exists that during the evolution of one serotype of the feline coronavirus, recombination with the canine coronavirus has occurred. The high frequency of recombination in coronaviruses may be related to the unusual mechanism of coronavirus replication, involving discontinuous transcription (reviewed by Lai [29]).
Porcine viruses that may damage the xenotransplant under reduced immuno-surveillance

Under conditions of reduced immuno-surveillance, some viruses could damage the xenotransplanted organ without necessarily posing a risk to public health. Such viruses are likely to be those that establish latent infections in the host; porcine cytomegalovirus is the prime example.

Transmission of human viruses to the xenotransplant

Some human viruses may be able to infect and damage the integrity of the xenotransplant. These can be divided into several categories, as follows:

a) viruses known to infect pigs, e.g. human influenza
b) viruses where serological evidence exists of infection which could be productive or abortive, e.g. human adenoviruses and rhinoviruses
c) viruses that have been transmitted experimentally to pigs, e.g. human isolates of hepatitis E virus.

The complexity of the latter situation is revealed by the recent description of a naturally occurring porcine hepatitis E virus which is capable of causing disease in humans (36).

A further complication in xenotransplantation is that the complement regulating transgenes may also serve as receptors for human viruses. Decay-accelerating factor cluster of differentiation antigen 55 (CD55) has been identified as the receptor for echovirus 7 (58) and CD46 is one of the receptors required by measles virus to infect cells. However, while some murine cells expressing human CD46 transgenes have not been shown to attach to and fuse with the cells (20).

Unknown viruses

Certain virus families (e.g. the Hepadnaviridae) or certain virus groups (e.g. the morbilliviruses) have never been reported in the pig. However, new virus diseases are being recognised or are emerging, for example, Menangle and Nipah viruses (12, 50). No simple solution exists to identify these viruses which have not been identified. However, one approach is to identify risk categories of viruses and then attempt to demonstrate that the virus genus or family is absent, using redundant polymerase chain reaction (PCR) and related techniques. For instance, redundant PCR has been used to identify the porcine cytomegalovirus in tissue and in turn the amplified sequence has enabled the development of specific assays for this virus (Q-One Biotech, unpublished data). Similarly, representational difference analysis (RDA) may be useful in some contexts to exclude the presence of exogenous DNA (or RNA) in a target tissue. For instance, in a particular line of pigs, the target organs within a donor breeding herd could be used to screen for unknown agents by RDA (14, 32). Practical experience of RDA indicates that an extensive work load is involved in sequence analysis, but the technique is probably worth considering as a final validation of the quality of a particular line. The probability of detecting novel viruses using this technique might be enhanced by immunosuppression of the pigs before analysis.

Endogenous retroviruses: a special case

Retroviruses belonging to the Gammaretrovirus group (formerly type C oncovirus group) are associated with the induction of leukemias, lymphomas and other degenerative conditions (44). A feature of the replication of retroviruses is the integration of an RNA copy of the virus genome into chromosomal DNA. Over evolutionary history, retroviral genomes have become integrated into the germ line so that some are transmitted as Mendelian elements termed endogenous proviruses (15). Many of these proviruses are defective and are unable to be expressed as functional viruses while others can be reactivated to produce infectious virions. In other instances, the endogenous sequences are not expressed as infectious virus but may serve as substrates for recombination with exogenous retroviruses. For instance, a cat-to-cat transmitted retrovirus, feline leukaemia virus subgroup A (FeLV-A) has been demonstrated to undergo recombination with endogenous retroviral elements to generate a new subgroup called FeLV-B (51). The subgroups of retroviruses are defined by the receptor used to enter cells, which is governed by the major envelope glycoprotein (surface unit: SU). The use of DNA probes to regions outside the envelope gene or long-terminal repeat (LTR) will detect all viruses belonging to the group.

The porcine endogenous virus

Host range of porcine endogenous viruses

Porcine endogenous retroviruses (PoERVs or PERVs) are spontaneously produced from certain primary and secondary porcine cell cultures or may be induced to do so by various mutagenic events (4, 8, 31, 39, 53, 56, 61). Initial studies failed to show that porcine endogenous retroviruses were able to infect human cells. In 1996, D.E. Onions and colleagues reported to the United States Food and Drug Administration (FDA), the infection of human Raji and 293 cells by porcine kidney (PK) 15 cells, and independently, Robin Weiss and colleagues demonstrated the infection of human cells by PoERVs (19, 30, 47, 60).

Three subgroups of PoERV (A, B and C) are recognised, based on interference properties. By definition, this indicates that these viruses use separate receptors to enter cells. Both subgroups A and B have infected a range of human cell types (30), but with an efficiency approximately $10^2$ to $10^3$ times lower than that for amphotropic murine leukaemia virus which is widely used as the basis of human gene therapy vectors (45, 55). Subgroup C has only been shown to infect one human cell line, HT-1080-1 cells, and whether this virus has a general ability to infect human cells is not yet clear (55). A further feature limiting the spread of these viruses in vitro is...
that upon infection, the proviruses frequently become transcriptionally silent.

Sequence of the porcine kidney 15 virus

The complete nucleotide sequence of two PoERVs has been obtained (1, 19), in addition to envelope sequences of other PoERVs (5). Some of these may represent type variants within subgroups A, B and C, but many have yet to be analysed by pseudotype analysis to confirm subgroup status.

The PoERV-B1 and C strains share 90.1% identity within the gene gag and 96.8% identity within pol; the strains are also closely related to the gibbon ape leukaemia virus (GaLV). Classification of retroviruses has been performed on the basis of primer binding sites. Surprisingly, PoERV-B1 uses a transfer RNA (tRNA) binding site complementary to glycine 2, a feature which has not been recorded in any other retrovirus. PoERV-C utilises a proline tRNA primer.

The long terminal repeat of the PoERV-B1 virus contains enhancers within the U3 region that have motifs indicative of expression in lymphoid or haematopoietic cells. These motifs include LyF-1, E47 and ETS-1 (25, 33). Interestingly, spontaneous expression of PoERVs has been observed from activated primary lymphocytes and from endothelial cells (35, 60).

Relevance of sequencing PoERV for the development of porcine xenotransplantation

The acquisition of the complete PoERV sequence has enabled a number of tools to be developed for the detection and analysis of the expression of PoERVs in porcine and human tissue.

The development of clinical trials using porcine tissue must exclude the possibility that infection of human cells will occur, with subsequent production of mutated or recombinant proviruses that may spread through the human population (52). The poor infectivity and limited expression of PoERV in vitro indicate that in vivo the virus would probably be unable to establish a productive infection. However, confounding factors are the effects of iatrogenic immunosuppression and the probable loss of the protective complement-dependent lysis of retroviruses in pigs transgenic for human complement regulatory genes. Consequently, whole organ xenografts may pose a higher risk than tissue transplants (e.g. islet cells) for the following reasons:
- immunosuppression is required
- the tissue is likely to be transgenic for complement regulatory genes
- a wider range of tissues including endothelial cells are transplanted.

A decision tree for progressing to clinical trials might encompass several elements, as follows:

1. Expression of the virus in pigs

It is necessary to ascertain whether proviruses are present in every pig and whether these proviruses are expressed. All pigs examined to date appear to contain multiple copies of PoERV A and B (30), whereas subgroup C is more polymorphic with some pigs lacking a prototype subgroup C env sequence and others having approximately fifty copies (45). Expression of PoERV sequences appears to be common in many tissues, but may be restricted to certain cells, e.g. endothelial cells or lymphocytes (G. Langford, personal communication). However, recent evidence indicates that some pigs have low level viraemias (G. Langford and D. Galbraith, personal communication).

2. Analysis of primate models with porcine xenotransplants

Provided the cells of primates can be shown to be permissive for infection by PoERV, they are potentially valuable models for assessing the safety of porcine xenotransplantation. If the confounding factor of micro-chimaerism can be controlled, confirmation of whether PoERV infects the primate tissues should be possible. In the event that PoERV genomes can be identified in primate cells, extreme caution would be needed before considering clinical trials of whole organ transplants.

3. Analysis of patients who have received porcine xenografts or xenotransplants

A number of patients have already received porcine islet cell xenografts (48). These patients have been examined for PoERV and no evidence of infection has been observed (23). Analysis can take the form of examination of the peripheral blood mononuclear cells (PBMCs) of these patients for the presence of PoERV sequences. This process is more complex than it appears as micro-chimaerism from porcine cells present in peripheral blood can complicate the analysis. An approach to solve this problem is to use quantitative PCR techniques and to compare the PoERV copy number to a reference porcine gene sequence. Ratios of PoERV copy number to control gene copy number outside a defined range would be indicative of infection.

However, analysis of natural models of gamma-retrovirus infection indicates that a broader range of assays is required to define all infection states. For instance, in cats infected with FeLV, the majority eliminate the infection and develop antibodies to the virus. This is in contrast to infections with human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV) where antibodies indicate active infection. Persistently infected animals, which develop disease and transmit the virus, exhibit plasma viraemias and plasma antgenaemia. However, other infection states exist,
for example, the virus can be sequestered in epithelial sites. These animals usually lack virus or viral genomes in the circulation but may have viral antigen and possibly anti-viral antibody. In a true latent state, FeLV genomes are detectable in the bone marrow, and to a variable extent, in the peripheral blood. Such animals usually have detectable anti-viral antibody but no viral antigen in the plasma. Consequently, caution should be exercised in the use of a single assay system to define the viral status of patients. Western blot systems using both purified virions and recombinant viral proteins have been validated for use in screening for human antibody to PoERV (D. Galbraith and D.E. Onions, unpublished findings), and highly sensitive fluorescent product-enhanced reverse transcriptase assay (F-PERT) and reverse transcriptase-PCR systems have been developed to screen for plasma viraemias (34).

The future of porcine xenotransplantation

With the caveats expressed above, progress to clinical trials may be possible, with a cautious, step-by-step approach. Rigorous analysis of the recipient patients in these trials will play an important part in validating the safety of the procedure. Should the endogenous retrovirus pose an unacceptable level of risk, longer-term solutions also exist. Vaccination of patients before transplantation is not unrealistic as effective vaccines are available against gamma-retroviruses (in contrast to the lentiviruses). The effects of vaccination on a transplanted organ which could express PoERV proteins would have to be evaluated before this strategy could be applied. The polymorphism of PoERV loci within the pig population indicates that the breeding of pigs lacking functional loci might be possible, although current data does not favour this solution. Alternatively, the development of new cloning technologies for the pig may permit the application of either conventional gene knock-out techniques or the introduction of negative regulatory transgenes to control proviral expression.

Unknown agents

As seen from the above overview, a number of known infectious agents can cause disease in humans and could be transmitted during xenotransplantation. In all probability, however, other agents of animal origin also exist. These agents are not currently recognised, but could present microbiological risks both to the immediate xenotransplant recipient, and to human contacts of the recipient and the general public. Experience gained in the past when transmitting previously-unknown human pathogens through allotransplantation (i.e. HIV, hepatitis C), demonstrates the need for caution when assessing the risk of unknown, but potentially existent animal-origin agents (38). The assumption that unknown microbial agents may exist and could have a negative impact on the safe practice of xenotransplantation implies a need to create a robust programme to develop and refine diagnostic capabilities for identifying new or previously unrecognised infectious agents.

Microbiological risk management for xenotransplant source animals

In general, before a decision is made to proceed with a technology, the microbiological risks associated with that technology should be identified and quantified, and measures to manage and reduce those risks should be demonstrated to be effective. In the case of xenotransplantation, however, while such a risk assessment and management scheme is possible using what is understood about the traditional zoonotic agents and using the allotransplantation model, the process is not possible for all potentially relevant agents, as yet unknown agents, or for all xenotransplantation-associated transmission scenarios (43). Many unanswered questions remain.

Therefore, during this period of xenotransplantation investigation, risk management schemes should favour precaution. The goal of a risk management scheme for xenotransplant source animals is to produce and maintain animals which are as free as possible from the microbiological agents that could have an adverse impact on the health of xenotransplant recipients, their contacts and the general public. The design and implementation of effective mechanisms to prevent xenozoonotic infections is essential, including the monitoring, prompt response to, and correction of any suspected or identified xenozoonotic occurrence. The implementation of any instituted risk management practices must be paired with a continuous assessment and validation process for the ability of the practices to adequately and appropriately prevent or control microbiological risks.

Consequently, the authors suggest that a microbiological risk management scheme for xenotransplantation should include the following:

- the development of 'xenotransplant-grade' source animals which meet the exacting microbial agent status believed necessary for xenotransplantation
- the implementation of a comprehensive health surveillance and clinical investigation programme for the timely detection and response to infectious disease occurrences in xenotransplant source animals and colonies
- the strict application and monitoring of husbandry and biosecurity practices designed to prevent source animal exposure to microbial agents relevant to xenotransplants
- support for the development and refinement of diagnostic procedures appropriate for the accurate detection and identification of microbial agents, both known and as yet unknown, which are relevant to the xenotransplantation setting.
The effectiveness of the biosecurity required for 'xenotransplantation-grade' animal colonies can be monitored through programmes of assurance and clinical investigation. 'Xenotransplantation-grade' animal health maintenance needs of xenotransplant source animals (17). Between colony animals. Other 'barrier' practices may also be required to support the specific husbandry and health infectious diseases which could be transmissible to and from animals housed. These persons are expected to be free of overalls, surgical masks and caps, single-purpose footwear and gloves. These persons are expected to practice such 'barrier' procedures as wearing overalls, surgical masks and caps, and single-purpose footwear and gloves, which serve as vectors for inanimate objects which could serve as vectors for microbial agents, and the monitoring for breaks in the physical and procedural integrity of the 'barrier' in which the animals are housed.

Under ideal conditions, the only infectious agents permitted in xenotransplant source animals would be those which are necessary for the normal biology of the animal and which cannot be transmitted through the act of transplantation. In reality, such a highly restrictive definition of 'xenotransplantation-grade' may not be attainable. Nevertheless, a scientifically-based and precautionary standard should be expected, established and maintained.

Husbandry practices for 'xenotransplant-grade' animals

A 'xenotransplantation-grade' animal colony should be maintained under strict biosecurity conditions which ensure the complete isolation of animals from the microbial agents potentially relevant to xenotransplantation. These conditions could be met by a 'barrier'-type facility where the structure and operation of the facility prevent the inadvertent entrance of unauthorised personnel or animals, microbial agents, or inanimate objects which could serve as vectors for contaminations in the facility. In such facilities, all items used for the care and support of the animals (e.g., water, feed, bedding, medical, diagnostic and husbandry supplies and equipment), are autoclaved or otherwise sterilised before being brought into the 'barrier'. The in-coming air supply to animals is exclusively high-efficiency-particulate-air, filtered to prevent air-borne contaminations. Persons entering the 'barrier' are expected to practice such 'barrier' procedures as showering-in-and-out, wearing sterilised protective garments such as overalls, surgical masks and caps, single-purpose footwear and gloves. These persons are expected to be free of infectious diseases which could be transmissible to and between colony animals. Other 'barrier' practices may also be required to support the specific husbandry and health maintenance needs of xenotransplant source animals (17).

'Xenotransplantation-grade' animal health assurance and clinical investigation programmes

The effectiveness of the biosecurity required for 'xenotransplantation-grade' animal colonies can be monitored and assured through the implementation of an animal health surveillance programme designed to quickly and accurately detect the appearance in the colony of any microbial agents relevant to xenotransplants. The exact design of the surveillance programme will depend on the natural history of the agents under survey, the strengths and weaknesses of the diagnostic testing systems technically available, the history of the colony, and the size and form of the animal housing arrangements (18). The elements of the surveillance programme should include both the monitoring of the animals (either through cohorts or as individuals) for microbial agents, and the monitoring for breaks in the physical and procedural integrity of the 'barrier' in which the animals are housed.

Agents presenting a direct microbiological risk for xenotransplantation should be monitored at an interval which will allow for rapid detection and elimination from the colony. The exact frequency of testing should be determined by the expected turn-over rate of the animals in the colony, and the expected incubation and transmission periods of the microbial agents under surveillance.

In the event of an illness in a source animal, an immediate and thorough clinical investigation should be initiated and continued until the cause of the illness is specifically determined. If the aetiology of the illness is infectious, timely measures must be taken to contain, control and remove the infection from the colony. This may require the destruction of economically and scientifically valuable animals (54).

In addition to the above, in a closed xenotransplantation-grade colony, the assurance of biosecurity will require a rigorous application of quality-controlled and validated animal care and barrier maintenance procedures. Such procedures, when combined with animal health and microbiological surveillance can serve to increase confidence in the overall process used for producing xenotransplantation-grade animals.

To aid future animal health surveillance and clinical investigations, archives of biological samples from source-animals, records of animal health surveillance results and records of procedural quality control activities should be maintained and be accessible for review.

Research and development of xenozoonotic diagnostic technology

Research and development of methods to detect, diagnose and, if practical, treat xenozoonoses is essential for the management of the microbiological risks associated with xenotransplantation (27). Research is needed to expand the knowledge-base of the microbiological risk factors potentially associated with xenotransplantation. These risk factors include the following:

- source-animal microbial flora
known microbial agents which could impact on xenotransplantation

- new or currently unknown agents which might emerge as a result of xenotransplantation.

Current diagnostic capability should be continuously assessed and, if necessary, refined, to ensure the validity and meaningfulness of the diagnosis in the xenotransplantation setting. New technology should be developed where current technology is not able to fulfil xenotransplantation risk management needs (62).

Quality assurance of management and prevention strategies

As with any effective microbiological risk management scheme, periodic evaluations should be made of the effectiveness and appropriateness of implemented procedures (such as animal selection, and infectious disease prevention and control measures). These evaluations should lead to the formulation of recommendations for the further research and investigation of xenozoonosis risk management measures and strategies.

As the associated risks become more fully characterised and quantifiable, it will clearly be necessary to continuously update the risk assessment assumptions regarding xenotransplantation. Similarly, the risk management schemes which have been implemented on the basis of these assumptions will need to be re-evaluated and revised. A dynamic and continuous risk assessment and management review and refinement process will be essential components of any safe implementation of this technology. The review will need to be grounded in sound professional judgement and up-to-date knowledge of the microbiological risks involved, in addition to a thorough understanding of the capabilities and limitations of the management methods being used or under consideration. With careful preparation and foresight, the microbiological risks associated with xenotransplantation can be reduced, and if the technology proves efficacious, the decision could be made to implement xenotransplantation as a useful treatment modality for reducing human morbidity and mortality.

Conclusion

As discussed above, many, as yet unquantifiable microbiological risks are associated with xenotransplantation, and given the current understanding of these risks, a comprehensive and quantifiable risk assessment is not possible. However, assumptions can be made about these risks, and prudent and reasoned risk management schemes, which could help to reduce the microbiological risks potentially associated with xenotransplantation, can be suggested for consideration.

As the associated risks become more fully characterised and quantifiable, it will clearly be necessary to continuously update the risk assessment assumptions regarding xenotransplantation. Similarly, the risk management schemes which have been implemented on the basis of these assumptions will need to be re-evaluated and revised. A dynamic and continuous risk assessment and management review and refinement process will be essential components of any safe implementation of this technology. The review will need to be grounded in sound professional judgement and up-to-date knowledge of the microbiological risks involved, in addition to a thorough understanding of the capabilities and limitations of the management methods being used or under consideration. With careful preparation and foresight, the microbiological risks associated with xenotransplantation can be reduced, and if the technology proves efficacious, the decision could be made to implement xenotransplantation as a useful treatment modality for reducing human morbidity and mortality.

Xénogreffes : le point sur les risques microbiologiques et les perspectives en matière de gestion du risque

D.E. Onions & C.J. Witt

Résumé

La xénogreffe ou xénotransplantation consiste à greffer sur l'homme des organes, des tissus ou des cellules provenant d'espèces animales, afin de traiter diverses affections. Lorsque l'efficacité de cette technique aura été démontrée, son utilisation permettra de limiter le déséquilibre existant actuellement entre la demande croissante en organes, tissus et cellules pouvant être greffés et la disponibilité en greffons d'origine animale dans le monde. Outre la démonstration...
de l’efficacité de cette méthode et l’examen des diverses questions pratiques liées à son utilisation, il convient de bien apprécier les risques éventuels de transmission de maladies infectieuses. S’il reste encore beaucoup à apprendre sur les risques microbiologiques associés à la xénotransplantation, il est possible de définir certains des éléments qui devront figurer dans un programme de gestion des risques d’infection, en se fondant sur l’état actuel de nos connaissances sur les agents infectieux qui pourraient être impliqués lors d’une xénogreffe.

Mots-clés

Xenotransplantes: resumen de los riesgos microbiológicos y posibilidades de gestión de riesgos
D.E. Onions & C.J. Witt

Resumen
El xenotransplante consiste en trasplantar órganos, tejidos o células animales a seres humanos con el fin de tratar condiciones patológicas varias. Si se llega a demostrar su eficacia, esta técnica puede servir para paliar el enorme déficit mundial de órganos, tejidos y células de origen humano para trasplantes y cubrir parcialmente una demanda que no cesa de crecer. Al igual que deben investigarse su aplicación práctica y su eficacia, es preciso analizar el riesgo de enfermedades infecciosas asociadas que esta técnica conlleva. Aunque todavía queda mucho por aprender acerca de los riesgos microbiológicos ligados a los xenotransplantes, sí es posible, a partir de lo que ya se sabe sobre los agentes infecciosos potencialmente implicados en un xenotransplante, considerar los elementos que habría de incorporar un sistema de gestión de esos riesgos microbiológicos.

Palabras clave

References


