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Genetically-engineered pig kidney transplantation in a brain-dead human subject

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Abstract

In September 2021, a kidney (with donor-specific thymic tissue) from an α1,3-galactosyltransferase gene-knockout (GTKO) pig was transplanted into the groin (with anastomoses to the femoral vessels) of a brain-dead subject by a surgical team at New York University Langone Health (NYU). It was reported to function immediately, passing urine and excreting creatinine. The experiment was terminated after 54 hours and, during this period, the kidney did not show macroscopic features of rejection.

Does this experiment provide information not available to us previously and does it move the field forward to clinical trials? The information provided was very limited, but the following points are worthy of note. (i) Numerous in vivo studies in nonhuman primates have predicted that the pig kidney would function immediately. (ii) Numerous in vitro studies have predicted that a GTKO pig kidney would not be rejected within the first few days after transplantation into a human subject. (iii) GTKO kidneys are not optimal for clinical transplantation, and the transplantation of a triple-knockout (TKO) pig kidney would have been more relevant. (iv) There was no purpose in transplanting a 'thymokidney' without pre-transplant conditioning therapy and follow-up for several months. (v) Because the native kidneys were retained, it is difficult to determine whether the function of the graft was sufficient to support life. (vi) The experiment was announced to the media rather than published in a peer-reviewed medical journal (although hopefully this will follow), suggesting that it was primarily carried out to gain attention to the great potential of xenotransplantation (and/or possibly to NYU). In this respect the experiment was successful.

Because of the very limited period of time for which a brain-dead subject can be maintained in a metabolically and hemodynamically stable state, the value of experiments in such subjects will remain very limited. It is hoped that any future similar experiments will be planned to be more relevant to the clinical situation. Nevertheless, the report has stimulated public attention towards xenotransplantation which, unless there is an adverse response to what some might consider to be a Frankenstein-like experiment, should be of significant benefit to future progress.

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Conflict of interest statement

The author is a consultant to eGenesis Bio, Cambridge, MA, USA, but the opinions expressed in this article are his own and do not necessarily represent the views of eGenesis Bio.

Keywords

brain death; humans; kidney; pig; xenotransplantation

Introduction

The news that (I believe on Friday, September 24, 2021) a genetically-engineered pig kidney had been transplanted into a brain-dead human subject by the New York University Langone Health (NYU) transplant team (1) had been anticipated for some time. To my knowledge, there are at least three groups in the USA that have been planning such an experiment. The NYU team was the first to identify a family who generously agreed for the experiment to be carried out on their deceased relative, for which altruism they must receive much credit and our profound gratitude.

To my knowledge, the first proposal to carry out such an experiment was by Tom Starzl in the early years of the present century. (Some years later, Joe Tector also considered such a study.) When I joined the Thomas E. Starzl Transplantation Institute at the University of Pittsburgh in 2005, Dr Starzl asked me to submit such a proposal to the university committee that considered research on deceased humans. Eventually, we did not pursue this experiment because at that time there was considerable concern about the potential risk of the transfer of porcine endogenous retroviruses (PERVs) to the staff who would care for the brain-dead subject. The monitoring of the staff that might have been required, possibly for the rest of their lives, seemed an insuperable problem.

In addition, there were concerns that public opinion might be adverse because xenotransplantation itself seemed to many to be science fiction, and the transplantation of a pig organ into a brain-dead subject may have been perceived as a weird, Frankenstein-like experiment. Many members of the public and media would not have understood the reasons for the study, and any adverse publicity might have hindered progress in xenotransplantation research and delayed the ultimate goal of using organs from pigs to overcome the problem of an inadequate number of donor organs for clinical transplantation.

Times have changed and recent surveys and focus groups by Paris and his colleagues suggest that clinical xenotransplantation is more acceptable to the public than it was 15 years ago (2–9). It will be of interest to see if there is any adverse comment on the recent experiment – other than from the ‘animal rights’ groups. Hopefully, the ‘success’ of the experiment, using a genetically-engineered pig kidney provided by Revivicor (Blacksburg, VA), will be accepted by the public as one more step towards introducing pig organ, tissue, and cell transplantation as therapeutic options for patients with terminal organ failure or conditions such as type 1 diabetes, corneal blindness, or Parkinson’s disease, and even for red blood cell transfusion. Any step that increases the public’s awareness of the potential that xenotransplantation has to offer is surely welcome.

An assessment of the experiment

The NYU surgical team was led by Dr Robert ('Bob') Montgomery, who has made significant contributions to kidney *allo*transplantation (and who has himself received a heart transplant). He described the experiment as allowing them "… to answer a really important question: Is there something that's going to happen when we move this from a primate to a human that is going to be disastrous?" He described the event as "a transformative moment in organ transplantation." Others who were reported in the *New York Times* or elsewhere to have commented on the experiment described it as "this incredible scientific achievement", "a tour de force", "monumental", "a huge breakthrough", "a big, big deal", and "a watershed moment".

Are these comments justified or is this just so much hyperbole, possibly resulting from the euphoria of the moment? Has the success of this experiment been such a major step in taking us closer to the clinic?

Unfortunately, because the experiment was announced to the media (with limited information) and has not yet been reported in the medical literature, we know very few details about it. We have been informed that an α1,3-galactosyltransferase gene-knockout (GTKO) pig kidney was transplanted into the groin, anastomosing the graft renal vessels to the femoral vessels (a technique used in the very first kidney *allo*transplant carried out by Yu Yu Voronoy in 1933 [10]) and that it functioned well, producing urine and creatinine, for 54 hours before the experiment was terminated.

What we do not know is whether the recipient's two native kidneys (that appear to have been retained) were still functioning well. In one media report it was erroneously stated that the graft maintained a normal serum creatinine, but this cannot be known because the native kidneys may well have contributed significantly to the maintenance of a normal serum creatinine.

Even if the pig kidney was functioning well, can the experiment be described as "an incredible scientific achievement"?

Over the past several years, researchers have accumulated a great deal of evidence from in vitro laboratory studies that demonstrate that many human subjects do *not* have antibodies against cells from triple-knockout (TKO) pigs, i.e., pigs that do not express any of the three known pig carbohydrate antigens against which humans have anti-pig antibodies (11–13). These observations are important because organs from TKO pigs will almost certainly form the basis for initial clinical trials.

So strong is this evidence that it is probably not necessary to confirm it by transplanting a TKO pig kidney into a human subject, whether brain-dead or not. However, such an in vivo experiment would at least confirm that hyperacute rejection does not occur when a TKO pig kidney is transplanted into a recipient who does not have any preformed anti-TKO pig antibodies.

Unfortunately, this was not tested in the recent experiment. The kidney was taken from a pig in which only expression of galactose-α1,3-galactose (Gal) had been deleted (a GTKO pig). The in vitro evidence is that this pig would *not* be the optimal source of organs for clinical trials (14), and so the opportunity to confirm that a TKO pig kidney would not be rapidly rejected by a human recipient was not tested. This is a lost opportunity because assessing the survival of TKO pig kidneys in nonhuman primates (NHPs) is beset with difficulties because *all* Old World NHPs have preformed antibodies against cells from these pigs, making in vivo studies almost certainly less successful than they would be in human recipients (14,15).

Hyperacute rejection of a GTKO pig organ has very rarely occurred in NHP models, but early rejection would be more likely to occur in a human recipient (14). Whether any immunosuppressive or other therapy, e.g., a complement inhibitor, was administered to the brain-dead recipient remains unreported at present.

The pig kidney was wrapped in donor-specific thymic tissue - I feel sure based on the extensive studies by Yamada and his colleagues (16) - aimed to ‘re-educate’ the T cells to accept the kidney graft. This approach has not yet proved to be effective in inducing tolerance to a pig xenograft in a NHP but, to be successful, some pre-transplant conditioning treatment of the potential recipient is required (of which no mention was made in the media reports). Furthermore, it would be necessary to follow the recipient for some months to ascertain the outcome, and so it is not clear why a ‘thymokidney’ was transplanted in the NYU experiment. We must presume that it was the only pig kidney that was available at the time and the thymic tissue transplant was not an integral part of the experiment.

The potential impact of brain death on the experiment

The greatest limitation of the experiment was that follow-up was for only 54 hours. Why it was terminated was not made clear. The regulatory authorities might be reassured to learn of this short experience, but what they really want to know now is whether a pig kidney will function well for at least 6 or 12 months, or even longer. It is in providing these data that we are struggling in the pig-to-NHP model (13,14). The recent experiment does not contribute to this goal, and it is unrealistic to anticipate that any experiment in a brain-dead subject can be pursued for more than a few days or weeks.

Brain-death leads to dramatic biochemical changes that greatly impact organ function, particularly of the heart, leading to hemodynamic instability requiring considerable pharmacologic support (17–19). No mention is made in the media reports as to whether any hormonal therapy was administered to correct the metabolic imbalance that follows brain death. Although it is good to know that the recipient must have remained sufficiently hemodynamically stable for 54 hours not to impact kidney graft function, this concern would obviously not be a factor in a trial in a living patient.

My major concern is that this metabolic instability, together with the knowledge that both brain death (20) and xenotransplantation (21) generate systemic inflammatory responses, almost certainly augmenting the immune response, could have resulted in graft injury and/or in some degree of graft dysfunction. If this had occurred, it would have been necessary

to explain the reasons to the regulatory authorities, which could possibly have delayed us making progress towards the first clinical trials. The wisdom of carrying out an experiment with a kidney from a less-than-optimal genetically-engineered pig in a model where there is a risk of graft injury, dysfunction, or even failure (from causes that will *not* be faced in a proper clinical trial), is therefore questionable. There is a risk that any adverse outcome would impair future progress. Fortunately, my fears appear to be unfounded in this initial experiment.

Future experiments in brain-dead subjects

We can now ask whether this type of experiment will be carried out again, either by the NYU group or by the other centers that we know are planning to do so. If further experiments take place, they should be designed to provide maximal information that is more relevant to what we need to know, e.g., by transplanting a TKO pig kidney graft, by following the recipient, if possible, for a longer period of time, by assessing the intensity of the inflammatory response by measurement of serum cytokine levels etc., but I suggest that the information gained will remain very limited. This first experiment has stimulated public interest, but the positive impact on the public that follows subsequent experiments may be less.

Motivation

Let us consider what motivated the NYU surgical team, who to my knowledge have carried out no previous research in the field of xenotransplantation, to undertake this experiment. Reluctantly, one is persuaded that obtaining some attention from the media must have been a factor. If not, why announce the results of the experiment through a national newspaper *(USA Today, October 19, 2021)* rather than in the usual way in a peer-reviewed journal? It is possible, though unlikely, that this was the true purpose of the experiment – simply to draw attention to the potential of xenotransplantation. If so, it was successful. But, in view of the limitations of the experiment, Bob Montgomery's question of whether "there is something that's going to happen when we move this from a primate to a human that is going to be disastrous" has not really been answered.

Positive impact

However, let us consider the positive aspects of the experiment. Although I do not believe the experiment, as successful as it was in its very limited goal, has added much to our knowledge of xenotransplantation or towards obtaining regulatory approval for a clinical trial, it has drawn more attention to the potential of xenotransplantation in resolving the critical shortage of organs for clinical transplantation. Unless there is a media or public backlash against what may be conceived to be a bizarre experiment, this alone will certainly be beneficial.

It may be that the NYU team has collected a great deal of data from the experiment that will help us progress further. If not, I would hope that any future experiments of this nature will be directed more towards the aim of seriously adding to our knowledge, despite the

limitations of the model. I would also hope that the results will be reported in a reputable medical journal after peer-review.

How can we best proceed?

At present, we are trying to prove that TKO pig kidneys will function for (relatively) long periods, i.e., months or even years, in patients (many of whom have *no* serum anti-pig antibodies) by carrying out TKO pig kidney transplants in NHPs (*all* of which have anti-TKO pig antibodies). Surely this is a ridiculous situation. If we had in vitro data indicating that a certain drug worked well in humans, but not in baboons, would we be expected to demonstrate that it would be effective in humans by administering it in vivo to baboons? I suggest that we would not.

Instead of persevering with an animal model (that does not mimic the clinical situation) or carrying out pig kidney transplantation in brain-dead subjects (from which only extremely limited information can be obtained), we should surely be working urgently towards a small initial clinical trial in living patients who are in desperate need of a kidney transplant but who will *never* receive a deceased human donor kidney (22). I contend that we will progress much more quickly on the basis of a limited clinical trial in carefully-selected patients than by any other approach (23). Perhaps that is what Bob Montgomery is trying to tell us.

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Abbreviations:

Gal	galactose-α1,3-galactose
GTKO	α1,3-galactosyltransferase gene-knockout
NHP	nonhuman primate
NYU	New York University Langone Health
TKO	triple-knockout (i.e., a pig in which expression of all three known carbohydrate xenoantigens has been deleted by genetic-engineering)

References

1. Rabin RC. In a first, surgeons attached a pig kidney to a human, and it worked. New York Times. 10 19, 2021. Updated Oct 21, 2021.
2. Paris W, Seidler RJH, Fitzgerald K, Padela AI, Cozzi E, Cooper DKC. Jewish, Christian and Muslim theological perspectives about xenotransplantation. Xenotransplantation 2018 5;25:e12393. Doi: 10.1111/xen.12393. [PubMed: 29655276]
3. Mitchell C, Lipps A, Padilla L, Werkheiser Z, Cooper DKC, Paris WD. Meta-analysis of public perceptions towards xenotransplantation. Xenotransplantation. 2020; 1 19:e12583. Doi: 10.1111/xen.12583. [PubMed: 31957115]
4. Padilla LA, Hurst DJ, Jang K, et al. Racial differences in attitudes to clinical pig organ xenotransplantation. Xenotransplantation. 2020 10 25:e12656. [PubMed: 33099814]

5. Padilla LA, Hurst DJ, Lopez R, Kumar V, Cooper DKC, Paris W. Attitudes to clinical pig kidney xenotransplantation among medical providers and patients. Kidney 360. 2020;1:657–662.
6. Padilla LA, Rhodes L, Sorabella RA, et al. Attitudes toward xenotransplantation: a survey of parents and pediatric cardiac providers. Pediatr Transplant. 2020 10 6:e13851. Doi:org/10. 1111/petr.13851. [PubMed: 33022840]
7. Padilla LA, Sorabella RA, Carlo WF, et al. Attitudes to cardiac xenotransplantation by pediatric heart surgeons and physicians. World J Pediatr Congenit Heart Surg. 2020; 121:426–430.
8. Hurst DJ, Padilla LA, Cooper DKC, Walters W, Paris WD. The attitudes of religious group leaders towards xenotransplantation: a focus group study. 2021. Submitted.
9. Hurst DJ, Padilla LA, Cooper DKC, Paris WD. Factors influencing attitudes toward xenotransplantation clinical trials: a report of focus group studies. Xenotransplantation. 2021 3 7:e12684. Doi: 10.1111/xen.12684.
10. Hamilton DN, Reid WA Yu Yu.. Voronoy and the first human kidney allograft. Surg Gynecol Obstet, 1984;159:289–294. [PubMed: 6382662]
11. Estrada JL, Martens G, Li P, et al. Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/β4GalNT2 genes. Xenotransplantation 2015;22:194–202. [PubMed: 25728481]
12. Martens GR, Reyes LM, Li P, et al. Humoral reactivity of renal transplant-waitlisted Patients to cells from GGTA1/CMAH/B4GalNT2, and SLA class I knockout pigs. Transplantation 2017;101:e86–e92. [PubMed: 28114170]
13. Yamamoto T, Iwase H, Patel D, et al. Old World monkeys are less than ideal transplantation models for testing pig organs lacking three carbohydrate antigens (triple-knockout). Sci Rep. 2020 6 17;10:9771:doi.org/10.1038/s41598–020-66311–3. [PubMed: 32555507]
14. Cooper DKC, Hara H. "You cannot stay in the laboratory forever.: Taking pig kidney xenotransplantation from the laboratory to the clinic. eBioMedicine 2021 9 10: 71: 103562. Doi: 10.1016/j.ebiom.2021.10. [PubMed: 34517284]
15. Iwase H, Jagdale A, Yamamoto T, et al. Evidence suggesting that deletion of expression of N-glycolylneuraminic acid (Neu5Gc) in the organ-source pig is associated with increased antibody-mediated rejection of kidney transplants in baboons. Xenotransplantation. 2021. 25 5 2021. E12700. [PubMed: 34036638]
16. Yamada K, Ariyoshi Y, Pomposelli T, Sekijima M. Co-transplantation of vascularized thymic graft with kidney in pig-to-nonhuman primates for the induction of tolerance across xenogeneic barriers. Methods Mol Biol 2020;2110:151–171. [PubMed: 32002908]
17. Novitzky D, Wicomb WN, Cooper DKC, Rose AG, Fraser RC, Barnard CN. Electrocardiographic, haemodynamic and endocrine changes occurring during experimental brain death in the Chacma baboon. J Heart Transplant 1984; 4:63–69.
18. Novitzky D, Cooper DKC, Morrell D, Isaacs S. Change from aerobic to anaerobic metabolism after brain death, and reversal following triiodothyronine therapy. Transplantation 1988;45:32–36. [PubMed: 3336918]
19. Cooper DKC, Novitzky D, Wicomb WN. The pathophysiological effects of brain death on potential donor organs, with particular reference to the heart. Ann R Coll Surg Engl 1989;71:261–266. [PubMed: 2774455]
20. Barklin A, Hvas CL, Toennesen E. The inflammatory response to brain death. In: The Brain-Dead Organ Donor: Pathophysiology and Management. (Eds. Novitzky D, Cooper DKC). New York: Springer, 2013. pp. 107–119.
21. Ezzelarab MB, Ekser B, Azimzadeh A, et al. Systemic inflammation in xenograft recipients precedes activation of coagulation. Xenotransplantation 2015;22:32–47. doi: 10.1111/xen.12133. [PubMed: 25209710]
22. Jagdale A, Kumar V, Anderson DJ, et al. Suggested patient selection criteria for initial clinical trials of pig kidney xenotransplantation in the USA. Transplantation. 2021; 1 20. doi: 10.1097.0000000000003622. Online ahead of print. (2021;105)
23. Cooper DKC. Moving xenotransplantation to the clinic. (The Keith Reemtsma Lecture) Xenotransplantation 2021. In press.