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COMMENTARY

First transplantation of a pig heart from a multiple gene-modified donor, porcine cytomegalovirus/roseolovirus, and antiviral drugs

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The porcine cytomegalovirus (PCMV) is actually a porcine roseolovirus (PRV).[1] As the official International Committee on Taxonomy of Viruses (ITCV) name, suid betaherpesvirus 2 (SuBHV2),[2] is not widely used, the abbreviation (PCMV/PRV) will be used to make clear that it is a herpesvirus not closely related to the human cytomegalovirus (HCMV), but to the human herpesviruses 6 and 7 (HHV-6, HHV-7), which are also roseoloviruses.[3] PCMV/PRV was shown to reduce significantly the survival time of pig xenotransplants in non-human primates. Yamada et al.[4] and Sekijima et al.[5] reported a reduction of the survival time of pig kidneys from PCMV/PRV-positive donor pigs when transplanted in cynomolgus monkeys and baboons, respectively, in 2014. Already in 2002 Mueller et al.[6] reported an activation PCMV in a pig-to-primate model of xenotransplantation in animals with short survival times. In 2016 for the first time an active replication of PCMV following transplantation of a pig heart into a baboon despite undetected virus in the donor pig was reported.[7] Denner et al.[8] reported a reduction of the survival time of genetically modified pig hearts in baboons. For detailed reviews of these and other studies see Denner.[9,10] PCMV/PRV was also transmitted to the human recipient who received a pig heart in January 2022 in Baltimore, Maryland, USA.[11] The clinical features and the steadily increasing virus load observed in the patient in Baltimore are very similar to the features and high virus load observed in baboons, which received a PCMV/PRV-positive heart in Munich.[8] Therefore, it is likely that the virus contributed together with other factors to the death of the patient.[12–16] These additional factors were surgical problems, problems with immunosuppression, application of the IVIG, and the antiviral drugs (see below).

After transplantation the patient was treated six times with antiviral drugs developed mainly against HCMV (Figure 1). There were early publications indicating that antiviral drugs against HCMV are less efficient against PCMV/PRV.[17,18] Since some of these drugs, for example, cidofovir are also used successfully against other DNA viruses, for example, monkeypox or mpox virus,[19] it is important to analyze, how effective the applied antivirals were against PCMV/PRV.

The PCMV/PRV infection was observed in the patient at day 20.[11] Ganciclovir and valacyclovir were given starting with day 0, and 19, respectively (Figure 1), possibly as a prophylactic measure to prevent HCMV activation, however, in the publication by Griffith et al.[11] HCMV was not indicated as present in the patient.

Ganciclovir is a nucleoside analogue, it is a derivative of aciclovir and exhibits in vitro activity against HCMV and herpes simplex virus types 1 and 2 (HSV-1, HSV-2), and to a lesser degree against Epstein-Barr virus (EBV), varicella zoster virus (VZV), human herpesvirus - 6 (HHV-6), and with a very low efficiency against human adenoviruses.[20] Common adverse drug reactions include: granulocytopenia, neutropenia, anemia, thrombocytopenia, and others. Thrombocytopenia was a marked feature in the Baltimore patient.[11] Ganciclovir is considerably more potent than aciclovir against HCMV. Valaciclovir, the L-valyl ester

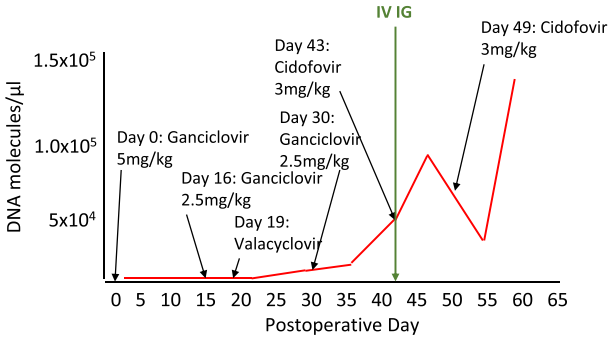

FIGURE 1 Virus load of PCMV/PRV (red line) in the plasma of the Baltimore patient and treatment of the patient by antiviral drugs according to Griffith et al.[11] The days of changing the antiviral drugs or their dose (black arrows) and the day of application of the IVIG preparation (day 43, green arrow) are indicated.

of aciclovir, is a prodrug, which is active after being converted to aciclovir in the human body.[21] Aciclovir is used for the treatment of HSV infections, chickenpox, and shingles. Like aciclovir, ganciclovir cannot eradicate a latent herpesvirus infection.[20]

When ganciclovir was applied to baboons given PCMV/PRV-positive pig hearts, it did not decrease the virus load of PCMV/PRV, i.e., it had no therapeutic efficacy in vivo in achievable concentrations.[17] In vitro, PCMV/PRV was relatively resistant against ganciclovir and aciclovir. Only cidofovir and another drug, foscarnet, were found to have a therapeutic efficacy in vivo in achievable concentrations. However, cidofovir and foscarnet were often significantly toxic in transplant recipients.[17] Similar results were also obtained in another study.[18] In this cell culture system, ganciclovir and cidofovir were effective against PCMV/PRV, however, some toxicity was associated with the highest concentration of cidofovir. Aciclovir and foscarnet were not effective in this system.[18]

After detection of PCMV/PRV at day 20, a permanent increase in the virus load was observed in the Baltimore patient, showing that ganciclovir and valacyclovir were not effective against PCMV/PRV in the patient. Consequentially, the antiviral therapy was changed from ganciclovir to cidofovir on day 43. Cidofovir is an acyclic phosphonate nucleotide analogue of deoxycytidine monophosphate with a broader spectrum of activity against double-stranded DNA viruses, including human herpesviruses.[22] It was approved for the treatment of HCMV induced retinitis in AIDS patients, which was the reason that some newspapers reported that the Baltimore patients was treated with a drug against AIDS. The major dose-limiting side effect of cidofovir is nephrotoxicity,[23] its half-life is between 2.6 and 9.5 h.[24]

But how effective was cidofovir in the Baltimore patient? After the first application at day 43 the virus titer was still increasing (Figure 1). The delay may be explained by the fact that cidofovir is acting on the viral polymerase and therefore on the de novo production of the virus, but not on already produced virus. Starting with day 47 the virus titer was decreasing, and a second application of cidofovir was given on day 49. The titer was further decreasing until day 55, then it was increasing rapidly to a very high virus load. Was cidofovir causing the decline? It seems possible that in addition to cidofovir another factor was involved in the reduction of the virus load: On day 43, together with cidofovir, intravenous immune globulin (IVIG) was administered. IVIG are usually isolated from pooled plasma that has been donated by 1000–100 000 people and they are used as replacement therapy in primary and acquired humoral immunodeficiency and as an immunomodulatory therapy in autoimmune disease and transplantation.[25] We recently have shown that antibodies against HHV-6 are cross-reacting with PCMV/PRV.[26] There are two HHV-6 species, HHV-6A and HHV-6B, which cannot be discriminated serologically. HHV-6 has a high seroprevalence in the human population of 95%–100% in humans older than 2 years of age.[27,28] The Baltimore patient was also HHV-6 positive.[11] Therefore, it is likely that the IVIG preparations contain antibodies against HHV-6 considering the high number of donors. Consequentially, it cannot be excluded that the reduction in the virus load of PCMV/PRV in the Baltimore patient after day 47 was due to neutralizing antibodies and antibodies contributing to opsonization and clearing of the virus.[29]

To summarize, the antiviral drugs (especially ganciclovir and valacyclovir) applied to the Baltimore patient had only a very limited effect on PCMV/PRV. It is likely that PCMV/PRV and certain antibodies in the IVIG preparation found to be directed against pig tissues[13] contributed to the death of the patient together with other factors. At present, it remains unclear whether the antiviral drugs and their toxic effects may have contributed.

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CONFLICT OF INTEREST STATEMENT

The author has no conflict of interest.

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REFERENCES

1. Gu W, Zeng N, Zhou L, Ge X, Guo X, Yang H. Genomic organization and molecular characterization of porcine cytomegalovirus. *Virology*. 2014;460-461:165-172.
2. https://ictv.global/report/chapter/herpesviridae/herpesviridae/roseolovirus
3. Denner J, Bigley TM, Phan TL, Zimmermann C, Zhou X, Kaufer BB. Comparative analysis of roseoloviruses in humans, pigs, mice, and other species. *Viruses*. 2019;11(12):1108.
4. Yamada K, Tasaki M, Sekijima M, et al. Porcine cytomegalovirus infection is associated with early rejection of kidney grafts in a pig to baboon xenotransplantation model. *Transplantation*. 2014;98(4):411-418.
5. Sekijima M, Waki S, Sahara H, et al. Results of life-supporting galactosyltransferase knockout kidneys in cynomolgus monkeys using two different sources of galactosyltransferase knockout Swine. *Transplantation*. 2014;98(4):419-426.

6. Mueller NJ, Barth RN, Yamamoto S, et al. Activation of cytomegalovirus in pig-to-primate organ xenotransplantation. *J. Virol*. 2002;76(10):4734-4740.
7. Morozov VA, Abicht JM, Reichart B, Mayr T, Guethoff S, Denner J. Active replication of Porcine Cytomegalovirus (PCMV) following transplantation of a pig heart into a baboon despite undetected virus in the donor pig. *Ann Virol Res*. 2016;2(3):1018.
8. Denner J, Längin M, Reichart B, et al. Impact of porcine cytomegalovirus on long-term orthotopic cardiac xenotransplant survival. *Sci Rep*. 2020;10(1):17531.
9. Denner J. Xenotransplantation and porcine cytomegalovirus. *Xenotransplantation*. 2015;22(5):329-335.
10. Denner J. Reduction of the survival time of pig xenotransplants by porcine cytomegalovirus. *Virol J*. 2018;15(1):171
11. Griffith BP, Goerlich CE, Singh AK, et al. Genetically modified porcine-to-human cardiac xenotransplantation. *N Engl J Med*. 2022;387(1):35-44.
12. Denner J. The porcine cytomegalovirus (PCMV) will not stop xenotransplantation. *Xenotransplantation*. 2022;29(3):e12763.
13. Cooper DKC, Yamamoto T, Hara H, Pierson RN 3rd. The first clinical pig heart transplant: was IVIg or pig cytomegalovirus detrimental to the outcome? *Xenotransplantation*. 2022;29(4):e12771.
14. Mueller NJ, Denner J. Porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV): a threat for xenotransplantation? *Xenotransplantation*. 2022;29(5):e12775.
15. Fishman JA. Risks of infectious disease in xenotransplantation. *N Engl J Med*. 2022;387(24):2258-2267.
16. Denner J, Schuurman HJ. Early testing of porcine organ xenotransplantation products in humans: microbial safety as illustrated for porcine cytomegalovirus. *Xenotransplantation*. 2022;29(6):e12783. Epub 2022 Nov 6.
17. Mueller NJ, Sulling K, Gollackner B, et al. Reduced efficacy of ganciclovir against porcine and baboon cytomegalovirus in pig-to-baboon xenotransplantation. *Am J Transplant*. 2003;3(9):1057-1064.
18. Fryer JF, Griffiths PD, Emery VC, Clark DA. Susceptibility of porcine cytomegalovirus to antiviral drugs. *J Antimicrob Chemother*. 2004;53(6):975-980.
19. Harapan H, Ophinni Y, Megawati D, et al. Monkeypox: a comprehensive review. *Viruses*. 2022;14(10):2155.
20. Faulds D, Heel RC. Ganciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. *Drugs*. 1990;39(4):597-638.
21. Kausar S, Said Khan F, Ishaq Mujeeb Ur Rehman M, et al. A review: mechanism of action of antiviral drugs. *Int J Immunopathol Pharmacol*. 2021;35:20587384211002621.
22. Ahmed A. Antiviral treatment of cytomegalovirus infection. *Infect Disord Drug Targets*. 2011;11(5):475-503.
23. Jacobsen T, Sifontis N. Drug interactions and toxicities associated with the antiviral management of cytomegalovirus infection. *Am J Health Syst Pharm*. 2010;67(17):1417-1425.
24. Caruso Brown AE, Cohen MN, Tong S, et al. Pharmacokinetics and safety of intravenous cidofovir for life-threatening viral infections in pediatric hematopoietic stem cell transplant recipients. *Antimicrob Agents Chemother*. 2015;59(7):3718-3725
25. Looney RJ, Huggins J. Use of intravenous immunoglobulin G (IVIG). *Best Pract Res Clin Haematol*. 2006;19(1):3-25.
26. Fiebig U, Holzer A, Ivanusic D, et al. Antibody cross-reactivity between Porcine Cytomegalovirus (PCMV) and Human Herpesvirus-6 (HHV-6). *Viruses*. 2017;9(11):317.
27. Braun DK, Dominguez G, Pellett PE. Human herpesvirus 6. *Clin. Microbiol. Rev*. 1997;10, 521-567.
28. Gustafsson R, Svensson M, Fogdell-Hahn A. Modulatory effects on dendritic cells by human herpesvirus 6. *Front Microbiol*. 2015;6:388.
29. Hetherington SV, Giebink GS. Opsonic activity of immunoglobulin prepared for intravenous use. *J Lab Clin Med*. 1984;104(6):977-986.

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