NEW TECHNIQUE FOR CHEST OPENING IN MICE: U-STERNOTOMY

SONJA SCHREPFER, M.D.,* TOBIAS DEUSE, M.D.,
AND HERMANN REICHENSPURNER, M.D., Ph.D.

In cardiac xenotransplantation, transfections with recombinant adeno-associated viruses could be a promising way of modulating the cardiomyocyte genome. Proteins like TRAIL, hDAF, Il-10, Il-4, and β-galactosidase may be expressed on cardiomyocyte surfaces in order to prevent cellular rejection processes. However, after intracoronary perfusion with transfecting viruses, it takes up to 4 weeks before expression of the transgene can be detected. Mouse models have been established to create reliable transfection protocols. Currently, the major problem involves performing the intraaortic injection in mice without causing a lethal pneumothorax. Here we describe a new technique for chest opening to safely reach the mouse ascending aorta without opening the pleural space. This U-sternotomy reduces the risk of animal death and therefore the amount of quite expensive virus solutions needed.

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The increasing shortage of donor organs is a worsening problem of transplantation surgery. Mostly affected are the fields of heart and lung transplantation: approximately 30% of patients on the list die within the first 9–12 months before receiving an organ. Xenotransplantation might be a solution for the organ shortage.[1] The mouse model is widely used for experimental studies. But cardiomyocyte transfection through intraaortic injection, using viruses with surface protein-encoding DNA sequences, has never been performed.

MATERIALS AND METHODS

Male C57/B6 mice weighing 19–25 g are anesthetized with ether. Then the skin cephalad of the xyphoid is incised and tipped up. The underlying muscle is mobilized from the xiphoid cranially and also fixed. A transverse sternotomy is done at the height of the third intercostal space. The incision is then extended on both sides of the sternum up to the first rib. The cranial sternal part can now be mobilized, giving room for dissection of the thymus as much as necessary and for aortic cross-clamping using a straight vascular clamp (Fig. 1). The transfection solution may now be injected proximal to the aortic clamp for antegrade coronary perfusion (Fig. 2). A 0.5-ml syringe was used (0.30 × 8 mm/30G × 5/16''; Becton-Dickinson, Switzerland). The clamp must be removed within 60 sec, since longer cross-clamp times may not be tolerated. Hemostasis of the aortic needle hole is achieved with a 10-0 Ethilon stitch, since a blood loss of ≥0.8 ml leads to shock and death of the mouse. The sternum is then stabilised with two 7-0 Prolene stitches, and the overlying muscle and skin are adapted.

RESULTS

Weeks later, expression of the transgene will have taken place, and the heterotopic cardiac transplantation may be performed.

DISCUSSION

Immunologic processes after xenotransplantation may be divided into hyperacute, acute, and chronic rejections.[2,3] Hyperacute rejection is mediated by preformed natural antibodies (PNAb) of the recipient. These antibodies recognize gal-epitopes on the surface of xenogeneic cells, and mediate organ destruction within minutes or hours via complement activation.[4] To overcome hyperacute rejection, the following methods are used:

1. Transgenic animals are bred whose endothelial cells express complement-inhibiting factors such as human DAF or CD59.[5] However, until now it was impossible to breed animals expressing high levels of more than one of these transgenes.

Department of Thoracic and Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

*Correspondence to: Sonja Schrepfer, M.D., Department of Cardiovascular Surgery, University Hospital Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. E-mail: sschrepfer@uke.uni-hamburg.de

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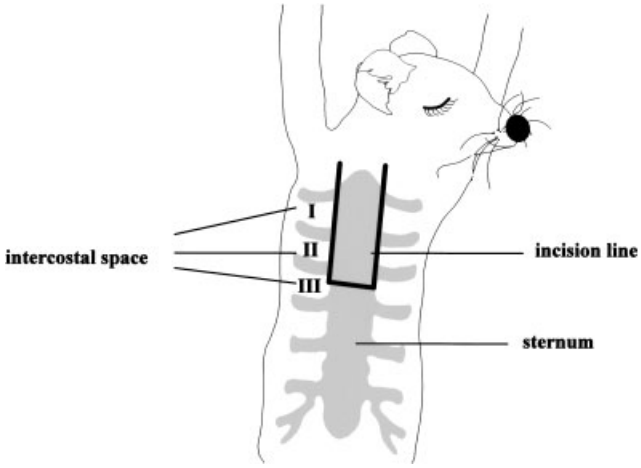

Figure 1. Incision line of the U-sternotomy.

Figure 2. Operation field after sternum opening.

2. A more elegant method is to selectively modulate the donor organ by transfecting cardiac myocytes with different transgenes, which are then expressed on their cell surfaces. Though a stable transfection of more than 50% of cardiomyocytes is possible, the transfection process lasts up to 4 weeks.[6]

CONCLUSIONS

Until now, cardiomyocyte transfection through intraaortic injection using viruses with surface protein-encoding DNA sequences has never been performed. Lethal pneumothoraxes are common, with donor animals dying before organ harvesting can be performed 4 weeks after cardiac transfection.

REFERENCES

1. Rogers NJ, Dorling A, Moore M. Xenotransplantation: steps towards a clinical reality. Immunol Today 1998;19:206–208.
2. Bach FH, Robson SC, Winkler H, Ferran C, Stuhlmeier KM, Wrighton CJ, Hancock WW. Barriers to xenotransplantation. Nat Med 1995;1:869–873.
3. Platt JL. New directions for organ transplantation. Nature 1998;392:11–17.
4. Lin SS, Weidner BC, Byrne GW. The role of antibodies in acute vascular rejection of pig-to-baboon cardiac transplants. J Clin Invest 1998;101:1745–1756.
5. Schmoeckel M, Nollert G, Shahmohammadi M, Muller-Hocker J, Young VK, Kasper-Konig W, White DJ, Hammer C, Reichart B. Transgenic human decay accelerating factor makes normal pigs function as a concordant species. J Heart Lung Transplant 1997;16:758–764.
6. Svensson EC, Marshall DJ, Woodard K, Lin H, Jiang F, Chu L, Leiden JM. Efficient and stable transduction of cardiomyocytes after intramyocardial injection or intracoronary perfusion with recombinant adeno-associated virus vectors. Circulation 1999;99:201–205.