Of Mice and Men (and Effects of Gene Silencing)

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In this issue of *Circulation*, Zheng and coworkers demonstrate the efficacy of specific small interfering RNAs (siRNAs) added to University of Wisconsin solution in protecting donor hearts against cold ischemic injury during storage and subsequent reperfusion. siRNAs targeted tumor necrosis factor-α, C3, and Fas. The study design was well rationalized and performed systematically to confirm the efficacy of these siRNA constructs in silencing their respective targets, first in cell culture and subsequently in whole mouse hearts. Quantitative polymerase chain reaction was used to demonstrate knockdown of each of these proteins after ischemia and reperfusion in contrast to their strong upregulation in control hearts. Beneficial effects were confirmed by enhanced function, decreased apoptosis, diminished neutrophil activation and infiltration, and less histological evidence of tissue damage in the treated hearts.

First discovered as a method for gene silencing in plants, siRNA effectively silences or reduces posttranscriptional efficacy of messenger RNA, thereby interfering with protein synthesis. In the model used in this study, proteins upregulated with ischemia and reperfusion and thought to play a critical role in the inflammatory response to ischemia and reperfusion were chosen as the focus for gene silencing. This work extends prior observations of this group using similar techniques for renal preservation after prolonged storage. It differs importantly in that the siRNA was introduced into the myocardiocytes through a combination of cold flushing of the coronary system and addition to the storage medium. Presumably, effectiveness occurred by directly targeting the endothelial cells and cardiomyocytes.

This is an intriguing approach to long-term heart preservation but should be viewed as representing more “proof of concept” than a pragmatic regimen for organ preservation. Several considerations need to be addressed when considering the potential clinical applicability of this strategy for heart preservation.

First, there is, in fact, little need to preserve hearts for such long periods of time. The regional recipient demand for hearts is so high that it is rarely necessary to transport hearts for such extended times. In this study, hearts were preserved for 48 hours. The authors do not indicate why 48 hours was chosen as the study time. It may have been chosen to provide a very rigorous challenge to the preservation technique. Alternatively, the rate of cell entry and subsequent efficacy of siRNA into intact hearts are not described, and shorter storage times may have resulted in lesser incorporation efficiencies. Second, although the treated hearts maintained a rhythm after abdominal transplantation and generated good aortic ejection velocities, these are not very stringent indicators of cardiac function and need to be assessed in a large-animal model. Third, as one progresses to larger and larger animals, the amount of siRNA, as admitted by the authors, becomes huge. Fourth, although targeting only 3 important components of the deleterious ischemia/reperfusion pathway, it is likely that other important targeting mediators are activated (generated) in larger-animal models that may result in significant injury. It would have been instructive to understand why the well-established key activator and effector caspases (eg, caspase-8 and caspase-3) of the death-mediated apoptosis pathway cascade were not silenced directly. Fifth, one must be certain that the siRNA effects were confined to silencing of the targeted gene expression. siRNA, although specifically constructed, may nonspecifically interfere with translation of unrelated genes that share similar base sequences. Sixth, in this study, syngeneic mice were used to eliminate the confounding effects of the immune response normally seen with allogeneic transplantation. Immune mediators are thought to interact with tissues subjected to targeting injury. Seventh, and perhaps the most important conceptual concern, this model does not reflect clinical allogenic cardiac graft transplantation in that there was no use of or need for extracorporeal circulation. Extracorporeal circulation, independent from any ischemic challenge, induces an inflammatory response that results in activation of complement, neutrophils, and endothelial cells. Although targets for inflammation in the transplanted hearts may be reduced, it is unlikely that siRNA treatment can completely eliminate them, and amplification of ischemia/reperfusion injury through liberation of free oxygen radicals and other important immunologic (cell-triggered rejection) mediators (ie, alternate pathways) may still occur. For example, induction of hypoxia-inducible factor-1α and inducible nitric oxide synthase with siRNA was shown to protect the ischemic/reperfused murine myocardium.

Nonetheless, this basic study is extremely important in helping us understand that adverse changes in hearts subjected to similar preservation methods are not immutable given novel molecular tools that can modify programmed biological responses.

Disclosures

None.

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