Toll-like receptor 4 inhibition attenuates ischemia-reperfusion injury in rats: Will it work in human beings?

Chadrick E. Denlinger, MD

From the Division of Cardiothoracic Surgery, Medical University of South Carolina, Charleston, SC.

Disclosures: Author has nothing to disclose with regard to commercial support.

Received for publication Sept 14, 2015; accepted for publication Sept 16, 2015; available ahead of print Oct 16, 2015.

Address for reprints: Chadrick E. Denlinger, MD, 25 Courtenay Dr, ART Suite 7018, Charleston, SC 29425 (E-mail: denlinge@musc.edu).

J Thorac Cardiovasc Surg 2016;151:507-8

0022-5223/\$36.00

Copyright © 2016 by The American Association for Thoracic Surgery

<http://dx.doi.org/10.1016/j.jtcvs.2015.09.075>

Ischemia-reperfusion injury (IRI) of transplanted lungs remains a critical clinical problem that appreciably afflicts at least 25% of all patients undergoing this operation. One immediate manifestation of IRI is primary graft dysfunction, and its delayed manifestation may include bronchiolitis obliterans. These 2 conditions contribute to the majority of early and late posttransplant deaths, respectively. In this issue of the *Journal*, Merry and colleagues¹ have again demonstrated the important role that alveolar macrophages and expression of the toll-like receptor 4 (TLR4) in the induction of IRI. Selective inhibition of TLR4 with small interfering RNA (siRNA) potentially diminished the inflammatory response and surrogate markers for IRI. Most importantly, systemic administration of siRNA in a rat model also attenuated the effects of ischemia and reperfusion. Although the central theme of the article of Merry and colleagues¹ focuses on the importance of TLR4 in an in vivo experimental model, it is interesting to speculate about the potential for clinical translation of these data.

The paradigm of gene therapy for various clinical syndromes remains in its infancy. A number of gene therapy clinical trials have previously been conducted in the United States and around the world. The majority of these trials have focused on rare syndromes with the etiology stemming from a clearly identifiable single enzymatic deficiency. Enthusiasm for these trials waned after the death of 1 trial participant, a death that was attributed to an intense inflammatory reaction to the adenovirus delivery vector. In a different study, 2 children had a lymphoma-like monoclonal lymphocyte proliferation develop, which was attributed to integration of the retroviral vector's DNA into the host genome.² Despite some doubts, gene therapy clinical trials have persisted and trials with siRNA have also been conducted, with the majority of these trials relying on topical application of the delivery vector. At least 2 phase I clinical trials relying on the systemic delivery of siRNA have also been conducted.³

As pointed out by Merry and colleagues,¹ an advantage of the use of siRNA to target TLR4 activity is the transient nature of this therapy, which could be applied precisely at the time

Chadrick E. Denlinger, MD

Central Message

It is time to move TLR4 inhibition for lung ischemia-reperfusion injury into the clinical arena.

See Article page 499.

when the IRI is most likely to occur. Theoretically, precise temporal treatment would broaden the therapeutic window in which the therapy provides the intended benefit without causing detrimental side effects. There are a few concerning issues with prolonged systemic suppression of TLR4 related to the potential for adverse side effects. Macrophage-mediated TLR4 signaling plays an important role in wound healing, and this has been demonstrated in several studies including transgenic TLR4 knockout mice.^{4,5} The clinical importance of TLR4 in wound healing has also been supported by the implication of TLR4 downregulation among patients with diabetes, which may have a role in the delayed wound healing that is seen in this population.

Currently, there are no pharmacologically available direct inhibitors of TLR4. TLR4 is only a single link, however, in a long chain of an inflammatory cascade that includes other targetable components. Recently, pharmacologic inhibition of heat shock protein 90 was shown to inhibit activation of TLR4, resulting in a diminished IRI in a renal IRI model⁶ that had many similarities to the lung IRI model demonstrated by Merry and colleagues.¹ In addition, clinical trials with TAK-242 with blocks downstream second messengers of TLR4 have shown some efficacy in abrogating the inflammatory response of gram negative bacterial sepsis.⁷ These agents may provide other avenues for TLR4 inhibition to be explored in patients

undergoing lung transplants provided untoward side effects of wound and anastomotic healing are not observed with transient abrogation of the TLR4 signaling pathway. Taken together, in vivo studies demonstrating that TLR4 inhibition attenuates IRI in lung and kidney models, along with the availability of a pharmacologic inhibitor of the TLR4 pathway that has already been investigated in a clinical trial, suggest the potential for a clinical investigation of TLR4 inhibition in patients receiving lung transplants.

References

1. Merry HE, Phelan P, Hwang B, Mulligan MS. Validating the use of siRNA as a novel technique for cell specific target gene knockdown in lung ischemia-reperfusion injury. *J Thorac Cardiovasc Surg*. 2016;151:499-506.
2. Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2007—an update. *J Gene Med*. 2007;9:833-42.
3. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. *Nat Rev Drug Discov*. 2009;8:129-38. Erratum in: *Nat Rev Drug Discov*. 2009;8:516.
4. Suga H, Sugaya M, Fujita H, Asano Y, Tada Y, Kadono T, et al. TLR4 rather than TLR2, regulates wound healing through TGF- β and CCL5 expression. *J Dermatol Sci*. 2014;73:117-24.
5. Cheng TL, Lai CH, Chen PK, Cho CF, Hsu YY, Wang KC, et al. Thrombomodulin promotes diabetic wound healing by regulating toll-like receptor 4 expression. *J Invest Dermatol*. 2015;135:1668-75.
6. O'Neil S, Humphries D, Tse G, Marson LP, Dhaliwal K, Hughes J, et al. Heat shock protein 90 inhibition abrogates TLR4-mediated NF- κ B activity and reduces renal ischemia-reperfusion injury. *Sci Rep*. 2015;5:1-11.
7. Matsunaga N, Tsuchimori N, Matsumoto T, Li M. TAK-242 (resatorvid), a small molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR 4 and interferes with interactions between TLR4 and its adaptor molecules. *Mol Pharmacol*. 2011;79:34-41.