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Acute, renally restricted siRNA-mediated gene silencing/adeno-associated virus gene rescue through a novel renal subcapsular approach

Pedro A. Jose

University of Maryland School of Medicine, USA

Abstract

Essential hypertension is the consequence of genetic dysfunctions that are modified by the environment. The importance of the kidney in the regulation of blood pressure is supported by renal transplantation studies in humans, rats, and mice.Gene silencing has led to important discoveries in the roles of genes in physiology and pharmacology. However, competing or compensatory systems in conventional knockout mice can occur during pre- and postnatal development. An ideal system with which to study the effect of a gene on a particular phenotype would be one in which the loss of gene expression is both inducible and completely reversible. Such a system would not be susceptible to compensatory mechanisms that may arise in germline mutant models. The role of a certain gene on the function of specific tissues can also be studied by tissue specific gene silencing. While such gene silencing could be made inducible, it is not reversible. These methods can also not be used to determine the role of the kidney as a whole in the regulation of blood pressure, as one would have to selectively silence the gene of interest in renal blood vessels and nephron segments, by inserting vascular and nephron gene promoters. The role of genes of interest on the physiology of one kidney can be studied by doing cross-renal transplantation. However, this procedure is laborious and costly. Drugs can also be delivered into the kidney of rats via the suprarenal artery, so as not to interfere with renal blood flow but this is not possible in mice. Selective gene silencing in one or both kidneys can be done by interstitial infusion of antisense oligonucleotides in rats and by the renal subcapsular infusion of siRNA or shRNA in mice, a method developed in our laboratory (Hypertension. 2012;59:446-52, PLoS One. 2012;7:e38745,Free Radic Biol Med. 2012;53:437-46,J Biol Chem. 2013;288:152-63). This method allows the silencing of the gene(s) of interest in the ipsilateral kidney and studying the consequences of this maneuver in the contralateral kidney. Our method also allows a reversible knockdown of gene expression (efficiency has ranged from 40-70% in the renal cortex only). Gene expression reverts back to normal levels after 7 days from cessation ofsiRNA infusion. Because siRNA/shRNA effects may not be totally specific, another method of gene silencing is needed to confirm the observed effects. Therefore, gene silencing should be complemented by the renal subcapsular infusion of AAV vectors. The infusion of such viral vectors retrogradely via the ureter affords gene silencing in the renal medulla.Current gene manipulation methods, including transduction with viral vectors to achieve inducible/reversible gene knockout or rescue, cannot be restricted to one kidney without direct injection (which can be damaging) into the renal artery with or without renal vein clamping. This subcapsular infusion may also be used for the delivery of viral vectors for gene rescue or knock-in selectively to one kidney. The infusion of agents into one kidney also allows studies on the contralateral, non-infused kidney. The silencing of genes in other organs of interest can also be done by the infusion of siRNA into the artery that supplies the organs, other than the kidney, so long as blood supply is not impeded. We have silenced *Gast* in the stomach and proximal duodenum of mice by the intraceliac arterial infusion of gastrin-specific siRNA (unpublished).Our novel subcapsular or arterial siRNA or AAV infusion offers the advantage of restricted gene silencing or rescue of the gene of interest in one kidney only or the organ of interest that offers advantages over germline mutant models.

pjose@medicine.umaryland.edu