Systemic biopolymer-delivered vascular endothelial growth factor promotes therapeutic angiogenesis in experimental renovascular disease

Alejandro R. Chade[1,2,3], Maxx L. Williams[1], Erika Guise[1], Luke J. Vincent[1], Taylor W. Harvey[1], Marija Kuna[4], Fakhri Mahdi[5] and Gene L. Bidwell III[4,5]

[1]Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi, USA; [2]Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA; [3]Department of Radiology, University of Mississippi Medical Center, Jackson, Mississippi, USA; [4]Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi, USA; and [5]Department of Neurology, University of Mississippi Medical Center, Jackson, Mississippi, USA

We recently developed a therapeutic biopolymer composed of an elastin-like polypeptide (ELP) fused to vascular endothelial growth factor (VEGF) and showed long-term renoprotective effects in experimental renovascular disease after a single intra-renal administration. Here, we sought to determine the specificity, safety, efficacy, and mechanisms of renoprotection of ELP-VEGF after systemic therapy in renovascular disease. We tested whether kidney selectivity of the ELP carrier would reduce off-target binding of VEGF in other organs. *In vivo* bio-distribution after systemic administration of ELP-VEGF in swine was determined in kidneys, liver, spleen, and heart. Stenotic-kidney renal blood flow and glomerular filtration rate were quantified *in vivo* using multi-detector computed tomography (CT) after six weeks of renovascular disease, then treated with a single intravenous dose of ELP-VEGF or placebo and observed for four weeks. CT studies were then repeated and the pigs euthanized. *Ex vivo* studies quantified renal microvascular density (micro-CT) and fibrosis. Kidneys, liver, spleen, and heart were excised to quantify the expression of angiogenic mediators and markers of progenitor cells. ELP-VEGF accumulated predominantly in the kidney and stimulated renal blood flow, glomerular filtration rate, improved cortical microvascular density, and renal fibrosis, and was accompanied by enhanced renal expression of VEGF, downstream mediators of VEGF signaling, and markers of progenitor cells compared to placebo. Expression of angiogenic factors in liver, spleen, and heart were not different compared to placebo-control. Thus, ELP efficiently directs VEGF to the kidney after systemic administration and induces long-term renoprotection without off-target effects, supporting the feasibility and safety of renal therapeutic angiogenesis via systemic administration of a novel kidney-specific bioengineered compound.

Correspondence: *Alejandro R. Chade, Department of Physiology and Biophysics, Medicine, and Radiology, University of Mississippi Medical Center, 2500 North State Street, Jackson, Mississippi 39216-4505, USA. E-mail: achade@umc.edu*

Received 15 May 2017; revised 20 September 2017; accepted 28 September 2017

Kidney International (2018) ■, ■–■; https://doi.org/10.1016/j.kint.2017.09.029

KEYWORDS: drug delivery; elastin-like polypeptide; imaging; renal hemodynamics; renal injury; renovascular disease; vascular endothelial growth factor

The outcomes of renovascular disease (RVD) are still poor, and there is a noticeable lack of consensus regarding the best therapeutic strategy for these patients, which adds a burden of uncertainty to the treatment selection and course. Regardless of the chosen therapy (medical, interventional, or combined therapy), patients with RVD improve in ~30% of the cases.[1,2] Furthermore, the results of the CORAL study support the notion that pharmacologic or interventional (e.g., renal angioplasty to resolve the obstruction) strategies do not show significant differences in renal recovery to support one treatment over the other,[3] although secondary evaluations of the ASTRAL study suggest that interventional strategies may still be beneficial in selected populations.[4] These controversies feed a pressing need for novel and more effective therapeutic strategies for the growing population of patients suffering from RVD that are at higher cardiovascular risk and at risk of the development of chronic kidney disease (CKD).

It is possible that the vascular obstruction in RVD may be a major instigator of renal injury, and it may also exacerbate pre-existing renal damage.[5,6] However, the dynamic and progressive nature of RVD may be a driving force for evolving renal injury distal to the vascular obstruction and may determine the chances of renal recovery after therapeutic interventions. Thus, it is possible that the poor recovery in RVD results from a combination of doing too little or acting too late with the possibility of neglecting the stenotic renal parenchyma.

Renal microvascular (MV) dysfunction, remodeling, and even loss are hallmarks of CKD irrespective of the etiology.[7,8]

We have shown that damage of the small vessels in the kidney correlates with a significant deterioration of renal hemodynamics, filtration, and tubular function in a swine model of chronic RVD.[9–11] We also demonstrated that MV disease in the stenotic kidney is associated with blunted renal angiogenesis, which is driven by a progressive decrease in renal vascular endothelial growth factor (VEGF).[10,12] The pivotal role of this proangiogenic cytokine in the kidney is supported by proof of concept studies showing that intrarenal replenishment of VEGF ameliorated renal MV rarefaction and attenuated renal dysfunction and damage.[11,13,14]

We recently extended and refined renal VEGF therapy. We developed a novel fusion of a bioengineered protein for drug delivery with $VEGF_{121}$. We used elastin-like polypeptides (ELPs), which are genetically encoded drug-delivery vectors with long plasma half-life, low immunogenicity, and adaptability to be fused to nearly any therapeutic. Furthermore, ELPs naturally accumulate in kidney.[15–18] The ELP-VEGF construct displayed a prolonged circulation and tissue residence time, improved stenotic kidney targeting, and long-term efficacy of VEGF therapy in the RVD model (compared with unconjugated VEGF) after a single intrarenal administration.[18] However, whether *systemic* administration of ELP-VEGF targets and protects the kidney is unknown and is important to determine from a clinical/translational perspective. Thus, we first seek to establish the renal specificity of the ELP-VEGF construct after systemic administration, as we also aim to determine the safety and efficacy in the RVD model through this route. We hypothesize that the fusing of VEGF to the ELP biopolymer carrier will lead to renal tissue specificity and kidney accumulation without decreasing therapeutic efficacy even after systemic administration. Finally, we intend to determine potential off-target effects (a concern from a clinical/translational perspective) and underlying mechanisms of long-term renoprotection after systemic ELP-VEGF therapy.

RESULTS

Characterization and labeling of the ELP-VEGF construct with fluorescent probes

Labeling was performed on primary amine residues, including the protein's N-terminus, its 1 lysine residue near the N-terminus, and its 8 lysine residues on the surface of $VEGF_{121}$ as highlighted in Supplementary Figure S1. Labeling did not alter VEGF potency. For more details, see Supplementary File. The stability of the ELP-VEGF biopolymer was determined *in vitro*, as described in the Supplementary File. As shown in Supplementary Figure S2A, ELP-VEGF was present as a single band migrating at 74 kDa, and the free rhodamine label migrated below the 10-kDa marker. When incubated in phosphate-buffered saline, very little ELP-VEGF degradation was observed for the first 24 hours (quantified in Supplementary Figure S2B). Degradation of the protein began between 24 and 48 hours of incubation and proceeded to nearly complete loss of the full-length band after 4 days. A similar slow degradation was observed when ELP-VEGF was incubated in plasma. The kinetics of the degradation in plasma were different from the PBS incubation, beginning more quickly. However, after 5 days in plasma, more full-length protein remained intact than in the phosphate-buffered saline incubation, possibly reflecting a stabilizing decoy effect of other plasma proteins occupying proteases. Detection of free dye by using trichloroacetic acid to precipitate the protein component of each sample mirrored the gel electrophoresis data in the phosphate-buffered saline incubation, with free dye slowly being released over a period between 20 and 96 hours. However, the amount of free dye peaked at only ~25%, indicating that most of the dye was still bound to a protein component and consistent with the presence of a band of ~10 kDa in Supplementary Figure S2A. After incubation in plasma, almost no free dye was detectable, although it is possible that some free dye bound to albumin or other plasma proteins and was thus precipitated by trichloroacetic acid. These analyses reveal that ELP-VEGF does degrade under physiologic conditions, but the rate of degradation is quite slow relative to the rate of clearance from the body observed here and in other studies.[18,19]

In vivo biodistribution of fluorescently labeled ELP-VEGF after a single i.v. administration

To determine the pharmacokinetics and biodistribution of ELP-VEGF, the protein was fluorescently labeled, and plasma levels and organ biodistribution were determined 4 hours after a single i.v. administration (ear vein catheter) in the swine. The fluorescently labeled protein was administered i.v. in swine at a bolus dose of 1 mg/kg, plasma was sampled intermittently, and organ fluorescence was determined at killing 4 hours after injection. Whole-organ imaging revealed that ELP-VEGF predominantly accumulated in the kidney (Figure 1a). When the kidneys were cut in cross section, fluorescence imaging revealed ELP-VEGF localized at high levels in the renal cortex, with additional focal medullary localization in what are likely the large vascular branches. Retention of ELP-VEGF in the kidney was 3.2-fold higher than in the next most abundant organ, the liver. Additionally, ELP-VEGF levels in the kidney were 14.7-fold higher than in the lung, and the protein was undetectable in the heart and spleen at this dose (Figure 1b). Direct measurement of plasma fluorescence revealed a biphasic clearance of ELP-VEGF from the blood. A rapid distribution phase was evident within 30 minutes of injection, followed by a very slow clearance phase (Figure 1c). The slow clearance of ELP-VEGF is consistent with our observations of this protein after intrarenal administration, where we observed a half-life of ~13.5 hours.[18] However, the short duration of this experiment did not provide enough clearance time to achieve an accurate fit of the terminal half-life following i.v. administration.

Overall, these results demonstrate that ELP-VEGF is sufficiently stable under physiologic conditions, clears slowly, and most of the injected protein is retained in the kidney even after systemic injection using an ear vein route, suggesting that ELP-VEGF is renal selective and that systemic