Another Reason to Eat Your Greens
Cardiopulmonary Protection by Dietary Delivery of Angiotensin-Converting Enzyme-2 and Angiotensin-(1-7) Made in Plants

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Pulmonary arterial hypertension (PAH), first described in 1891, is a devastating disorder involving increased blood pressure in the pulmonary circulation leading to right heart failure, typically resulting in a survival of only 2 to 3 years if left untreated. Drug therapies for PAH are broadly limited to phosphodiesterase inhibitors, and soluble guanylate cyclase activators. Recent evidence from the research team led by Dr Mohan Raizada (University of Florida) supports the addition of activators of angiotensin-converting enzyme-2 (ACE2) and its downstream product, angiotensin-(1-7) [Ang-(1-7)], to this list.1 A clinical trial (ClinicalTrials.gov; NCT01884051), the primary outcome of which is to assess the mechanism, safety, and efficacy of ACE2 in the treatment of PAH, is currently underway. As with every hypertensive disorder, cost of therapy and compliance with therapy are major complicating factors for effective clinical management.

In the current issue of Hypertension, Shenoy et al examined the effect of feeding recombinant ACE2 or Ang-(1-7) protein to rats with monocrotaline-induced PAH. Monocrotaline treatment is a well-established method to induce PAH in rodents that is equivalent to New York Heart Association/World Health Organization Class III to IV human PAH. Although the model system and the concept that ACE2/Ang-(1-7) stimulation is protective are both already established by this team and others, the current study should excite the hypertension research community for its unique mode of delivery, the tobacco plant chloroplast. Most will remember the chloroplast as the specialized organelle required for photosynthesis. However, the chloroplast genome is amenable to genetic manipulation, and with 10,000 copies of the chloroplast genome per plant cell, plentiful amounts of foreign proteins can be produced.2 Recent advances in the development of sophisticated vectors for delivery of proteins and peptides in plants have helped overcome the challenges of inefficient delivery because of the rigors of the gastrointestinal tract. Bioencapsulation prevents degradation of the therapeutic proteins by gastric enzymes. In the current study, 2 novel features were incorporated into the design of the delivery vector. First, the authors fused a transmucosal carrier protein, cholera nontoxic B subunit, to the N terminal of both ACE2 and Ang-(1-7) to aid in systemic absorption by directly targeting the proteins to specific monosialotetrahexosylganglioside receptors present on the intestinal epithelium. Second, a small hinge peptide and a ubiquitous processing protease (furin) cleavage site were engineered between the transmucosal carrier and ACE2 and Ang-(1-7) to cleave the carrier and aid in the systemic release of the therapeutic proteins after it is internalized. Delivery becomes as simple as feeding the test subject (rats in the current study) with powder derived from liquid nitrogen frozen chloroplast-lead leaves. Thus, these advances challenge some long-held pharmacokinetic principles that the oral delivery of peptides is generally assumed to be poorly bioavailable. A plant-based pharmaceutical has been approved for the treatment of Gaucher disease, a lysosomal storage disorders resulting from glucocerebrosidase deficiency.3

In the current study, Shenoy et al observed significant functional and structural cardiopulmonary improvements with oral delivery of ACE2 and Ang-(1-7) in both prevention (simultaneous administration of plant proteins and induction of PAH) and reversal (administration of plant-derived proteins after established PAH) protocols. Evidence of improved cardiac function and hemodynamics and reduced cardiac remodeling and fibrosis was observed in both protocols. In the reversal protocol, combination therapy with ACE2 and Ang-(1-7) was better than single therapy, particularly at the higher combined dose. Both treatments were associated with reduced expression of proinflammatory cytokines and a marker of autophagy normally induced in PAH. Moreover, the increase in the ACE2/ACE2 and angiotensin type 1 receptor/angiotensin type 2 receptor mRNA ratio caused by monocrotaline was corrected by ACE2 and Ang-(1-7).

These data clearly challenge the assumption that oral delivery of peptides and proteins will not be beneficial therapeutically. In fact, the article provides a salient proof-of-concept demonstration that potent, orally active cardiopulmonary protective peptides can be produced in large quantities in plants and easily administered. Nearly one third of patients with chronic illnesses such as hypertension or chronic obstructive pulmonary disease in the National Health Institute Survey are unable to afford food, medications, or both.5 This statistic underscores the growing need for cost-effective alternative production methods for pharmaceuticals and highlights the economic significance of the current findings. Pharming...
or molecular farming is not an entirely new concept because Sijmons et al. produced human serum albumin in plants as early as 1990. Even vaccines can be produced in plants, and such delivery methods may represent the only realistic way to deliver therapies to the developing world because of the ease of production, simple administration, and low cost. Although definitive population-level statistics are not available, PAH is associated with schistosomiasis, sickle cell disease, acute pulmonary embolism, and hypoxia because of lung diseases such as chronic obstructive pulmonary disease or as a result of high altitude, supporting the concept that PAH may be more prevalent in developing countries. Thus, the current article is remarkable for demonstrating that inexpensive, transgenic plant-based production of beneficial protein products—at levels which can significantly affect cardiopulmonary disease progression in vivo—is possible.

Many questions still remain to be answered. Ideally, the level of the orally delivered peptide could be quantified in the plasma or tissues of the treated animal to definitively illustrate absorption and integration of the pharmaceutical. Although increased levels of both ACE2 (increased by 37% in the circulation) and Ang-(1-7) (increased 2-fold in the circulation) were achieved, it is unclear whether this is the result of absorption of the product (as the authors contend) or is the result of de novo synthesis induced by the treatment. The current study design largely precludes this determination because the sequence of Ang-(1-7) is identical across species, but future studies including radiolabeled or similar tracers may help clarify this point. Moreover, how this treatment affects the gut microbiota or the gastrointestinal renin–angiotensin system remain unknown. Nonetheless, whether the delivered enzyme and product are absorbed or achieve their effects by some indirect action within the gastrointestinal tract, the point that this maneuver improves cardiopulmonary structure and function in monocrotaline-treated animals, remains impressive. This demonstration simultaneously highlights the use of targeting the ACE2/Ang-(1-7) axis in PAH, the feasibility of oral delivery of peptide-based therapeutics in PAH, and the success of cost-effective production of such peptide-based therapeutics in transgenic plants. Now, if they can transition the tobacco-based system described in the current article to something more palatable, lettuce for instance (and then make lettuce taste good), perhaps we can combat the ever-present complicating factor of compliance…

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Disclosures
None.

References
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