

EDITORIAL COMMENT

# Inhalable Therapies for the Heart

## Take the “Lung” Way Home?\*

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**M**olecular therapies have the potential to revolutionize the way we are managing patients with cardiovascular diseases. Many targets are involved in abnormal ion balances, deficient calcium cycling, or activation of pathologic signaling pathways in different cardiac cells, and thereby represent appealing therapeutic targets. But it remains a major challenge to deliver these therapies directly and efficiently to the heart.

Researchers around the world intensely develop gene therapy, microRNA therapeutics, and gene editing for the heart, relying on viral and nonviral vectors and continuously refining uptake and transfection or transduction efficacies. In human applications, these innovative therapies are mostly delivered by systemic intravenous administration, as for microRNAs,<sup>1</sup> or direct intracoronary infusions, as performed in preclinical<sup>2</sup> and clinical research programs using adeno-associated viruses.<sup>3,4</sup> A more recent strategy consists of targeting the liver to limit the secretion of pathologic proteins for the heart, for example, for hypertension treatment.<sup>5</sup> Recent developments in the treatment of transthyretin amyloidosis with RNA-targeting and gene-editing therapies have also shown the efficacy of this

strategy.<sup>6,7</sup> But wouldn't it be nice to access the heart differently from conventional avenues, using a high uptake and first-pass delivery system that could greatly enhance opportunities?

Researchers from Berlin, Milan, and Parma did just that. In this issue of the *Journal of the American College of Cardiology*, Alogna et al<sup>8</sup> demonstrate that inhaling a dry powder (dp) composed of mannitol-based microparticles embedding mimetic peptide (MP)-loaded calcium phosphate nanoparticles (dpCaP-MP) led pigs with rapid pacing-induced heart failure to improve their ejection fraction by an absolute 17% compared with pretreatment ejection fraction, by correcting the localization of L-type calcium channels (LTCCs) via interfering MPs. In other words, they demonstrated that daily inhalation can be an easy and efficient way to deliver a peptide to the heart (**Figure 1**).

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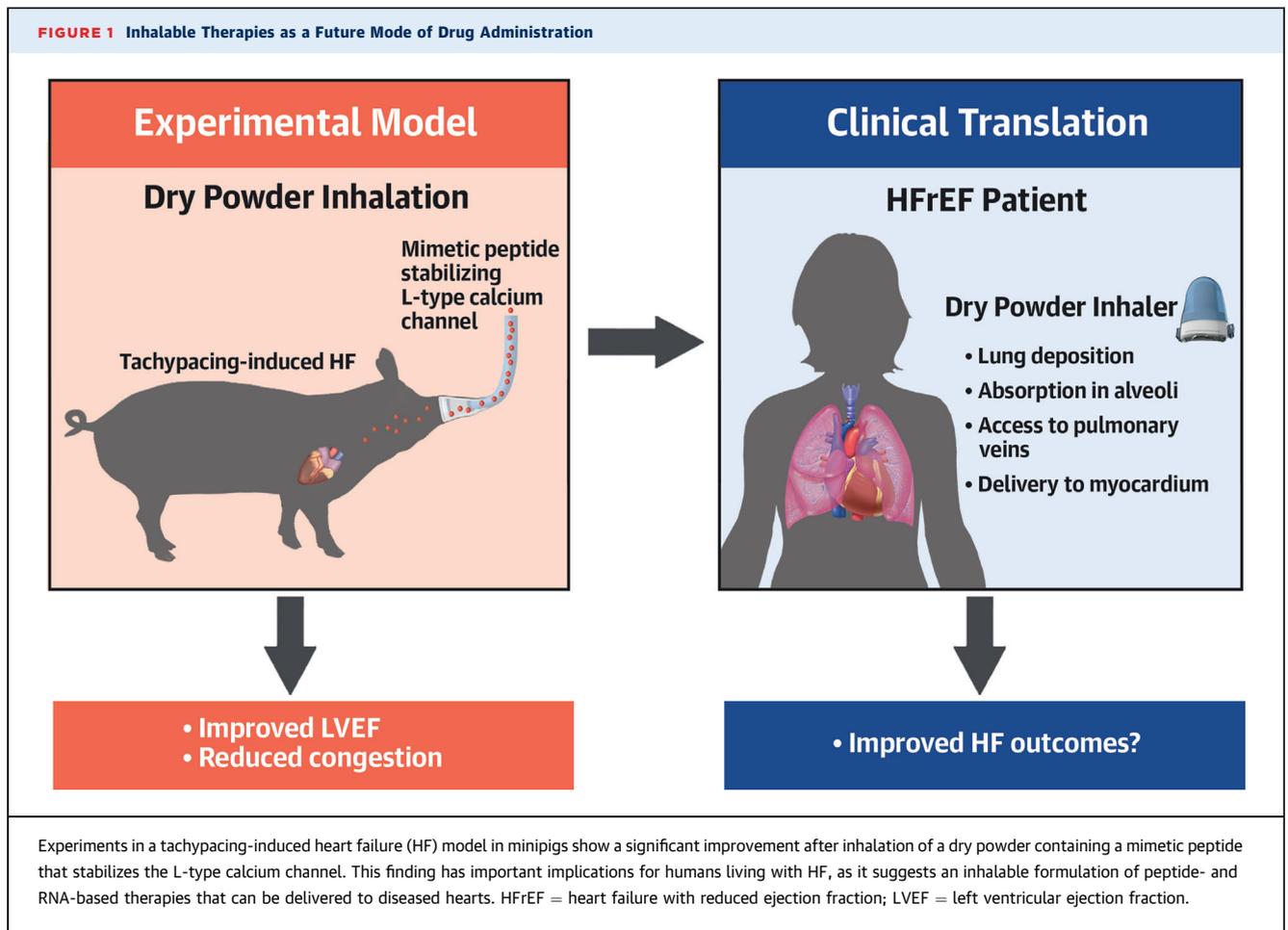
Obviously, this finding is rich in implications and prerequisites. First, the mechanism targeted should be of crucial relevance for cardiac force development. Indeed, LTCCs rely on phosphorylation C-terminus of the  $\text{Ca}_v\beta_2$  subunit, which binds to the SH3-domain of the same subunit and stabilizes interaction with the  $\text{Ca}_v\alpha_1$  subunit and stabilizes the channel. This function is lost because of a lack of phosphorylation of the C-terminus. The MP (DQRPDREAPRS) provides the same function as the phosphorylated C-terminus, thereby correcting a heart failure-induced ion imbalance, as shown before.<sup>9</sup>

Second, the peptide-uptake formulation should be robust and otherwise on target. Here, it is worthwhile to turn to the model: heart failure with reduced ejection fraction (HFrEF) was induced in Göttingen minipigs by tachypacing over 6 weeks. In a setting of overt HFrEF (left ventricular ejection fraction  $30\% \pm 8\%$ ), animals were randomized and

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treatment started after 4 weeks of tachypacing. HFrEF animals inhaled either dpCaP-MP or the calcium phosphate nanoparticles only (dpCaP without MP). Efficacy was evaluated by means of echocardiography, invasive hemodynamics, and biomarker assessment. In the pigs, dpCaP-MP was applied at a dose of 0.015 mg/kg/d for 14 days. The dose is lower than that in mice (0.25 mg/kg/d) that led to MP detection and improvement of diabetic cardiomyopathy after 10 days of treatment.<sup>10</sup> However, pilot studies in pigs indicated that the lower dose suffices, and the study was conducted with the lower dose.

Third, the contamination of other organs, such as liver and spleen, should be minimal in order to concentrate the effect to the heart. Indeed, inhalation of 50  $\mu$ Ci of technetium-loaded CaP revealed less liver and spleen contamination than intravenous injection into mice. Whereas brain and kidney contained more radioactive CaP in the early hours after intravenous application (1-24 hours) rather than intratracheal application, the opposite was true for the heart,

where tracer peptide accumulation lasted for 24 hours in the inhalation group, but not in the intravenous group. Importantly, future pharmacokinetics studies will be needed to specify the exact distribution, metabolism, and excretion of therapies delivered through the proposed inhalation route.

These data exceed the findings of others. Adeno-associated viruses encoding for SERCA2a have been successfully aerosolized and thereby used to treat a pig model of pulmonary hypertension.<sup>11,12</sup> Along these lines, therapeutic aerosols have been used to apply trimannose-coupled miR21 inhibitors<sup>13</sup> for curbing COVID-19-associated acute inflammatory lung damage. Again, the heart was not a target in that study. In the present study, Alogna et al<sup>8</sup> used a dry powder microparticle that does not require water dilution and nebulization, but is administered to the lungs via inhalation (ie, 15 minutes of inhalation through a mask) and will then dissolve in the lung fluid and then access the cardiopulmonary circulation after getting through the alveolocapillary membrane.

The aerodynamics seems improved, but the trans-ferability of the inhalation protocol to humans will require further development.

Altogether, this first-in-class lung-to-the heart technology represents an appealing alternative for the delivery of peptide- and RNA-based therapies to diseased hearts. The demonstration of efficient delivery of an MP stabilizing LTCC trafficking in a large animal preclinical model paves the way for future clinical applications (Figure 1).

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#### REFERENCES

1. Täubel J, Hauke W, Rump S, et al. Novel anti-sense therapy targeting microRNA-132 in patients with heart failure: results of a first-in-human Phase 1b randomized, double-blind, placebo-controlled study. *Eur Heart J*. 2021;42(2):178-188.
2. Hinkel R, Batkai S, Bähr A, et al. AntimiR-132 Attenuates myocardial hypertrophy in an animal model of percutaneous aortic constriction. *J Am Coll Cardiol*. 2021;77:2923-2935.
3. Greenberg B, Butler J, Felker GM, et al. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial. *Lancet*. 2016;387:1178-1186.
4. Hulot J-S, Salem J-E, Redheuil A, et al. Effect of intracoronary administration of AAV1/SERCA2a on ventricular remodelling in patients with advanced systolic heart failure: results from the AGENT-HF randomized phase 2 trial. *Eur J Heart Fail*. 2017;19:1534-1541.
5. Addison ML, Ranasinghe P, Webb DJ. Novel pharmacological approaches in the treatment of hypertension: a focus on RNA-based therapeutics. *Hypertension*. 2023;80(11):2243-2254.
6. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *N Engl J Med*. 2021;385:493-502.
7. Aimo A, Castiglione V, Rapezzi C, et al. RNA-targeting and gene editing therapies for transthyretin amyloidosis. *Nat Rev Cardiol*. 2022;19:655-667.
8. Alogna A, Berboth L, Faragli A, et al. Lung-to-heart nano-in-micro peptide promotes cardiac recovery in a pig model of chronic heart failure. *J Am Coll Cardiol*. 2023;83(1):47-59.
9. Rusconi F, Ceriotti P, Miragoli M, et al. Peptidomimetic targeting of Cav $\beta$ 2 overcomes dysregulation of the L-type calcium channel density and recovers cardiac function. *Circulation*. 2016;134:534-546.
10. Miragoli M, Ceriotti P, Iafisco M, et al. Inhalation of peptide-loaded nanoparticles improves heart failure. *Sci Transl Med*. 2018;10.
11. Agüero J, Ishikawa K, Hadri L, et al. Intratracheal gene delivery of SERCA2a ameliorates chronic post-capillary pulmonary hypertension: a large animal model. *J Am Coll Cardiol*. 2016;67:2032-2046.
12. Bikou O, Tharakan S, Yamada KP, et al. Endobronchial aerosolized AAV1.SERCA2a Gene therapy in a pulmonary hypertension pig model: addressing the lung delivery bottleneck. *Hum Gene Ther*. 2022;33:550-559.
13. Beck C, Ramanujam D, Vaccarello P, et al. Trimannose-coupled antimiR-21 for macrophage-targeted inhalation treatment of acute inflammatory lung damage. *Nat Commun*. 2023;14:4564.

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