CA Dreamin': Carbonic Anhydrase Inhibitors, Macrophages, and Pulmonary Hypertension

Although it is a rare condition, a diagnosis of pulmonary arterial hypertension (PAH) confers a risk of significant morbidity and mortality. Characterized by vascular cell hyperproliferation, formation of occlusive lesions, and extension of smooth muscle to the typically nonmuscular regions of the arterial tree, the vascular remodeling that occurs during PAH is well described, but it is poorly understood from a mechanistic standpoint. Consequently, available treatments for PAH rely on manipulating pathways involved in vasoconstriction, and no current therapies are aimed primarily at slowing or reversing the vascular remodeling. Thus, a major focus in PAH research has been to elucidate pathways involved in the initiation and progression of remodeling, with the goal of identifying druggable targets for which therapies (i.e., small-molecule inhibitors) can be developed. Given the long time frame associated with drug discovery, a more practical and certainly time-effective approach might be to use repurposed drugs, with known safety profiles, to treat PAH. However, with an incomplete understanding of the mechanisms that control the remodeling process, achieving this goal has remained closer to a dream than a reality.

Immune cells have been long recognized as being present in vascular lesions of patients with PAH, and recent investigations have moved inflammation from a bystander to a central player in the pathogenesis of PAH (1). Although roles for mast cells, T cells, and B cells have been proposed, accumulating work is focusing on the macrophage as a viable target that modulates vascular remodeling during PAH (2). Macrophages can sense the local tissue microenvironment and transduce that signal via metabolic reprogramming to regulate their functional phenotype (3). In broad strokes, M1 (classically activated) macrophages are proinflammatory, whereas M2 (alternatively activated) macrophages are antiinflammatory. Both phenotypes have been described in PAH (4) and are believed to be driven by metabolic derangements in vascular wall cells that may influence the local milieu.

It is against this background that Hudalla and colleagues (pp. 512–524) tested the hypothesis that carbonic anhydrase inhibition (CAI) with acetazolamide could induce metabolic acidosis and ameliorate pulmonary hypertension in the SU5416 plus hypoxia (SuHx) rat model of PAH, as reported in this issue of the *Journal* (5). CAI was initially developed with primary action on the kidney, interfering with acid–base regulation and causing diuresis. Presently used to treat glaucoma, metabolic alkalosis, epilepsy, and acute mountain sickness, CAI has been shown to have a beneficial effect on pulmonary hypertension in preclinical models with hypoxia as the stimulus (6). The authors of the current report used a straightforward study design in a relevant preclinical model of PAH, and a comprehensive approach that included the use of samples from patients with PAH and mechanistic *in vitro* studies. When added to the drinking water either early or late in the course of the SuHx model, CAI reduced measures of pulmonary hypertension. Similar results were observed when metabolic acidosis was induced by ammonium chloride. The authors also showed that expression of proinflammatory mediators (i.e., *Tnfa* and *Il-6*) were upregulated in both the lungs and macrophages of SuHx rats and in tissues from patients with PAH. Interestingly, macrophages from SuHx animals exhibited both increased proinflammatory markers and increased markers of alternative activation.

One of the most intriguing findings of this study is that macrophage mediators, such as TNF-α and IL-1β, promoted dedifferentiation in pulmonary arterial smooth muscle cells (PASMCs), leading to a synthetic (i.e., hyperproliferative) phenotype. Indeed, supernatant from SuHx macrophages promoted PASMC dedifferentiation *in vitro*, which was prevented if the rats were treated with CAI. These results, coupled with other recent findings (7, 8), firmly position the macrophage as a mediator of vascular remodeling in pulmonary hypertension.

Although the current work clearly demonstrates a novel immunomodulatory role for CAI in macrophage function and links macrophage activation with the PASMC phenotype, several issues remain to be addressed. First and foremost is the mechanism by which CAI exerts its effects. The authors found that carbonic anhydrase II (CAII) was abundantly expressed and upregulated in macrophages from SuHx rats and lung tissue from patients with PAH. Given that activation of both M1 and M2 was suppressed by CAI, it is possible that a compensatory upregulation of CAII is required for macrophage metabolic reprogramming. Surprisingly, inducing acidosis with ammonium chloride suppressed only M1 activation. These dichotomous results raise an interesting question about mechanisms associated with CAI that are unrelated to the acid–base balance.

It is also not clear that macrophages are the sole or primary target that can alleviate pulmonary hypertension *in vivo*. For example, acetazolamide directly inhibits pulmonary vasoconstriction, attenuates Ca^{2+} signaling in PASMCs, and has been suggested to act as an antioxidant or stimulant of nitric oxide production (9–12). Some of these effects are independent of CAI; for example, an acetazolamide analog with a methylation in place of the sulfonamide moiety (N-methylacetazolamide) was shown to lack CAI, yet similarly repressed Ca^{2+} signaling and pulmonary contraction (9, 10). The non-CAI effects of acetazolamide are not well understood, and whether any of these effects played a role in the findings noted in the current paper awaits further exploration.

Regardless of remaining questions regarding the exact mechanisms by which CAI exerts beneficial effects in the SuHx

model and modulates macrophage function, this study provides intriguing new pieces in the PAH pathogenesis puzzle and demonstrates improved hemodynamics in the SuHx model. Whether similar effects will be observed in patients with PAH is unknown. A clinical trial (ClinicalTrials.gov Identifier: NCT02755259) examining the acute hemodynamic effects of acetazolamide in PAH is ongoing, but given the short exposure time (60 min) used in this trial, the results are unlikely to determine whether repurposing acetazolamide for treatment of PAH will become a reality. Nonetheless, the results from the current study encourage investigators to keep dreamin'. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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