A New Pathway to Airway Relaxation: Targeting the "Other" Cyclase in Asthma

Significant concerns have recently been raised about sole therapy with inhaled short-acting β_2-adrenoceptor agonists (SABAs) in poorly controlled asthma in the absence of prophylactic antiinflammatory corticosteroids (ICSs) (1, 2). Many individuals still have frequent exacerbations and persistent dyspnea despite escalation of combined ICSs and long-acting β_2-adrenoceptor agonists (LABAs). In both scenarios, higher doses of SABAs as a rescue medication can lead to potential receptor desensitization and loss of dilator efficacy. Overuse of SABAs has been associated with a higher risk of future exacerbations and hospital admissions (3, 4), and identified as one of several preventable factors that lead to higher asthma mortality with increasing disease severity (1, 5).

Given this background, it is important to identify alternative or adjunct bronchodilator therapies that are both safe and effective when responsiveness to β_2-adrenoceptor agonists has decreased. In a study reported in this issue of the *Journal,* Koziol-White and colleagues (pp. 43–48) provide evidence supporting soluble guanylate cyclase (sGC) as a novel therapeutic target (6). Using human precision-cut lung slices (PCLSs), the authors confirmed previously reported *in vitro* dilator responses to the sGC agonists BAY 41-2272 (BAY 41) and BAY 60-2270 (BAY 60) (7). These drugs increased cyclic guanosine monophosphate (cGMP) to similar levels and were comparable in terms of bronchodilator efficacy and potency to the LABA formoterol, which mediates relaxation via activation of the adenylate cyclase/cAMP/protein kinase A pathway. Notably, responsiveness to both BAY 41 and BAY 60 was maintained under experimental conditions that induced β_2-adrenoceptor desensitization, when formoterol-mediated relaxation of the airways in human PCLSs was almost completely abolished (6).

Relaxation of vascular smooth muscle via the nitric oxide (NO)/sGC/cGMP/protein kinase G pathway underpins the established use of sublingual NO donors, inhaled NO, and oral phosphodiesterase inhibitors in the treatment of angina and pulmonary hypertension. sGC activators have also shown benefit in numerous preclinical models of pulmonary hypertension. Riociguat, which is structurally similar to BAY 41, was the first in class to move beyond controlled clinical studies to approval (reviewed in Reference 8). However, the cGMP-dependent signaling cascade used by these drugs was previously believed to play only a relatively minor role in the regulation of airway smooth muscle tone, as exemplified by a study in which inhaled NO had no effect on airway conductance in either healthy subjects or patients with chronic obstructive pulmonary disease, and elicited only limited relaxation in subjects with asthma (9). Koziol-White and colleagues speculated that the marked dilator responses to BAY 41 and BAY 60 they observed in human lung slices may be driven by their more effective agonism of sGC compared with NO itself (6). Future comparisons of cGMP synthesis by these sGC agonists relative to NO-dependent dilators are required to clarify this possibility, as only BAY 41 causes direct NO-independent stimulation of sGC. Nevertheless, their results showed a distinct lack of cross-talk between cGMP and cAMP generation by sGC agonists versus formoterol (6), establishing a feasible mechanism whereby the *in vitro* dilator efficacy of BAY 41 and BAY 60 could be maintained when LABA responsiveness is impaired.

Two major mechanisms have been shown to contribute to the contraction of small intrapulmonary airways in PCLSs. The initiation of airway contraction, observed as agonist-induced Ca^{2+} oscillations localized to the sarcoplasmic reticulum, is termed Ca^{2+} signaling. Sustained contraction mediated via increased activity of protein kinase C and Rho-activated kinase is termed Ca^{2+} sensitivity, and is evident even when Ca^{2+} oscillations are abolished by prior treatment of the lung slices with caffeine and ryanodine (10). β_2-adrenoceptor agonists and sGC activators, as a consequence of their distinct second-messenger signaling pathways, could differentially oppose these regulatory mechanisms. A previous study using human PCLSs showed that formoterol effectively inhibited histamine-induced Ca^{2+} sensitivity, but only reduced Ca^{2+} oscillations when tested at higher concentrations (11). In contrast, in another study, both the NO donor NOC-5 and a stable cGMP analog inhibited serotonin-induced increases in the frequency of Ca^{2+} oscillations, but not contractions, due to increased Ca^{2+} sensitivity in mouse PCLSs (12). Because Ca^{2+} signaling and sensitivity may both be dysregulated in asthma (reviewed in References 13 and 14), it will be important to separately define the capacities of different dilators to oppose each pathway. These results may then help elucidate the potential benefits of sGC agonists relative to SABAs and LABAs, both separately and in combination, in asthma.

The novel findings of efficacy and resistance to desensitization of sGC agonists require validation in a disease context to support clinical translation. Given the limited availability of isolated airways from subjects with asthma, human PCLSs could be treated *in vitro* with asthma-relevant inflammatory cytokines such as IL-1, tumor necrosis factor-α, and IL-13, an approach that has previously been shown to increase contractile responses and reduce responsiveness to β_2-adrenoceptor agonists (reviewed in Reference 14). This would also complement the current study, in which overnight treatment with a high concentration of formoterol was used to cause β_2-adrenoceptor desensitization (6). When considering the therapeutic potential of sGC agonists as novel bronchodilators, one should remember that their efficacy has already been established *in vivo* in mouse models of allergic airways disease (7). Intratracheal administration of BAY 41 or BAY 60 before methacholine challenge in mice previously subjected to short-term exposure to ovalbumin or house dust mite extract afforded significant bronchoprotection. These findings are particularly pertinent with regard to the potential application of sGC agonists as an adjunct preventer therapy, but could be extended to longer-term models when airway

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hyperresponsiveness is driven by airway remodeling as well as inflammation.

Further intriguing possibilities of therapeutic benefit in response to sGC agonists arise as a consequence of their other reported actions (8). The combined bronchodilator and vasodilator effects of sGC activators could potentially counteract hypoxic vasoconstriction in the treatment of hypoxia-induced pulmonary hypertension, as improved ventilation has the potential to add benefit to the direct vasodilator effect of these drugs. sGC activation has also shown promise in limiting fibrosis, with inhibitory effects both on myofibroblast differentiation *in vitro* and on the development of bleomycin-induced pulmonary fibrosis (8). If similar findings could be demonstrated in fibroblasts from subjects with asthma, and with prophylaxis and treatment of mouse asthma models, this would clearly distinguish the activity profile of these drugs from that of β_2-adrenoceptor agonists, which relieve symptoms but not progressive airway fibrosis. sGC activation could have a dual effect in patients with asthma, by reducing fibrosis as well as promoting airway relaxation (8).

Clearly, the combined findings of Koziol-White and colleagues (6) and Ghosh and colleagues (7) support further investigation of sGC agonists as novel treatments for asthma. Because sole SABA therapy loses favor in the face of accumulating evidence of a greater benefit from combined LABA/ICS over ICS alone at all stages of asthma (1, 2), the limitations of bronchodilator therapy with β_2-adrenoceptor agonists still need to be addressed. Although a plethora of potential candidates, ranging from calcilytics and bitter-taste agonists to relaxin, have received significant support (14, 15), accumulating evidence indicates that targeting sGC also effectively opposes airway contraction (6, 7). Showing that sGC agonists are better bronchodilators than β_2-adrenoceptor agonists in a disease context is the next step toward establishing a new pathway, traveling via sGC rather than the more familiar adenylate cyclase route. ■

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