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Ozone-induced airway hyperresponsiveness: roles of ROCK isoforms

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Lambert JA, Song W. Ozone-induced airway hyperresponsiveness: roles of ROCK isoforms. Am J Physiol Lung Cell Mol Physiol 309: L1394–L1397, 2015. First published October 30, 2015; doi:10.1152/ajplung.00353.2015.—Acute ozone (O₃) inhalation has been shown to cause airway and pulmonary epithelial injury with accompanying inflammation responses. Robust evidence exists that O₃ induces airway hyperresponsiveness (AHR) in humans and in animal models. Several pathways exist that culminate in airway smooth muscle contraction, but the mechanism(s) by which O₃ elicits AHR are unclear. Here, we review the recent report by Kasahara et al. (Kasahara DI, Mathews JA, Park CY, Cho Y, Hunt G, Wurmbrand AP, Liao JK, Shore SA. Am J Physiol Lung Cell Mol Physiol 309: L736–L746, 2015.) describing the role of two Rho kinase (ROCK) isoforms in O₃-induced AHR utilizing a murine haploinsufficiency model. Compared with wild-type (WT) mice, the authors report that ROCK1⁻/⁻ and ROCK2⁻/⁻ mice exhibited significantly reduced AHR following acute exposure to O₃. Additionally, WT mice treated with fasudil, an FDA-approved ROCK1/2 inhibitor, recapitulated reduction in AHR as seen in ROCK haploptotypes. It was suggested that, although the two ROCK isoforms are both induced by Rho, they have different mechanisms by which they mediate O₃-induced AHR: ROCK1 via hyaluronan signaling vs. ROCK2 acting downstream of inflammation at the level of airway smooth muscle contraction. These observations provide an important framework to develop novel ROCK-targeting therapies for acute O₃-induced AHR.

Ozone; airway hyperresponsiveness (AHR); Rho kinase (ROCK); hyaluronan

OZONE (O₃) has long been known as an air pollutant originating from automobile emissions reacting with sunlight. Because of its potent oxidizing properties, O₃ inhalation results in airway and pulmonary injury leading to a severe state of inflammation characterized by recruitment of neutrophils and macrophages to the lung (10, 15, 21). One of the most highly characterized effects of O₃ inhalation is airway hyperresponsiveness (AHR), a key symptom of asthma (9). Although the consequences of O₃ inhalation are well characterized, the mechanism(s) by which O₃ induces AHR remain to be elucidated. In 2007, Williams et al. (22) demonstrated the important contribution of MAPK/JNK in O₃-induced inflammatory cell recruitment, gene expression, and AHR in mice, whereas Cho et al. (3) in 2008 implicated TNF-α receptors. Additionally, Garantziotis et al. (7) in 2009 reported hyaluronan as an integral mediator of AHR seen in O₃-exposed mice. Most recently, in 2015, Kasahara et al. (13) published their findings regarding Rho kinase’s (ROCK) mediation of AHR observed in allergic asthma. They reported that insufficiency of either ROCK1 or ROCK2 resulted in a reduction in AHR without having an effect on inflammation. The article reviewed herein (14) extends these findings and sheds additional light into the mechanism of O₃-induced AHR.

Two ROCK isoforms (ROCK1 and ROCK2) are presently identified. Both isoforms act as serine/threonine kinases, but they are thought to fulfill distinct roles due to their less homologous Rho binding domains (compared to their kinase domains). ROCK isoforms have been implicated in monocyte chemotaxis (18, 19) and fibronectin matrix assembly. Pertaining to the discussed article by Kasahara et al. (14), ROCK isoforms are also implicated in regulating smooth muscle contractility (20), as well as the allergic airway response in mice (26). In addition, both ROCK isoforms have been shown to stimulate proliferation of vascular smooth muscle cells (VSMC) through the ERK pathway. Furthermore, leptin activation of RhoA/ROCK is known to lead to the development of VSMC hypertrophy (8, 23, 24).

Kasahara et al. (14) utilized wild-type (WT), ROCK1⁺/⁺, and ROCK2⁺/⁺ haploinsufficient mice as well as the FDA-approved ROCK1/2 inhibitor fasudil (3 or 10 mg/kg) to determine ROCK’s influence on O₃-induced AHR. In this study, mice were exposed to O₃ at 2 ppm for 3 h then returned to room air. At 24 h postexposure, the investigators measured airflow resistance prior to and following challenge with methacholine (MCh). Following challenge, various indexes of inflammation were also measured in the bronchoalveolar lavage (BAL) and lung tissue. Their results showed that O₃-induced AHR occurs at least in part because of ROCK1 and ROCK2 activity. At 24 h post-O₃ exposure, there was a significant increased of the lung mechanic indexes, including Newtonian resistance, coefficient of lung tissue damping, coefficient of lung tissue elastance as well as lung resistance values in WT mice compared with their air control. These findings are consistent with previous observations and indicate the development of AHR (7). However, these indexes were significantly lower in ROCK1⁺/⁺ and ROCK2⁺/⁺ mice exposed to O₃. These results impressively showed that ROCK plays an important role in O₃-induced AHR. For the four parameters of lung mechanics analyzed, the authors found that the ROCK1⁻/⁻ and ROCK2⁻/⁻ mice exposed to O₃ had statistically different responses from O₃-exposed WT mice at the 30 mg/ml dose of MCh. However, it should be noted that no statistical significant difference was observed at any of the
lower doses of MCh or at the highest dose at 100 mg/ml. Since the sample numbers varied widely between groups (5–12), the lack or presence of significance may be reflective of unequal power in the comparisons, which could be remedied by equalizing the n per group. Alternatively, if differences persist after equalizing power of analysis, including additional MCh doses between 10 and 30 mg/ml as well as between 30 and 100 mg/ml would further elucidate a range of challenge in which ROCK insufficiencies attenuate AHR.

Following up on the ROCK1+/− and ROCK2+/− lung mechanic data, Kasahara et al. (14) utilized the ROCK1/ROCK2 inhibitor fasudil, which was administered intraperitoneally post-O₃ exposure (3 or 10 mg/kg, 30 min before flexivent measurement). Fasudil has been utilized extensively to elucidate ROCK’s roles in pulmonary hypertension and interactions with PPAR-γ, as well as in arterial endothelium of newborns (4, 8, 6). The authors observed similar lung mechanic data as that seen with the ROCK1+/− and ROCK2+/− mice with the exception that at the higher fasudil dose (10 mg/kg) there was a complete lack of response to all doses of MCh. These data are the highlights of the article, since, following acute O₃ exposure, Kasahara et al. put forth an easily mechanistic data, Kasahara et al. (14) utilized the ROCK1/ROCK2 activity following fasudil administration in WT mice, nor in the ROCK1+/− or ROCK2+/− mice, although these authors have shown previously that ROCK1+/− and ROCK2+/− mice have roughly 50% expression of the corresponding protein vs. WT type (26). Correlating protein levels and activity in the genetic models and in fasudil-treated mice with the functional effects would allow for further interpretation of the large differences in physiological response between the two approaches.

Previous reports have shown that O₃-induced AHR was influenced by IL-17A, TNF-α, and osteopontin (1, 3, 17). Pichavant et al. (17) claimed that repeated exposure of mice to O₃-induced AHR required the production of IL-17A. Kasahara et al.’s results do, in fact, demonstrate that IL-17A, TNF-α, and osteopontin are increased by exposure to O₃. However, all mice (i.e., WT, ROCK1+/−, and ROCK2+/−) exposed to O₃ had similar robust increases in IL-17A, TNF-α, and osteopontin.

In another study, Zhou et al. (25) reported increased pulmonary Grpr mRNA following O₃ exposure and concluded that GRPR signaling was required for O₃-induced AHR in WT mice. Kasahara et al. (14) noticed the same upregulation of Grpr mRNA by O₃ exposure. Again, no difference of Grpr exists between WT and ROCK1+/− and ROCK2+/− mice before and after exposure to O₃.

Although this was not spelled out in the discussion, the most plausible explanation to these findings is that ROCK isoforms act downstream of IL-17A, TNF-α, osteopontin, and Grpr in mediating AHR.

In addition to the aforementioned mediators, the authors’ data also revealed a significantly lower BAL hyaluronan level in O₃-exposed ROCK1+/− mice compared with O₃-exposed WT mice. Kasahara et al. (14) thus concluded that ROCK1 insufficiency attenuated AHR by lowering airway hyaluronan levels, which have been associated with the development of lung injury. This is an interesting development in the field since multiple investigators have found a role for hyaluronan in lung injury. For example, Garantziotis et al. (7) demonstrated that low-molecular-weight hyaluronan, formed by the fragmentation of high-molecular-weight hyaluronan by reactive intermediates, was necessary for the development of O₃-induced AHR. Most recently, Lazrak et al. (16) showed that low-molecular-weight hyaluronan was responsible for the depolarization of human airway smooth muscle cells and contributed to AHR in chlorine (Cl₂)-exposed mice. Lazrak et al. suggest that the depolarization caused Ca²⁺ influx and activation of RhoA and its downstream kinase (ROCK), which enhanced agonist-induced contraction via retention of myosin light chain in a phosphorylated state. Based on the data of Kasahara et al., the reduced AHR after O₃ exposure in ROCK1+/− mice was, at least, partially attributed to the lower level of hyaluronan, which was implied to be ROCK1 dependent. Interestingly, this is the first report to date that suggests the action of hyaluronan is downstream, rather than upstream of ROCK1. Therefore, the mechanism underlying this new finding warrants further study. On the other hand, Garantziotis and Lazrak also clearly showed that high-molecular-weight hyaluronan confers protection against O₃-specific as well as Cl₂-induced AHR. It should be noted that Kasahara et al. found no significant difference in BAL inflammatory cells and mediators.

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Fig. 1. Possible pathways by which Rho-associated coiled-coil forming kinase (ROCK) mediates ozone (O₃)-induced airway hyperresponsiveness (AHR). Following exposure to O₃, activated RhoA-GTP binds to and activates ROCK isoforms (ROCK1 and ROCK2). ROCK1 potentially mediates AHR via hyaluronan signaling following O₃ exposure. ROCK2 potentially mediates airway smooth muscle (ASM) contraction and subsequent AHR via phosphorylation of myosin light chain phosphatase (MLCP) independent of ROCK2’s mediation of O₃-induced lung injury. Solid black lines refer to previously established paths, dotted yellow lines refer to Kasahara et al.’s (14) proposed mechanisms by which each ROCK isoform is influenced by O₃ exposure. Although reducing ROCK2 increased airway injury, Kasahara et al. have proposed that ROCK2’s effect on airway injury is not the mechanism by which ROCK2 mediates the AHR.
ROCK2 expression (from lung homogenate). Thus they are the damage (increased BAL protein and epithelial cells) and inexperienced substantial pulmonary epithelium/endothelium production in ROCK1 variation that may be abrogated with a larger number of seemingly increase susceptibility to pulmonary inflammation.

Perspectives

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