Does UCP2 Couple Hyperoxia to Lung Injury?

First described for acute care treatment in 1885, supplemental inspired oxygen is routinely administered in critically ill patients to prevent or reverse hypoxemia. More recently, high levels of oxygen have been required in patients with severe cases of coronavirus disease (COVID-19). The obvious benefits of oxygen therapy, however, may be accompanied with complications when administered for prolonged periods at high concentrations. The untoward side effects of excessive oxygen administration include inflammatory cytokine production, atelectasis, coronary vasoconstriction, central nervous system toxicity, and several pulmonary disorders, including bronchopulmonary dysplasia in newborns, acute lung injury, and lung fibrosis (1). Recent data indicate an increase in mortality in patients experiencing hyperoxia in intensive care units (ICUs) (1, 2).

Oxygen toxicity is mediated through the production of reactive oxygen species (ROS), primarily via mitochondrial superoxide generation (3). Superoxide is produced in mitochondria by the donation of one electron to molecular oxygen owing to electron leakage from the electron transport chain during oxidative phosphorylation. UCPs (uncoupling proteins) are a family of mitochondrial inner membrane anion carrier proteins that are key players in regulating proton leak (4). UCPs uncouple electron transport from ATP synthase by leaking protons across the inner mitochondrial membrane back into the matrix. The mechanism by which UCP2 mediates ROS reduction and uncoupling function remain unclear; however, UCP2 is an attractive therapeutic target, especially in diseases in which excessive mitochondrial ROS generation mediates disease progression.

Although UCP2 expression has been described within the lung, its mechanistic role to exacerbate or protect against lung injury is incompletely understood (5). In this issue of the *Journal*, Raghavan and colleagues (pp. 323–336) reveal an interesting mechanism to explain the connection between superoxide generation and hyperoxia-mediated lung injury (6). The authors investigated the effects of hyperoxia-mediated reduction of UCP2 in mouse lung alveolar epithelial cells (MLE-12) and lungs from mice. The marked loss of mitochondrial UCP2 expression after exposure to hyperoxia (95% O_2) led to a robust increase in superoxide production and apoptosis in MLE-12 cells. Because UCP2 expression is known to be regulated by PGC-1α (PPAR-γ [peroxisome proliferator–activated receptor γ] coactivator) (7), the authors show that hyperoxia mediates the phosphorylation and nuclear localization of PGC-1α. Knowing that PGC-1α expression is increased in response to ROS and induces the production of antioxidant enzymes to reduce ROS levels (8), the authors demonstrate that the antioxidant protein Trx (thioredoxin) increases UCP2 expression by increasing the activation of PGC-1α and further increases its activity during hyperoxia. More importantly, Trx reduces hyperoxia-induced alveolar epithelial apoptosis. These results are interesting, especially in the context that Trx is not a superoxide scavenger (9).

Substantial *in vitro* data support their conclusion that hyperoxia mediates the phosphorylation and nuclear localization of PGC-1α, but the level of activation is not adequate to increase UCP2. Interestingly, treatment with recombinant human Trx during hyperoxia further enhanced PGC-1α activity to rescue UCP2 mitochondrial expression and reduce superoxide production. A lingering question pertains to the level of PGC-1α needed to increase UCP2. Particularly, the authors show that PGC-1α is required for UCP2 expression, as silencing PGC-1α inhibited UCP2 expression, and treatment with rhTrx did not rescue UCP2 mitochondrial expression. Although the precise phosphorylation site on PGC-1α was not determined, the authors established that Trx is needed for activation of p38 MAPK via MKK4 to mediate PGC-1α activation. Supporting their *in vitro* findings, the authors show that superoxide is increased in the lungs from hyperoxia-exposed (90% O_2, 48 hours) *UCP2*$^{-/-}$ mice compared with wild-type. The use of chemical uncouplers in hyperoxia-exposed wild-type and *UCP2*$^{-/-}$ mice helped establish that uncoupling protects against oxidative damage and potentially minimizes lung injury. Lending strong support for targeting UCP2 and its therapeutic potential, the authors use *Trx-Tg* (Trx transgenic) mice. Hyperoxia-exposed *Trx-Tg* mice showed a significant increase of UCP2 compared with normoxia-exposed (21% O_2) *Trx-Tg* mice, and the *Trx-Tg* mice maintained normal lung architecture, indicating protection against hyperoxia-mediated lung injury.

The study by Raghavan and colleagues provides new evidence into the mechanistic regulation of UCP2 in hyperoxia-induced lung injury, but many questions remained unanswered. Much debate remains regarding whether UCP2 functions as a bona fide uncoupler of mitochondrial electron transport from ATP synthesis. Although chemical uncouplers mediated a reduction in superoxide generation under hyperoxic conditions, it is unclear if ATP levels and mitochondrial membrane potential are similarly altered. This is particularly interesting because mild uncoupling has been suggested to protect against oxidative damage by decreasing mitochondrial membrane potential and oxygen availability to limit ROS generation (10). The authors focus on mitochondrial ROS driving hyperoxia-induced lung injury, but superoxide production may be from a source other than the mitochondria, such as the NADPH oxidases. Studies have confirmed the importance of mitochondrial- and nonmitochondrial-derived ROS in hyperoxia (11). This may have been more easily determined if the mice had expressed a conditional deletion of *UPC2* or overexpression of *Trx-Tg* in alveolar epithelial cells.

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These investigations raise exciting new possibilities for research directions. In addition to its uncoupling properties, UCP2 also regulates cellular bioenergetic processes, including fatty acid oxidation and glycolysis (4). Further exploration into the effects of UCP2 on cellular bioenergetics is warranted in hyperoxia exposure. Although UCP2 is ubiquitously expressed, it is the only UCP protein expressed within the lung (4, 12). The use of cell type–specific modifications of UCP2 may lead to further elucidation of the role UCP2 plays in governing hyperoxia-induced lung injury as well as its importance within alveolar epithelial cells. UCP2 may serve a protective role during normoxia, as the lungs are exposed to higher ambient concentrations of oxygen compared with other organ systems; however, hyperoxia mediates the downregulation of UCP2 and renders the lung susceptible to injury. Based on the morbidity and mortality that occurs with oxygen toxicity, this study provides compelling data in support of targeting the decrease of UCP2 by determining the mechanisms by which it is regulated during hyperoxia-induced lung injury. ■

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References

1. Chu DK, Kim LHY, Young PJ, Zamiri N, Almenawer SA, Jaeschke R, *et al.* Mortality and morbidity in acutely ill adults treated with liberal versus conservative oxygen therapy (IOTA): a systematic review and meta-analysis. *Lancet* 2018;391: 1693–1705.
2. Girardis M, Busani S, Damiani E, Donati A, Rinaldi L, Marudi A, *et al.* Effect of conservative vs conventional oxygen therapy on mortality among patients in an intensive care unit: the Oxygen-ICU Randomized Clinical Trial. *JAMA* 2016;316:1583–1589.
3. Freeman BA, Crapo JD. Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. *J Biol Chem* 1981;256: 10986–10992.
4. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab* 2005;2: 85–93.
5. Hough RF, Islam MN, Gusarova GA, Jin G, Das S, Bhattacharya J. Endothelial mitochondria determine rapid barrier failure in chemical lung injury. *JCI Insight* 2019;4:e124329.
6. Raghavan S, Kundumani-Sridharan V, Kumar S, White CW, Das KC. Thioredoxin prevents loss of UCP2 in hyperoxia via MKK4–p38 MAPK–PGC1α signaling and limits oxygen toxicity. *Am J Respir Cell Mol Biol* 2022;66:323–336.
7. Yoshihara E, Fujimoto S, Inagaki N, Okawa K, Masaki S, Yodoi J, *et al.* Disruption of TBP-2 ameliorates insulin sensitivity and secretion without affecting obesity. *Nat Commun* 2010;1:127.
8. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, *et al.* Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 2006;127: 397–408.
9. Das KC, Das CK. Thioredoxin, a singlet oxygen quencher and hydroxyl radical scavenger: redox independent functions. *Biochem Biophys Res Commun* 2000;277:443–447.
10. Cadenas S. Mitochondrial uncoupling, ROS generation and cardioprotection. *Biochim Biophys Acta Bioenerg* 2018;1859: 940–950.
11. Carnesecchi S, Deffert C, Pagano A, Garrido-Urbani S, Métrailler-Ruchonnet I, Schäppi M, *et al.* NADPH oxidase-1 plays a crucial role in hyperoxia-induced acute lung injury in mice. *Am J Respir Crit Care Med* 2009;180:972–981.
12. Pecqueur C, Alves-Guerra MC, Gelly C, Levi-Meyrueis C, Couplan E, Collins S, *et al.* Uncoupling protein 2, in vivo distribution, induction upon oxidative stress, and evidence for translational regulation. *J Biol Chem* 2001;276:8705–8712.