# Targeting AMPK and the Nrf2/HO-1 axis: a promising therapeutic strategy in acute lung injury

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**Therapeutic targeting of AMPK can improve acute lung injury *via* upregulation of the cytoprotective enzyme heme oxygenase-1** https://bit.ly/3DLf1xT

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Acute lung injury (ALI) is defined as pulmonary conditions leading to severe hypoxaemia, with human acute respiratory distress syndrome (ARDS) representing its most severe manifestation [1]. The primary causes of ARDS are trauma, bacterial and viral pneumonia, sepsis and adverse drug reactions [2]. The development of effective therapeutics for ALI/ARDS remains an urgent unmet need [3]. The overall crude incidence of ALI from all sources has been estimated at 76.9 per 100 000 person-years (86.2 after age adjustment) [4]; or ∼200 000 cases per year in the USA [1]. ALI is generally associated with endothelial and epithelial cell injury, loss of alveolar–capillary membrane integrity, neutrophil influx into the lung, and release of pro-inflammatory mediators [1]. Exposure to halogen gases, such as bromine ($Br_2$) and chlorine ($Cl_2$), as well as to halogenated organic compounds, can cause severe ALI. Although many experimental animal models of ALI have been developed [5], halogen gas-induced ALI has been less widely studied. Halogen gas-induced ALI/ARDS remains a public health concern as it can arise from industrial occupational and accidental exposures. Despite individual case reports, there is a lack of more precise epidemiological data on the incidence of halogen-gas specific ALI [6].

As reported in this issue of the *European Respiratory Journal*, Ahmad *et al.* [7], using a mouse model of $Br_2$-induced ALI, have identified 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an activator of 5′ AMP-activated protein kinase (AMPK), as a candidate therapy for ALI. When applied post-injury, AICAR improved lung histology, reduced $Br_2$-induced oedema, lung neutrophil influx, protein and cellularity of bronchioalveolar lavage (BAL) fluid and improved animal survival. AICAR treatment also restored phospho (p)-AMPK and p-liver kinase-B1 (LKB1)-levels in lung tissue, which were reduced by acute $Br_2$ exposure. These studies primarily concluded that AICAR treatment can reduce indices of ALI in mice after inhaled toxin exposure *via* stimulating AMPK (figure 1) [7].

In prior studies using a mouse model of LPS-induced lung injury, application of AICAR or the AMPK agonist metformin conferred lung protection *via* preserving endothelial barrier function. Corresponding *in vitro* assays identified AMPKα1 as the isoform responsible for endothelial cell protection [8]. Additional mechanistic studies revealed that application of AICAR protected against LPS-induced ALI *via* activation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) and superoxide dismutase 1 (SOD1) [9]. These results implicated *de novo* propagation of mitochondria *via* biogenesis, as well as activation of antioxidant defence, as possible mechanisms of AICAR action [9]. Intravenous application of AICAR was also shown to be effective at ameliorating intestinal ischaemia/reperfusion injury (IRI) in a rat model [10]. In this model, AICAR also prevented ALI secondary to intestinal IRI, by reducing lung apoptosis, neutrophil influx and production of pro-inflammatory cytokines and mediators [10]. Further, AICAR was also shown to be effective at reducing cisplatin-induced acute kidney injury in a rodent model, by inhibiting renal cell apoptosis which was attributed to activation of SOCS1 [11]. These results taken together support effective targeting of AMPK in animal models of organ injury. It should be noted that AMPK-independent effects of AICAR have also been described in a context-dependent manner [12].

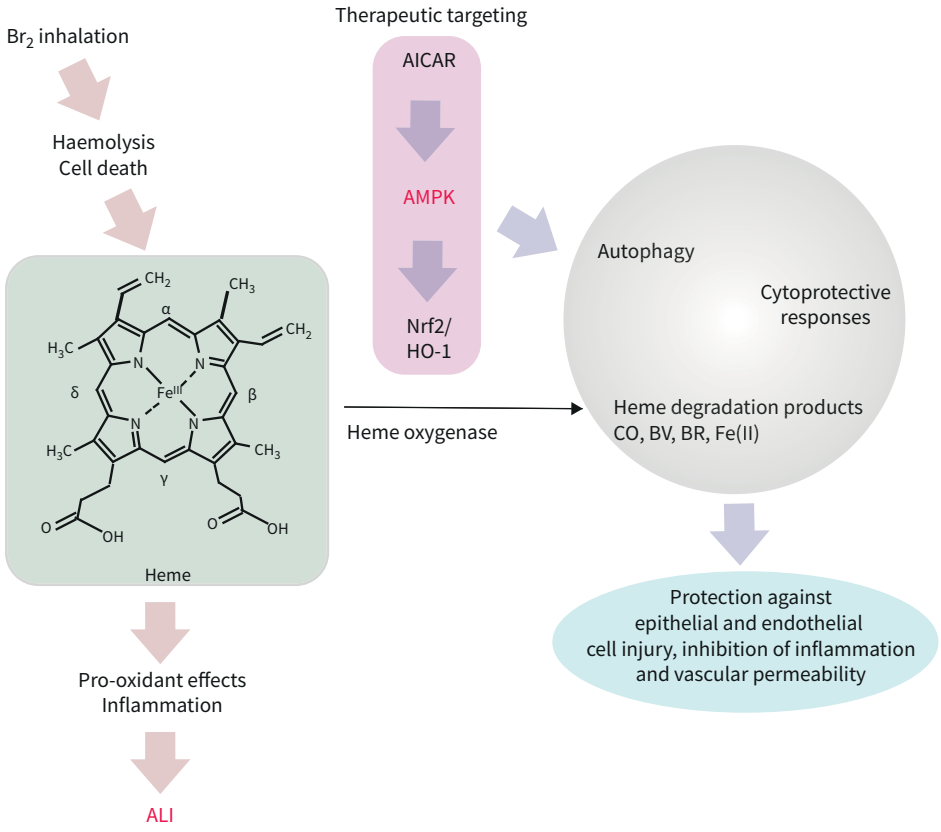


**FIGURE 1** AICAR ameliorates ALI *via* an AMPK/Nrf2/HO-1 axis. Ahmed *et al.* [7] propose the AMPK agonist AICAR as a therapy for ALI induced by halogen gas ($Br_2$) inhalation. $Br_2$ induces ALI *via* increased epithelial cell injury, by mechanisms potentially involving intravascular haemolysis, free heme accumulation, and associated pro-oxidant and pro-inflammatory effects. AICAR activated AMPK, leading the stimulation of HO-1 expression *via* Nrf2. Both autophagy and HO-1, regulated by AMPK, may contribute to cytoprotective pathways. HO-1 detoxifies heme *via* its enzymic removal, and by the generation of reaction products. The latter, namely, CO, BV, BR and Fe(II) may potentially contribute to cellular adaptive responses. AICAR: 5-aminoimidazole-4-carboxamide ribonucleotide; ALI: acute lung injury; AMPK: 5' AMP-activated protein kinase; BV: biliverdin; BR: bilirubin, CO: carbon monoxide; Nrf2: Nuclear factor E2-related factor 2; HO-1: heme oxygenase-1.

The authors of this meta-analysis cautioned against using AICAR, due to its potential off-target effects, to establish the role of AMPK in a particular signalling process, without further independent validation [12].

AMPK is associated with regulation of several vital cellular processes, including glucose transport and metabolism, fatty acid oxidation, regulation of lipogenesis and cholesterol biosynthesis, mitochondrial biogenesis, and autophagy [12–14]. AMPK regulates mitochondrial biogenesis, which is generally regarded as a protective process associated with repopulation of mitochondria, *via* PGC1α [15]. AMPK can also induce cellular (macro)-autophagy by several mechanisms, including the downregulation of the mammalian target of rapamycin complex 1 (mTORC1) *via* phosphorylation of the resident protein Raptor and phosphorylation of the upstream regulator tuberous sclerosis-2 (TSC2). Further, AMPK also inhibits autophagy by phosphorylating the mTORC1 substrate complex at unc-51-like kinase 1 (Ulk1/2) [16]. Upregulation of autophagy is generally associated with tissue protective effects through the lysosomal autodigestion of cellular components, including denatured proteins, lipids, and damaged organelles, the latter including mitochondria in a process called mitophagy [17].

In the current study, Ahmad *et al.* [7], using global *Hmox1* knockout mice as a model for heme oxygenase-1 (HO-1) deficiency, demonstrate that the mechanism of action of AICAR is largely dependent on HO-1 regulation [7]. *Hmox1* mice were also previously used to demonstrate a protective intermediate role for HO-1 in $Br_2$-induced ALI [18]. HO-1 represents the stress inducible isozyme of the heme

oxygenase enzyme system (EC: 1.14.14.18), which is responsive to a large spectrum of injurious agents, including oxidants and inflammatory mediators [19]. HO-1 transcriptional upregulation by various stimuli is largely regulated by nuclear factor E2-related factor-2 (Nrf2), a master regulator of the antioxidant response. Nrf2 is anchored in the cytoplasm by Keap1 and antagonised by the heme-binding protein Bach1 [20]. Mechanistic studies using knockdown of either Nrf2 or HO-1 confirmed that the protective effect conferred by AICAR in cultured lung cells was dependent on the Nrf2/HO-1 pathway [7].

HO-1 serves a vital metabolic function by catalysing the oxidative generation of heme to generate biliverdin-IXα (BV), which is converted to bilirubin-IXα (BR) by NAD(P)H biliverdin reductase [21]. HO-1 has been implicated as an anti-inflammatory cytoprotectant in rodent models of inflammatory injury [19]. The primary mechanism of action in these models, as well as models of sepsis [22] and malaria [23], is believed to be the removal of pro-oxidant heme. In a prior work by this investigative group, Aggarwal *et al.* [18] implicated free heme as a pro-pathogenic mediator of $Br_2$-induced ALI, which was found elevated in plasma and BAL fluid after $Br_2$ exposure, and which was shown to contribute to inflammation and pulmonary oedema formation in exposed mice. Heme is known as a pro-oxidant and proinflammatory effector molecule *via* iron-dependent catalysis and TLR-4-dependent pathways, respectively, as recently reviewed elsewhere [24]. Scavenging of heme by haemopexin, or transgenic overexpression of HO-1, were also shown to confer protection from $Br_2$-induced ALI in mice [18]. These results are consistent with a role for HO-1 in detoxifying heme in the context of inflammatory lung disease [25].

In addition to heme removal, HO-1 can generate cytoprotective intermediates through heme catabolism, including the generation of BV and BR, compounds with reputed systemic antioxidant properties, as well as by enhanced expression of the cytoprotectant ferritin *via* release of labile iron [24]. Carbon monoxide (CO), a byproduct of the HO-1 reaction, has been widely investigated as a potential anti-inflammatory agent and therapeutic modulator [19]. For example, exogenously applied CO has been shown to confer anti-inflammatory protection in rodent models of LPS- and hyperoxia-induced ALI, and in a baboon model of pneumonia-induced ALI [26–28]. The protective effects of HO-1 have also been associated with context-dependent non-canonical or enzyme-activity independent effects, as reviewed elsewhere [29]. Although not explored in the current study, further investigation of the role of endogenous CO production in the therapeutic effects of AICAR are warranted.

A relationship between AMPK activation and HO-1 induction has been previously delineated in endothelial cell injury. Liu *et al.* [30] demonstrated that AICAR treatment induced HO-1 *via* activation of Nrf2 in endothelial cells, and that HO-1 could confer protection against cytokine-mediated apoptosis. HO-1 activation could also be effectuated by other pharmacological activators of AMPK and ectopic overexpression of AMPKα1. The HO-1 activating potential of AICAR was also shown after *in vivo* application and demonstrated in rat carotid arteries [30]. It should be noted that compound C, an AMPK inhibitor, was also able to induce Nrf2-dependent HO-1 induction in endothelial cells. These observations suggest that care should be taken when interpreting findings using pharmacological agents alone, and that HO-1 is also potentially regulated by NRF2 *via* AMPK-independent pathways [31].

Previously, relationships have been proposed between HO-1 induction and the regulation of metabolism. For example, localisation of HO-1 to mitochondria has been suggested to contribute to stress mitigation and potentially influence mitochondrial bioenergetics [32]. HO-1 was found to be co-regulated with autophagy to provide protection in a model of acute liver injury *in vivo* and *in vitro* [33]. Similar observations were reported in LPS-stimulated macrophages, whereby HO-1 was responsible in part for triggering autophagy signalling [34]. Although autophagy was not directly studied as a mechanism of action in the current featured article [7], these observations place HO-1 in the context of a larger integrated stress response. Finally, HO-1 regulation has been proposed as a mechanism of action for the protective effects of pharmacological agents, including many natural antioxidants [35]. In summary the AMPK/mTOR and Nrf2/HO-1 pathways are implicated in protective responses against the development of ALI. These pathways and their downstream effectors may be further exploited for therapeutic targeting in various forms of ALI, including those caused by inhaled gases.

Conflict of interest: S.W. Ryter declares no conflict of interest.

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