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Editorial Focus

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On:

"Heme Oxygenase-1 Mitigates Ferroptosis in Renal Proximal Tubule Cells"

Heavy Metal Suicide

Andreas Linkermann[1], Brent Stockwell[2] and Tom Vanden Berghe[3,4]

[1]Division of Nephrology, Department of Internal Medicine III, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany
[2] Department of Chemistry, Columbia University, New York, NY 10027, USA
[3] VIB Inflammation Research Center, Ghent, Belgium.
[4]Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium.

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Correspondence:
PD Dr. med. Andreas Linkermann, FASN
Division of Nephrology - Department of Internal Medicine III
University Hospital Carl Gustav Carus at Technische Universität Dresden
Fetscherstr. 74, 01307 Dresden, Germany
Phone: +49-351-458-4233
Fax: +49-351-458-5333
Email: andreas.linkermann@ukdd.de
web: https://www.uniklinikum-dresden.de/

"…wheel of fortune, Sally Ride, heavy metal suicide…"

Billy Joel in "We didn´t start the fire" – September 1989

In acute kidney injury (AKI), the majority of dying tubular cells succumbs to an iron-dependent form of regulated necrosis, referred to as ferroptosis. Ferroptosis is essentially mediated by iron-catalyzed lipid peroxidation upon GPX4 dysfunction. Heme oxygenase 1 (HO-1) is a master regulator of intracellular free iron due to the conversion of heme to iron, carbon monoxide, and biliverdin(12), and therefore represents a potential regulator of ferroptotic cell death. In this issue of AJP - Renal Physiology, Adedoyin et al. demonstrate that the lack of HO-1 sensitizes renal tubular cells to ferroptosis(1).

Agarwal and colleagues incubated primary renal proximal tubular cells (PTCs) with different concentrations of the class I ferroptosis-inducing compound (FIN) erastin, or the class II FIN RSL3, which directly targets and inactivates glutathion-peroxidase 4 (GPX4)(16). Whereas 10µM RSL3 clearly induced profound ferroptosis within 16 hours, approximately 35% of PTCs died following treatment with 1µM erastin. Cleavage of capsase-3 remained absent over the investigated time of the experiment, ruling out the involvement of apoptotic cell death. Instead, the iron chelator desferoxamine (DFO), N-acetylcysteine (NAC) or the ferrostatin (Fer-1) were effective in preventing cell death, which underscores that both FINs induced ferroptosis in PTCs. Interestingly, both FINs upregulated the expression of HO-1 during ferroptotic cell death, which was reduced upon treatment with the ferroptosis inhibitors DFO, NAC and Fer-1. Functional characterization of HO-1 knockout PTCs also revealed an increased sensitivity to ferroptosis, suggesting a protective role of HO-1 in ferroptosis. In line with these data, it was previously demonstrated that genetic absence of HO-1 sensitizes mice to cisplatin-induced AKI, and that the induction of HO-1 expression by addition of the molecule hemin to human renal tubules reduced necrotic damage(13). Also in hepatocellular carcinoma cells, a protective role for the NRF2-HO-1-pathway was observed in response to erastin- and sorafenib-induced ferroptosis(14).

The extraordinary role of ferroptosis in renal tubules is highlighted by three hallmark observations. Firstly, inducible deletion of GPX4 in renal tubules results in massive tubular necrosis that is prevented by the ferroptosis inhibitor liproxstatin-1(3). Secondly, all renal clear cell carcinoma cell lines in the NCI tumor panel were sensitive to RSL3-mediated ferroptosis(16). Lastly, prevention of ferroptosis in murine models of acute kidney injury resulted in remarkable preservation of renal function, even upon otherwise lethal ischemic damage(9). In contrast to other clinically relevant forms of regulated necrosis, such as necroptosis or pyroptosis, ferroptosis exhibits the only known cell death pathway that is capable of inducing synchronized regulated necrosis (SRN) in functional units of

interconnected cells in a non-cell autonomous manner(8). Importantly, this effect does not appear to be limited to renal tubules. Recently, cell culture models have been employed to detect similar effects that are mediated by ferroptosis(6). With this growing mechanistic understanding of ferroptosis and its regulation, novel methodological approaches may allow more detailed research. In this sense, ACSL4 was recently demonstrated to mediate ferroptotic cell death(2, 5), providing the opportunity to generate tubular cell specific knockout of ACSL4.

Several obstacles remain related to the mechanisms of ferroptosis during acute kidney injury. One major question to address is the measurement of free or labile iron inside the cellular cytoplasm. This is of particular importance because the reaction that is catalysed by HO-1 releases free iron (Fe^{2+}) from heme and should increase the labile iron pool (LIP), which was postulated to contribute to the cytotoxicity already in 1999(15) More recently, this idea was further supported by the finding that HO-1 is accelerating erastin-induced ferroptosis in fibroblasts(7). This cytotoxic versus cytoprotective action of HO-1 might be related to the iron buffering capacity of the cells (such as ferritin) or the expression levels of the iron export protein ferroportin. Should this capacity be interfered with, e.g. by genetic targeting, tubular cells could no longer keep control about their free iron content, which may result in detrimental necrosis and systemic inflammation. Therefore, the results presented in the current work, together with previously published knowledge about HO-1 biology(11), suggest a faint and tightly regulated balance between ferritin, free iron and HO-1 activity. It will be important to investigate these balances in more detail to understand ferroptotic tubular necrosis and its inevitably associated inflammatory response – not only for AKI, but also for kidney transplantation and possibly ischemic injury in many other organ systems. In addition, the work by Adedoyin et al. provides an important *in vitro* link between HO-1 and ferroptosis. *In vivo*, HO-1 therefore should control local necroinflammation(10) and indirectly regulate systemic inflammation and distant organ effects in AKI(4).

"*We didn´t start the fire*".

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