ACUTE KIDNEY INJURY

Crystals spoil mitochondrial potential

The accumulation of oxalate crystals in the nephron as a result of acute oxalosis is known to trigger renal necroinflammation and cause acute kidney injury (AKI). Now, Shrikant R. Mulay, Hans-Joachim Anders and colleagues show that mitochondrial permeability transition contributes to renal cell necrosis caused by oxalate crystal deposition.

"Our previous work identified mixed lineage kinase domain-like protein (MLKL)-driven necroptosis as a molecular mechanism underlying crystal-induced cytotoxicity in a mouse model of acute oxalate-induced AKI," explains Anders. "However, the inhibition or knockdown of receptor-interacting serine–threonine-protein kinase 3 (RIPK3) or MLKL, key regulators of necroptosis, only partially protected mice from crystal-induced cytotoxicity, suggesting that additional pathways of regulated necrosis were involved," adds Mulay.

In their new study, the researchers report that exposure of L929 cells to calcium oxalate (CaOx) crystals in vitro induced mitochondrial swelling, loss of cristae and loss of mitochondrial outer membrane potential (MOMP). These effects were accompanied by the release of reactive oxygen species (ROS) and necrosis. Cyclosporin A, a drug known to inhibit mitochondrial permeability transition (MPT) by binding to cyclophilin, reversed the loss of membrane potential, in addition to inhibiting the release of ROS and necrosis. By contrast, the mitochondrial ROS inhibitor APDC prevented cell necrosis but not the loss of MOMP, suggesting that the loss of membrane potential precedes ROS release and necrosis.

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MPT-regulated cell death is characterized by the formation of an MPT pore that leads to swelling of mitochondria and necrosis. The PPIaseF isomerase (encoded by *Ppif*) is a key component of the MPT pore. Using their in vitro model of exposure to CaOX, the researchers demonstrated that when PPIaseF expression is suppressed, either in L929 cells transfected with *Ppif*-specific small interfering RNA or in primary tubular epithelial cells from PPIaseF-deficient mice, cells are protected from CaOx-induced necrosis.

Previous studies had shown that phagocytosis of CaOx crystals caused lysosomal disruption and the release of lysosomal proteases into the cytosol. Now, using confocal microscopy, the researchers were not only able to detect phagocytosed crystals within L929 cells incubated with CaOx but also a reduction in tetramethylrhodamine, ethyl ester (TMRE) staining compared with controls, which suggested mitochondrial depolarization. Inhibiting phagocytosis with cytochalasin D partially protected these cells from loss of MOMP and its downstream effects — ROS production and necrosis.

To investigate renal exposure to oxalate crystals in vivo, the researchers used a mouse model of hyperoxaluria-related AKI (induced by a single intraperitoneal injection of sodium oxalate). Serial block face-scanning electron microscopy confirmed the formation of crystal plugs within proximal and distal tubules, which were associated with mitochondrial ballooning and loss of inner membrane cristae. These signs of mitochondrial dysfunction were frequently observed in necrotic cells. Despite an identical accumulation of CaOx crystals, plasma creatinine levels and tubular necrosis were significantly lower in PPIaseF-deficient mice than in wild-type. "Our findings demonstrate the role of MPT-driven tubular cell necrosis in acute oxalosis," concludes Anders.

The researchers then compared mice that were either deficient for PPIaseF, MLKL or both to explore the relationship between necroptosis and MPT-induced necrosis in their in vivo model. All knockout mice exhibited some degree of protection from kidney injury, especially double knockouts. Moreover, in wild-type mice injected with sodium oxalate, treatment with cyclosporine A or necrostatin-1s (a necroptosis inhibitor) was renoprotective, but TUNEL staining and plasma creatinine levels were lowest in mice treated with both drugs. "Our results suggest that crystal-induced MPT and necroptosis occur in tandem and promote crystalline nephropathy," explains Mulay.

"Our study demonstrates that a broad range of crystalline particles induce necroinflammation through similar molecular mechanisms," remarks Mulay. "These newly discovered molecular pathways may qualify as therapeutic targets for the type 2 (tubular) forms of crystal nephropathies," adds Anders.

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