Unraveling the ceramide-calpain-caspase connection in cadmium-induced apoptosis: a novel role for ceramides as activators of calpains. Focus on “Cadmium-induced ceramide formation triggers calpain-dependent apoptosis in cultured kidney proximal tubule cells”

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Cadmium (Cd\(^{2+}\)) is a nonessential heavy metal that is known as an important industrial and environmental pollutant. In industrialized countries the Cd\(^{2+}\) burden on the environment has generally declined, once it became clear that Cd\(^{2+}\) represents a dangerous health hazard. This reduction of Cd\(^{2+}\) pollution occurred partly because industry renounced the use of Cd\(^{2+}\) for production but also because the Cd\(^{2+}\) manufacturing industry was transferred to emerging industrialized countries. In fact, globally Cd\(^{2+}\) pollution has increased, and in countries such as China and India Cd\(^{2+}\) pollution has grown to be a significant ecological problem.

Because Cd\(^{2+}\) has a long biological half-life (10–25 yr) and a low rate of excretion, the body becomes a “sink,” and Cd\(^{2+}\) accumulates until a threshold is reached and causes toxicity to many vital organs including the lungs, liver, and kidneys. The S1 segment of the proximal tubules is particularly sensitive to Cd\(^{2+}\) toxic effects, because in this part of the nephron transport and receptor systems for both free and bound Cd\(^{2+}\) are present (2, 8). For this reason, nephrotoxicity induced by Cd\(^{2+}\) may result in a general transport defect of the proximal tubules, which is manifested by proteinuria, aminoaciduria, glucosuria, and phosphaturia and mimics the De Toni-Debré-Fanconi syndrome. Human studies indicate that 7% of the Cd\(^{2+}\)-exposed population have renal dysfunction from Cd\(^{2+}\) exposure (3). The importance of an exact understanding of the cellular mechanisms involved in Cd\(^{2+}\) toxicity is therefore evident. Cd\(^{2+}\) modulates gene expression and signal transduction and reduces activities of proteins involved in antioxidant defenses (1). The cellular processes responsible for the development of nephrotoxicity culminate in triggering of cell death by either apoptosis or necrosis (7). Short exposure (3–6 h) to Cd\(^{2+}\) induces apoptotic cell death mediated by the Ca\(^{2+}\)-dependent proteases calpains (4). This pathway is distinct from longer (12–24 h) exposures to Cd\(^{2+}\), where mitochondrial damage results, followed by release of proapoptotic factors and thus leading to activation of caspase proteases to induce cell death (4). A further aspect of cell death signaling is the cross talk between calpains and caspases. It has been shown that calpains have the capacity to activate the executioner caspase, caspase-3, in a concerted effort to induce a “more efficient” apoptotic cell death (4).

In the recent paper entitled “Cadmium-induced ceramide formation triggers calpain-dependent apoptosis in cultured kidney proximal tubule cells” (6), Lee and colleagues describe the upstream processes underlying calpain-induced apoptosis caused by Cd\(^{2+}\). These are the most recent data that add to the ever-increasing knowledge about Cd\(^{2+}\)-induced cell death pathways, which has been substantially generated through previous work by this group (4, 5, 7). Even though ceramides have long been known to be involved in apoptotic signaling pathways, Lee et al. (6) show for the first time that exposure of kidney proximal tubular cells to low micromolar Cd\(^{2+}\) concentrations induces ceramide generation already after 3 h. Inhibition of ceramide synthase by fumonisin B\(_1\) abolished Cd\(^{2+}\)-induced ceramide formation after 3 h and also apoptosis after 3–6 h. According to these data, ceramide formation is attributed to a de novo synthesis pathway rather than to hydrolysis of the plasma membrane sphingomyelin. Since apoptosis at these time points involves calpain activation (4), the authors have investigated the hypothesis of whether ceramide formation and calpain activation are somehow connected. Indeed, it could be demonstrated that ceramide formation is a prerequisite for active calpains. But how do increased ceramide levels induce calpain activation? This question was answered through live Ca\(^{2+}\) imaging to monitor cytosolic Ca\(^{2+}\) concentration. Because of the Ca\(^{2+}\) dependence of calpains for activation, an increase in cytosolic Ca\(^{2+}\) was expected. By applying exogenous cell-permeant C\(_6\)-ceramide to fura-2-loaded cells, Lee et al. could show that cytosolic Ca\(^{2+}\) rapidly increases after application of C\(_6\)-ceramide, suggesting that ceramide stimulates calpain activity by increasing cytosolic Ca\(^{2+}\).

A further interesting observation, as well as corroboration of the ceramide-calpain-caspase connection, came from a series of experiments using C\(_6\)-ceramide. Maximal cell death by C\(_6\)-ceramide was found to be significantly different after 24-h exposure compared with exposure for 3–6 h. The authors hypothesized the involvement of caspases since they were found to be activated by Cd\(^{2+}\) after 24 h only (4). In fact, the presence of a caspase-3 inhibitor, z-DEVd-fmk, attenuated C\(_6\)-ceramide-induced cell death at 24 h, demonstrating partial caspase dependence in the propagation of cell death by ceramide.

To summarize, an early apoptotic signaling pathway induced by Cd\(^{2+}\) can be linked to late-onset signals by calpain activation.

This paper greatly contributes to the understanding of the apoptotic signaling pathways induced by Cd\(^{2+}\), explaining the early events implicated in apoptotic cell death and putting them in relation to late-onset events. Considering the growing exposure of humans to Cd\(^{2+}\), this issue is of special importance, in view of possible therapeutic intervention to treat Cd\(^{2+}\) poisoning by blocking early stages of apoptosis.
REFERENCES