Ultrastuctural investigation of lysosomal rupture, necroapoptotic interactions in neurons shortly after focal ischemia

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Several lines of evidence suggest that lysosomal rupture takes place within hours following focal ischemia [1]. However, its relation to apoptotic mechanisms in ischemic cells has not been investigated, although the independent role of each pathway in ischemic cell death has been studied by several groups, and a crosstalk between the apoptotic pathways and cathepsin-B has also been proposed [2, 3, 4, 5, 6]. The reported data suggesting a cross-talk between the two systems were drived from the whole brain tissue but not at cellular level. Therefore, in this study, we also assessed the potential for interactions between these pathways at the cellular level by evaluating the intracellular co-localization of their markers. We chose early time points (1, 4 and 12h) after 1h of MCA occlusion because, in this mild model of focal ischemia, neurons do not rapidly degenerate and provide a window of opportunity to assess the interactions between cysteine proteases.

Ischemic cell-death is a complex process and the initial distinction between apoptosis and necrosis appears to be an oversimplification. We previously reported that in ischemic neurons with disrupted plasmalemma, apoptotic mechanisms were also active. In the present study, we investigated cellular colocalization of another necrotic mechanism, lysosomal rupture, with apoptotic markers in the mouse brain and assessed the potential interactions between cysteine proteases.

The lysosomal enzyme, cathepsin-B, and the inner membrane protein LAMP-1 were spilled into the cytoplasm 1-4h after ischemia/reperfusion, suggesting that lysosomal membrane integrity was rapidly lost, as occurs in necrosis. The same neurons also exhibited caspase-3 and Bid cleavage, and cytochrome-c release. Electron microscopy and capsase-3p20 immunogold labeling confirmed these findings [Fig.1]. Caspase-3 activity preceeded cathepsin-B leakage in most neurons, and declined by 12h, while lysosomal leakage continued to increase. Moreover, within the same cell, cytoplasmic cathepsin-B immunoreactivity only partially overlapped with caspase-3 and Bid cleavage. These data suggest that necrotic and apoptotic mechanisms may act both in concert as well as independently within the same cell, beginning at the onset of ischemia, to ensure the demise of damaged neurons. Combined inhibition of cysteine proteases might abrogate potential shifts between alternative death pathways and improve the success of stroke treatments.

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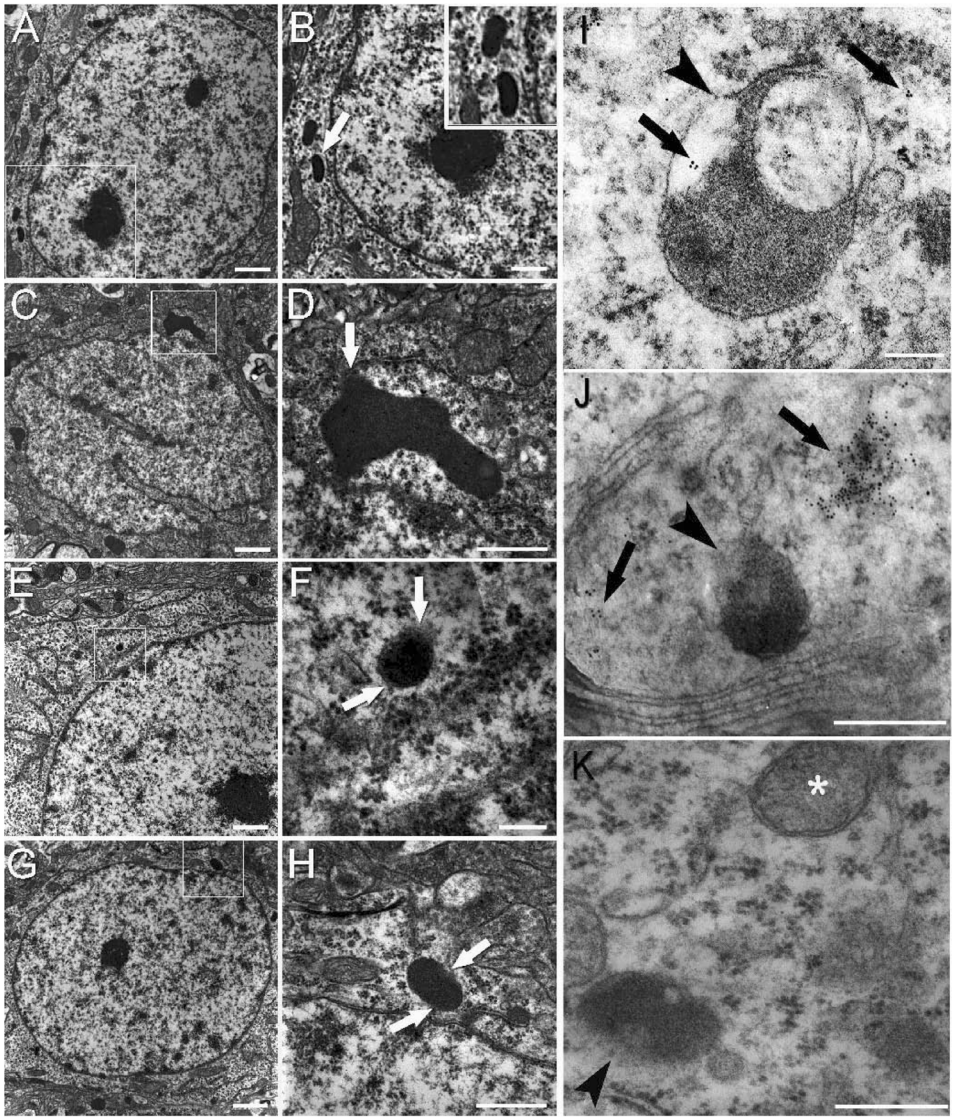

Figure 1. Electron microscopy showed that lysosomes lost their membrane integrity in the perifocal (C) and core (E and G) areas after 1-hour ischemia and 4 hours of reperfusion. A and the enlarged image of the boxed area (B) illustrate 2 intact lysosomes (arrow, see the inset for higher magnification) in a neuron from non-ischemic hemisphere. D, F & H are enlarged images of the boxed areas in C, E & G, illustrating the lysosomes that lost their membrane integrity in 3 separate cortical neurons (identified by the abundance of polyribosomes). In D, the electron-dense lysosomal content bulged into the surrounding cytoplasm after rupture of lysosomal membrane (arrow), whereas lysosomes in F and H exhibit membrane discontinuity and limited leakage of the lysosomal content into the cytoplasm (arrows). They illustrate the presence of caspase-3 activity (arrows mark immunogold particles labeling caspase-3-p20) in the vicinity of lysosomes that have lost their membrane integrity (arrowheads). K is a negative control section not incubated with immunogold particles, illustrating an ischemic neuron with a degenerating lysosome (arrowhead) and mitochondrion (*). Scale = 10 μm for A, C, E, G; 5 μm for A, B, F, I, J and K; 2 μm for D

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