Oxidative damage in reperfusion after stroke: ferroptosis and the role of the mitochondrial sodium/calcium exchanger NCLX

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Ferroptosis is a form of regulated cell death driven by iron-dependent accumulation of lipid hydroperoxides that cause membrane damage. It plays a role in pathological mechanisms such as ischemic cerebral stroke, where brain iron concentration and lipid peroxides levels are increased.

Lipid peroxidation can be initiated by reactive oxygen species (ROS), which are produced by the mitochondria during hypoxia - reoxygenation. The mitochondrial sodium/calcium exchanger NCLX is involved in this process, since its activation during acute hypoxia drives superoxide production at complex III. The inhibition of Na+ import through NCLX is enough to block this pathway and inhibit ROS production during hypoxia.

Our hypothesis is that NCLX could participate in cell death by ferroptosis, playing a role in lipid peroxidation and iron metabolism in ischemic stroke.

We used SK-N-DZ human neuroblastoma cells and subjected them to 2 hours of oxygen-glucose deprivation, followed by 24 hours of reperfusion with or without treatment with NCLX inhibitors, and we measured several ferroptosis hallmarks. We determined lipid peroxidation by labelling cells with Bodipy 581/591 C11, a lipophilic fluorescent ratio-probe used for indexing levels of lipid peroxides. We measured ROS levels using the fluorogenic dye Dihydroethidium. We also assessed levels of different proteins related to ferroptosis, such as GPx4 and transferrin receptor via western blot. Lastly, we quantified mRNA levels of genes involved in ferroptosis and iron metabolism, such as transferrin receptor, ferritin, IRP-1 and IRP-2.

We observed that lipid peroxidation and ROS levels decrease when using NCLX inhibitors. Moreover, NCLX seems to participate in the regulation of the expression of genes involved in iron metabolism, since iron-related protein and mRNA levels change when treating the cells with NCLX inhibitors during reperfusion.

Overall, our results suggest that NCLX plays a role in ferroptosis after ischemia-reperfusion.