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Mass spectrometry analysis of cardiolipin profile after experimental ischemic stroke

Background: Ischemic stroke is the most common form of stroke, accounting for 1 in 6 cardiovascular deaths in the United States. Ischemia-reperfusion injury is associated with oxidative damage to numerous cellular organelles, with the mitochondria being particularly vulnerable to oxidation by reactive oxygen species. Cardiolipin (CL), a tetra-acylated phospholipid, is located in the inner mitochondrial membrane, functioning as a stabilizer for the respiratory chain. CL is moved to the outer mitochondrial membrane following mitochondrial destabilization, where it induces cell death and has also been shown to be critical modulators of inflammasome activation. We investigated whether ischemic stroke altered the CL lipidome in the ischemic core and ischemic penumbra regions of the brain and tracked these alterations over several days after stroke.

Methods: Male Sprague Dawley rats (280-300g) were subjected to 2h middle cerebral artery occlusion (MCAo) using the intraluminal filament model. Composite neurological battery (normal score=0, maximal deficit=12) was performed 60 minutes after the onset of MCAo to confirm occlusion and on days 1, 2, 3, or 5. Brains were removed 1 (n=4), 3 (n=3), or 5 (n=4) days after MCAo, divided into right and left hemispheres, and the penumbra (cortex) and core (subcortex) were dissected and stored at -80°C for lipidomic analysis. Lipids were extracted according to the Folch method, and the total lipid content was analyzed by liquid chromatography-mass spectrometry (LC-MS, instrument: Xevo G2-XS QTof).

Results: Total neurological scores showed deficit 60 minutes after MCAo (10.8±0.3) with consistent deficits from 1-5 days after MCAo (1 day: 9.5±0.5, 3 day: 9.3±0.5, 5 day: 9.3±0.5). LC-MS operating in negative ion electrospray ionization detected stereotypical cardiolipin peaks between 1400 – 1650 m/z that matched previously published literature. Several CL species showed notably different shifts in percentage composition between the ipsilateral and contralateral cortex and subcortex. Specific CL changes include: decreased 1520 m/z (mass/charge ratio; 78:14/76:0, acyl chain # carbon: # double bonds) in the ipsilateral cortex (1 day post MCAo) and subcortex (3 days post MCAo); increased 1552 m/z (80:12) in the ipsilateral cortex and subcortex (5 days post MCAo), and alterations in 1470 m/z (74:11), 1492 m/z (76:14/74:0), 1504 m/z (76:8), 1526 m/z (78:11), 1548 m/z (80:14/78:0), 1576 m/z (82:14/80:0) between the ipsilateral and contralateral cortex/subcortex after MCAo.

Conclusions: We have identified several cardiolipin species altered in regions of the brain affected by transient ischemia. We are currently developing an MS/MS method based on fragmentation analysis of the parent ion to characterize the acyl chains on the CL species of interest. Fatty acyl chains such as docosahexaenoic acid found on cardiolipin may be highly susceptible to oxidation, while arachidonic acid may participate in inflammatory signaling. Profiling of cardiolipin oxidation or other alterations may further elucidate the molecular changes in cerebral ischemia and uncover novel targets for therapeutic intervention using beneficial lipid mediators.