STROKE

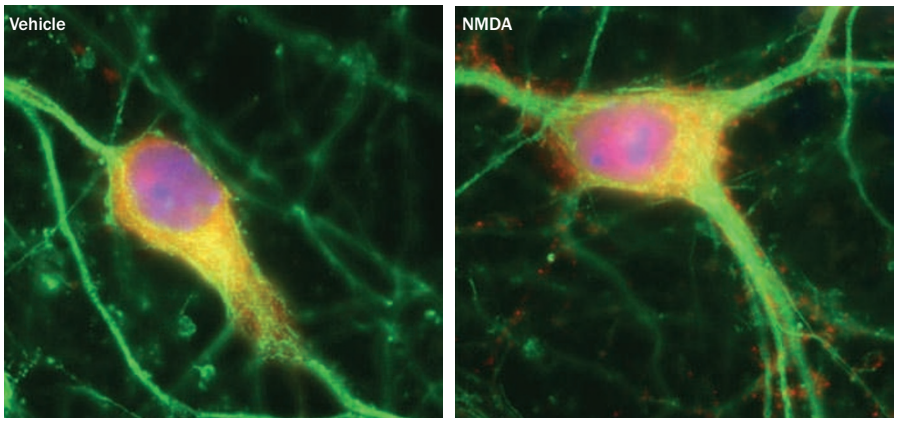
Disruption of the nNOS–PSD-95 complex is neuroprotective in models of cerebral ischemia

A study published in *Nature Medicine* shows that disruption of the interaction between neuronal nitric oxide synthase (nNOS) and postsynaptic density protein-95 (PSD-95)—a scaffolding protein that also binds to *N*-methyl-D-aspartate receptors (NMDARs)—can prevent cerebral ischemic damage in rodents. ZL006, a drug developed in the investigation to inhibit the formation of the nNOS–PSD-95 complex, could be a potential treatment for stroke, suggest the study's researchers.

Stroke causes disability and, in severe cases, death, and is a global public-health problem. Stroke-related ischemia can lead to excessive activation of NMDARs and nNOS and, in turn, excitotoxicity, which is the main cause of neuronal cell death in this condition. Selective NMDAR or nNOS antagonists have the potential to ameliorate stroke-related neuronal damage. These proteins both have important physiological functions, however, and drugs that block NMDAR or nNOS activity are associated with severe adverse effects.

Activation of nNOS, which exists in both soluble and particulate forms, is dependent on the interaction of the enzyme with PSD-95 (probably in a membrane-bound macromolecular complex) and NMDAR-mediated calcium influx. Ischemia substantially reduces the solubility of nNOS, indicating that under such conditions, this enzyme exists predominantly in insoluble protein complexes. These findings prompted Zhou and colleagues to examine whether ischemia-induced neuronal cell death results from NMDAR-mediated translocation of nNOS—through the interaction of nNOS with PSD-95—from the cytosol to the cell membrane.

The researchers conducted a series of *in vitro* and *in vivo* experiments to investigate this hypothesis. Coimmunoprecipitation experiments performed using cortices from mice that were subjected to middle cerebral artery occlusion (MCAO) and reperfusion showed that ischemia caused a substantial increase in the levels of the nNOS–PSD-95 complex. Levels of this complex also rose when cultured neurons were treated with glutamate and glycine, indicating that NMDAR activation might cause nNOS and PSD-95 to interact.



NMDA treatment enhances nitric oxide synthase immunofluorescence (red) in the cell membrane of cultured mouse neurons. Image provided by Prof. Dong-Ya Zhu.

Cultured neurons infected with a lentiviral vector selectively overexpressing the amino terminus (amino acid residues 1–133) of nNOS showed a substantial reduction in the amount of nNOS–PSD-95 formed following glutamate treatment. Moreover, "in mice subjected to MCAO and reperfusion, infusion of the lentiviral vector into the cortex significantly decreased the infarct size and neurological deficits," explains senior author Dong-Ya Zhu. Indeed, among MCAO animals, those receiving the nNOS lentiviral vector also had a lower mortality rate than those receiving a control vector.

The researchers synthesized a series of compounds that could potentially disrupt the interaction between nNOS and PSD-95, and tests confirmed that several of these agents ameliorated glutamate-induced excitotoxicity *in vitro*. Of these compounds, ZL006—5-(3,5-dichloro-2-hydroxybenzylamino)-2-hydroxybenzoic acid—was the most effective agent for preventing excitotoxicity.

In mice subjected to MCAO and reperfusion, pretreatment with ZL006 attenuated the ischemia-induced increase in nNOS–PSD-95 complex levels, and in neurons treated with glutamate this drug ameliorated NMDAR-dependent nitric oxide synthesis. Furthermore, in MCAO mice, ZL006 treatment decreased infarct size, neurological deficits and mortality in a dose-dependent manner compared with vehicle. Of note, the beneficial effects of ZL006 were evident during a 3 h postreperfusion therapeutic time window.

"Interestingly, ZL006 did not inhibit NMDAR function or the catalytic activity of nNOS, or affect spatial memory or aggressive behaviors, which are both known to occur as a result of nNOS inhibition," says Zhu. Thus, according to the researchers, ZL006 might represent an effective novel stroke treatment that is not associated with major adverse effects.

Nick Jones

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