Journal Club

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Molecular Cross Talk in Traumatic Brain Injury

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Review of Lau et al. (http://www.jneurosci.org/cgi/content/full/26/45/11540)

Peroxynitrite (PN), a product of the near-diffusion-limited reaction between nitric oxide (NO) and mitochondrially derived superoxide (O_2^-), is a potent and versatile oxidant produced after brain trauma. PN can cause lipid peroxidation, DNA fragmentation, and protein nitration (Hall et al., 2004). Although the precise targets of posttranslational PN-mediated nitration and oxidation have not been fully elucidated, the activity of this radical contributes to the susceptibility of neurons to secondary injury posttrauma (Arundine and Tymianski, 2004). Recent work from Lau et al. (2006) in *The Journal of Neuroscience* reports novel, trauma-induced cross talk between peroxynitrite and caspases, traditional mediators of apoptotic neuronal death after traumatic brain injury (TBI) (Yakovlev et al., 1997; Clark et al., 1999, 2000). Lau et al. (2006) contend that peroxynitrite inhibits caspase-3-mediated apoptosis through cysteinyl oxidation after *in vivo* and *in vitro* neurotrauma, effectively switching the mode of cell death from apoptotic to oxidant mediated (Fig. 1).

To probe the relationship between peroxynitrite and apoptotic signaling, Lau et al. (2006) used an *in vitro* neuronal stretch model of TBI. The authors observed apoptotic-like cell death (characterized by terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling staining and DNA laddering similar to that observed with staurosporine) in neurons exposed to NMDA and sublethal neuronal stretch [Lau et al. (2006), their Fig. 1*B*,*C* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F1)]. Although the cells demonstrated cytochrome *c* translocation and PN formation [Lau et al. (2006), their Fig. 2*A*,*D* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F2)], they lacked activated caspase-3, a traditional feature of apoptotic neurons [Lau et al. (2006), their Fig. 2*B*,*C* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F2)]. Lau et al. (2006) hypothesized that PN directly inhibited apoptosis in addition to causing the observed DNA fragmentation in mechanically injured cells. The authors believed that this might underlie their observation of cytochrome *c* release in the absence of caspase-3 activation.

Lau et al. (2006) asked whether PN directly inhibited caspase-3 or whether it interfered with the steps between cytochrome *c* release and subsequent activation of caspase-3. Using an antibody to 3-nitrotyrosine, the authors immunoprecipitated the inactive form of caspase-3 (procaspase; a 32 kDa band) after trauma [Lau et al. (2006), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/26/45/11540/F3)]. In an *ex vivo* system, Lau et al. (2006) also observed direct inhibition of fluorogenic caspase-3 activity by the PN donor 3-morpholinosydnonimine (SIN-1) and a complete block by PN itself [Lau et al. (2006), their Fig. 4*A* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F4)]. Additional exposure of caspase-3 to PN in the presence of a sulfhydryl-reducing agent [dithiothreitol (DTT)] reduced the inhibition of caspase-3 activity by PN, indicating that an oxidative modification of cysteine residues, rather than tyrosine nitration, was primarily responsible for inhibition of caspase-3 [Lau et al. (2006), their Fig. 4*B* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F4)].

Could a PN donor inhibit caspase-3 activation in neurons destined to die from apoptosis? Using SIN-1, Lau et al. (2006) demonstrated robust inhibition of staurosporine-induced fluorogenic caspase-3 activity and immunoreactivity, as well as the attenuation of traditional downstream apoptotic activity such as PARP-1 [poly-(ADP-ribose) polymerase-1] cleavage and phosphatidylserine inversion [Lau et al. (2006), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/26/45/11540/F7)]. Cell death, however, was not inhibited. The authors thus concluded that cellular demise could be effectively shifted to a caspase-independent pathway through exposure to PN.

To examine the applicability of these findings to an *in vivo* model of cortical trauma, the authors used the lateral fluid percussion injury model. TBI induced robust 3-NT (3-nitrotyrosine) immunoreactivity in injured rat cortex [Lau et al. (2006), their Fig. 8*B*,*E* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F8)] in the absence of activated caspase-3 [Lau et al. (2006), their Fig. 8*F* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F8)]. When lysates from injured cortex, but not uninjured cortex, were incubated with DTT, fluorogenic

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caspase-3 activity markedly increased [Lau et al. (2006), their Fig. 10*A* (http://www.jneurosci.org/cgi/content/full/26/45/11540/F10)]. Thus, substantial caspase-3 existed in an inhibited, oxidized state as a direct result of the injury. Given that this could be reversed by DTT incubation, the authors concluded that the observed caspase-3 inhibition was a result of PN-induced cysteinyl oxidation.

Lau et al. (2006) describe a multifaceted role for trauma-induced PN formation. In addition to inhibiting classical apoptosis, PN still can play a lethal role in posttrauma neuropathology. PN inhibition may disinhibit proapoptotic substrates and thus underlie the variable cytoprotective efficacy of reactive oxygen species scavengers after brain trauma (Lewen et al., 2001; Marklund et al., 2001). The authors therefore suggest that combined anti-oxidant and anti-apoptotic strategies may be necessary in post-TBI treatment. In light of the evidence presented by Lau et al. (2006), what can we learn about controlling the activity of peroxynitrite and using it advantageously? Specifically, can PN-dependent cysteine oxidation of caspase-3 be preserved while reducing the damage inflicted by this radical?

The source specificity of PN formation might be important in harnessing the caspase-inhibiting activity of PN and using it appropriately. After brain injury, inducible nitric oxide synthase (NOS), endothelial NOS, and neuronal NOS all underlie PN formation (Wada et al., 1998, Gahm et al., 2005). However, we do not know that the radicals produced by all of these sources have identical downstream effects (e.g., caspase inhibition vs DNA fragmentation). Do the heterogeneous activities of PN identified by these authors come about because of the different cellular locations of production of the radicals? The endogenous source of NO responsible for this molecular switch is still unidentified, and the effects of inhibiting various combinations of NO sources on specific PN activities have not been mapped out. However, the interplay among NO synthases, PN levels, caspase activity, and radical-induced cell damage after traumatic brain injury can now be further investigated.

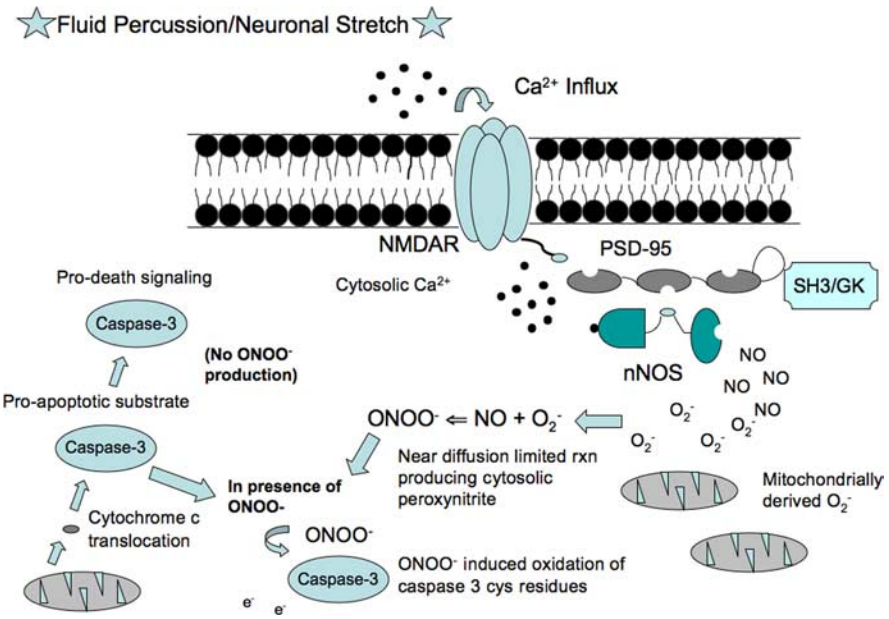

Figure 1. Lau et al. (2006) suggest that cysteine oxidation of caspase-3 by peroxynitrite occurs after traumatic brain injury. The authors propose that this cross talk between reactive oxygen species and proapoptotic substrates switches the mode of cell death after trauma from apoptotic to oxidant mediated. Peroxynitrite therefore could act as a molecular switch, usurping the responsibility for progressive neurodegeneration. GK, Guanylate kinase; NMDAR, NMDA receptor; nNOS, neuronal NOS; PSD-95, postsynaptic density-95; SH3, Src homology 3.

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