Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

The Utility of a New *In Vitro* Model of the Stroke Penumbra

Trisha A. Dwyer,[1] Damien E. Earl,[2] and Liping Wang[1]

Departments of [1]Neurology and [2]Physiology and Pharmacology, Graduate Program in Neurosciences and Neurological Disorders, University of Toledo College of Medicine, Toledo, Ohio 43614

Review of Kaushal and Schlichter (http://www.jneurosci.org/cgi/content/full/28/9/2221)

Ischemic stroke in the brain is produced by arterial occlusion, resulting in dramatically reduced levels of oxygen and glucose to the region supplied. In the stroke core, where perfusion is completely absent, irreversible loss of tissue (infarction) occurs within minutes. The surrounding tissue, known as the stroke penumbra, has limited perfusion and impaired function, which may either recover or progress to infarction over time. Penumbral damage might be mediated by glutamate-induced excitotoxicity, because Na^+-dependent glutamate transporters reverse uptake after loss of ATP and Na^+ pump function. However, damage might also be caused by immune responses, which can persist for days after an ischemic insult (Dirnagl et al., 1999).

The initial immune response after a stroke involves activation of microglia, resident CNS mononuclear phagocytes. After activation, microglia retract their processes, migrate to the site of injury, and release proinflammatory cytokines, nitric oxide (NO), neurotrophic factors, and chemokines that attract other microglia and immune cells (Lai and Todd, 2006). Although activation of microglia after stroke has been well documented (Gerhard et al., 2005), current *in vitro* models of microglial activation and the effects of microglia on the stroke penumbra are inconclusive.

Highlighting the importance of microglial activity in the stroke penumbra, a recent study by Kaushal and Schlichter (2008) published in *The Journal of Neuroscience* uses an innovative three-stage *in vitro* experimental paradigm of the stroke penumbra [Kaushal and Schlichter (2008), their Fig. 1 http://www.jneurosci.org/cgi/content-nw/full/28/9/2221/F1]. In the first stage, neuron and astrocyte cocultures were placed under oxygen and glucose deprivation (OGD) for various times, simulating the stroke core. In the second stage, these OGD-stressed cocultures (OGD-SCs) were coincubated for 24 h with microglia cultured on Transwell inserts allowing soluble factors released from OGD-SCs to activate the microglia. In the third and final stage, microglia were washed and incubated with naive neuron/astrocyte cocultures for 48 h. This approach allowed the authors to examine whether microglia were activated by OGD-SCs and whether activated microglia produced soluble signals that subsequently damaged naive cocultures. Thus, this new method provided a systematic approach to isolate and study key signaling factors that may contribute to cellular deterioration within the stroke penumbra.

Two key signaling pathways known for their production of proinflammatory signals, nuclear factor κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK), were assayed in microglia to determine whether their activity levels increased after exposure to OGD-SCs. Also assayed was the production of tumor necrosis factor α (TNF-α) and NO, which are downstream products of NF-κB and p38 MAPK signaling cascades, respectively. Interestingly, NF-κB activation and TNF-α production significantly increased, whereas neither p38 MAPK nor NO production were affected [Kaushal and Schlichter (2008), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/28/9/2221/F2)].

Kaushal and Schlichter (2008) next examined which soluble factors might be activating the NF-κB signaling cascade. Because *in vivo* evidence suggested that extracellular glutamate increased during ischemia (Graham et al., 1990), and *in vitro* evidence suggested that glutamate activated microglia via group II metabotropic glutamate receptors (mGluRIIs) (Taylor et al., 2005), the authors investigated whether glutamate activated the microglia resulting in production of TNF-α in their model of the stroke penumbra. Direct activation of microglia using a selective mGluRII agonist (DCG-IV [(2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine]) proved sufficient to induce release of TNF-α, but not NO [Kaushal and Schlichter (2008), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/28/9/2221/F4)]. Additionally, incubating naive cocultures with agonist-treated microglia revealed an increase in cell death, which was blocked by either an mGluRII antagonist [2-(*S*)-α-ethylglutamic acid] or a soluble TNF-α receptor (TNFR) (sTNFR1). Together, these data suggest that activation of microglial mGluRIIs induces release of TNF-α, contributing to the death of naive neurons/astro-

Received April 25, 2008; revised May 23, 2008; accepted May 28, 2008.

We thank Drs. Marthe Howard and Joseph Margiotta for their constructive criticism and guidance in the Journal Paper Review course in the Neurosciences Graduate Program and for their advice in preparing this manuscript.

Correspondence should be addressed to Trisha A. Dwyer, Department of Neurology, University of Toledo College of Medicine, Mail Stop 1195, 3000 Arlington Avenue, Toledo, OH 43614. E-mail: trisha.dwyer@utoledo.edu.

DOI:10.1523/JNEUROSCI.1841-08.2008

 0270-6474/08/286537-02$15.00/0

cytes. Although TNF-α release caused by activation of microglial mGluRIIs has already been reported (Taylor et al., 2005), Kaushal and Schlichter (2008) are the first to show that this occurs after activation of microglia by OGD-SCs. Demonstrating this point, naive neurons/astrocytes coincubated with microglia exposed to OGD-SCs in the presence of mGluRII antagonists exhibited reduced cell death [Kaushal and Schlichter (2008), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/28/9/2221/F5)].

One question that could be addressed in future studies is which signaling cascades TNF-α activates and through which TNFRs. TNF-α is known to activate TNFR types I (TNFRI) and II (TNFRII). TNFRI activation is linked to neurodegeneration, whereas TNFRII mediates survival during retinal ischemia (Fontaine et al., 2002). The study by Kaushal and Schlichter (2008) supports TNFRI stimulation on neurons/astrocytes indicated by caspase-8 activation [Kaushal and Schlichter (2008), their Fig. 3 http://www.jneurosci.org/cgi/content/full/28/9/2221/F3]. However, the neuroprotective properties of TNFRII have not been elucidated in the new stroke penumbra model. Experiments using the new model along with transgenic neuronal knock-outs of TNFRI and TNFRII would provide a clearer picture of the effects of TNF-α release on neurons and astrocytes.

The new model may also establish using microglial activation by OGD-SCs as a more relevant *in vitro* ischemic model than other microglial activators like lipopolysaccharide (LPS). Kaushal and Schlichter's (2008) current results differ from those studies using LPS to activate the microglia. A previous *in vitro* model using LPS implicated p38 MAPK and NO as mechanistic players in neurodegeneration (e.g., stroke) (Kaushal et al., 2007). In the current study, microglial activation by OGD-SCs did not induce p38 MAPK activity or production of NO. Thus, Kaushal and Schlichter (2008) have highlighted the importance of using OGD-SCs to activate microglia in stroke models, because activation using other molecules like LPS may not induce the same microglial signaling cascades as an ischemic event.

Although using OGD-SCs to activate microglia might be a more relevant model of stroke than LPS, there are still differences between the authors' findings and those of other studies using *in vivo* ischemia or *in vitro* hypoxia as models of stroke. First, there is *in vivo* evidence that microglial inducible NO synthase is elevated in the penumbra after transient middle cerebral artery occlusion (Vannucchi et al., 2007). Hence, there seems to be a disconnect between the current study and *in vivo* evidence regarding the production of NO. The difference may be attributable to other soluble factors that come from blood supply or other cell types not present in Kaushal and Schlichter's (2008) model, although the new model as of yet is the closest to simulate the stroke penumbra *in vitro*.

Contrasting the results of Kaushal and Schlichter (2008), a concurrent *in vitro* study (Lai and Todd, 2008) found increased production of NO and TNF-α in microglia incubated for 24 h in medium that was conditioned by neurons exposed to 30 min hypoxia, but not neuron-conditioned medium (NCM) from neurons subjected to longer durations of hypoxia (2, 4, and 6 h). The studies used similar methods: both exposed microglia to soluble factors from stressed cultures and then incubate naive cells with soluble factors produced by the microglia. Yet, Lai and Todd (2008) did not find increased microglial TNF-α production after longer periods of hypoxic treatment in neuronal cultures, whereas Kaushal and Schlichter (2008) showed increased microglial TNF-α production with increasing OGD insult in the cocultures. In addition, Kaushal and Schlichter (2008) did not show an increase in NO production from microglia with increasing OGD insult, whereas Lai and Todd (2008) reported an increase in microglia exposed to a shorter hypoxic NCM (30 min). The discrepancy in TNF-α and NO production between the two studies could be attributable to the severity of the treatment or the type of culture used. Specifically, Lai and Todd (2008) used hypoxia (reduced oxygen only) on >98% neuronal cultures, whereas Kaushal and Schlichter (2008) used OGD (reduced oxygen and glucose) on 70%/30% neurons/astrocytes. The amount or types of soluble factors activating the microglia could be different because of the disparities between cultures and/or treatment conditions.

Despite these differences, the new model of the stroke penumbra provides a useful approach for assessing soluble factors involved in ischemia. One advantage of the model is the fact that the cultures contained both neurons and astrocytes. Astrocytes are known to contribute to glutamate excitotoxicity (Seki et al., 1999) and likely add to microglial activation of mGluRIIs. Therefore, the representation of multiple cell types more accurately reflects the *in vivo* situation than using neurons or astrocytes alone. Nevertheless, the model could also be used with purified neuronal cultures for direct comparison with the results of Lai and Todd (2008). If the findings are the same as in their current paper, then the investigators may want to examine hypoxia versus OGD. The penumbra model will also prove useful for investigating neuron–neuron as well as astrocyte–neuron interactions and their contributions to damage in the penumbra. In summary, this new model should provide valuable information about the soluble factors and signaling pathways involved in ischemia and guide researchers toward a suitable therapeutic target to decrease the progressive damage occurring in the stroke penumbra.

References

Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22:391–397.

Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, Pfizenmaier K, Eisel U (2002) Neurodegenerative and neuroprotective effects of tumor necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. J Neurosci 22:RC216.

Gerhard A, Schwarz J, Myers R, Wise R, Banati RB (2005) Evolution of microglial activation in patients after ischemic stroke: a [^{11}C](*R*)-PK11195 PET study. NeuroImage 24:591–595.

Graham SH, Shiraishi K, Panter SS, Simon RP, Faden AI (1990) Changes in extracellular amino acid neurotransmitters produced by focal cerebral ischemia. Neurosci Lett 110:124–130.

Kaushal V, Schlichter LC (2008) Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. J Neurosci 28:2221–2230.

Kaushal V, Koeberle PD, Wang Y, Schlichter LC (2007) The Ca^{2+}-activated K^+ channel KCNN4/KCa3.1 contributes to microglia activation and nitric oxide-dependent neurodegeneration. J Neurosci 27:234–244.

Lai AY, Todd KG (2006) Microglia in cerebral ischemia: molecular actions and interactions. Can J Physiol Pharmacol 84:49–59.

Lai AY, Todd KG (2008) Differential regulation of trophic and proinflammatory microglial effectors is dependent on severity of neuronal injury. Glia 56:259–270.

Seki Y, Feustel PJ, Keller Jr RW, Tranmer BI, Kimelberg HK (1999) Inhibition of ischemia-induced glutamate release in rat striatum by dihydrokinate and an anion channel blocker. Stroke 30:433–440.

Taylor DL, Jones F, Kubota ES, Pocock JM (2005) Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor-induced neurotoxicity in concert with microglial-derived Fas ligand. J Neurosci 25:2952–2964.

Vannucchi MG, Bizzoco E, Corsani L, Gianfriddo M, Pedata F, Faussone-Pellegrini MS (2007) Relationships between neurons expressing neuronal nitric oxide synthase, degree of microglia activation and animal survival. A study in the rat cortex after transient ischemia. Brain Res 1132:218–227.