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The selective p38 inhibitor SB-239063 protects primary neurons from mild to moderate excitotoxic injury

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Abstract

Inhibition of the p38 mitogen-activated protein kinase (MAP Kinase) pathway reduces acute ischemic injury in vivo, suggesting a direct role for this signaling pathway in a number of neurodegenerative processes. The present study was designed to evaluate further the role of p38 MAP Kinase in acute excitotoxic neuronal injury using the selective p38 inhibitor SB-239063 (*trans*-1-(4hydroxycyclohexyl)-4-(fluorophenyl)-5-(2-methoxy-pyrimidin-4-yl) imidazole). Unlike the widely used p38 inhibitor, SB-203580 (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole), this second generation p38 inhibitor more selectively inhibits p38 MAP Kinase without affecting the activity of other MAP Kinase signaling pathways and provides a more accurate means to selectively assess the role of p38 in excitotoxicity that has not been previously possible. SB-239063 provided substantial protection against cell death induced by either oxygen glucose deprivation (OGD) or magnesium deprivation in cultured neurons. The ability of this compound to block excitotoxicity was not due to direct inhibition of *N*-methyl-D-aspartate (NMDA) receptor-mediated currents as SB-239063 did not alter NMDA electrophysiological responses. SB-239063 did not protect against a severe excitotoxic insult induced by 60-min exposure to NMDA. However, when tested against a less severe, brief (5 min) NMDA exposure, p38 inhibition provided substantial protection. These data demonstrate that inhibition of p38 MAP Kinase can confer neuroprotection in vitro against mild but not severe excitotoxic exposure, and suggests that other additional pathways/mechanism(s) may be involved in severe excitotoxic cell death.

Keywords: p38 MAP Kinase; Acute neuronal injury; Cell culture; Neuroprotection; Ischemic cell death; Excitotoxicity

1. Introduction

The mitogen-activated protein kinase family (MAP Kinase) regulate cell stress and survival-related signals by phosphorylating intracellular enzymes and transcription factors (Cobb, 1999). Specific MAP Kinases are involved in cell survival, apoptosis, inflammatory cytokine production, and possibly secondary ischemia (Barone and Parsons, 2000; English et al., 1999; McLaughlin et al., 2001). However, the exact role(s) of MAP Kinases in mediating excitotoxic central nervous system (CNS) injury have not been fully elucidated. While a number of reports have suggested a critical role for p38 activation following *N*-methyl-D-aspartate (NMDA) receptor overstimulation (Kawasaki et al., 1997; Stanciu et al., 2000), these studies have been restricted to the use of less selective inhibitors which can effect other MAP Kinase signaling pathways. Recently, the selective p38 MAP Kinase inhibitor, SB-239063 (*trans*-1-(4hydroxycyclohexyl)-4-(fluorophenyl)-5-(2-methoxy-pyrimidin-4-yl) imidazole), has been shown to provide significant neuroprotection in rodent focal stroke (Barone et al., 2001a,b; Legos et al., 2001). The aim of the present study was to further evaluate the direct neuroprotective effects of p38 MAP Kinase inhibition in various in vitro neuronal cell models of excitotoxic injury. This clarification of the direct contribution of p38 to excitotoxicity should improve our understanding of the mechanism(s) involved in ischemia-induced brain injury, and hopefully lead to more effective therapeutic strategies in ischemic and traumatic brain injury (Barone and Parsons, 2000; Lee and Young, 1996).

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2. Materials and methods

2.1. Preparation of neuron-enriched forebrain cultures

Neuron-enriched forebrain cultures were prepared from embryonic day 17 (E17) rat fetuses as previously described (McLaughlin et al., 1998). Dissociated cells were plated on poly-L-ornithine-treated tissue culture plates in a growth medium comprised of 80% Dulbecco's modified eagle's medium (DMEM) (high glucose with L-glutamine but without sodium pyruvate), 10% Ham's F-12 nutrients, 10% bovine calf serum (heat-inactivated) with antimycotic/antibiotic mixture (with amphotericin B and streptomycin sulfate). Cultures were maintained in an incubator at 37 °C, 95% air/5% CO_2. Glial cell proliferation was inhibited after 48 h in culture with 1–2 μM cytosine arabinoside. After 3 days in vitro, medium was replaced with a serum-free solution comprised of Neurobasal medium (without L-glutamine), B27 supplement, and antimycotic/antibiotic mixture. At 2 weeks in vitro, these cultures were >95% neuronal as assessed by Hoechst and glial fibrillary acidic protein staining.

2.2. Oxygen glucose deprivation injury

Oxygen glucose deprivation (OGD) was performed on 2-week-old cultures. Sixty minutes prior to ischemia, cells were rinsed in Minimal Eagle's Medium with Earle's salts solution, previously saturated with 10% H_2/85% N_2/5% CO_2. Cultures were kept in an anaerobic chamber for various times (45, 60, 120 min) at 37 °C and anoxia–ischemia was terminated by replacement of the Earle's balanced salt solution with oxygenated growth medium containing vehicle or SB-239063 (20 μM). Cell viability was assessed 20–24 h after oxygen glucose deprivation by measuring the extent of lactate dehydrogenase (LDH) release into the medium as previously described (Hartnett et al., 1997). Media samples (40 μl) were analysed spectrophotometrically (490:630 nm) according to the manufacturer's instructions.

2.3. Preparation of mixed cortical cultures for electrophysiology and NMDA exposure

Electrophysiology and toxicity experiments assessing the effects of SB-239063 on NMDA receptors were performed in mixed forebrain cultures of neurons and glia. Cerebral cortices were obtained from embryonic day 16 (E16) Sprague Dawley rat fetuses and dissociated as previously described (Hartnett et al., 1997). Briefly, cells were plated onto poly-L-lysine-coated glass coverslips at a density of 225,000 cells/ml of growth medium (v/v mixture of 80% DMEM, 10% Ham's F12, 10% calf serum, 25 mM HEPES, 24 U/ml penicillin, 24 μg/ml streptomycin, and 2 mM L-glutamine) and maintained at 37 °C in 95% air/5% CO_2. Cytosine arabinoside (2 μM) was added once at 15 days in vitro after which growth medium lacking F-12 and containing low serum (2%) was added. Medium was partially replaced with fresh growth medium three times per week. At 3–5 weeks in vitro, these cultures contain ~10–20% neurons (Rosenberg, 1991; Rosenberg and Aizenman, 1989).

2.4. Electrophysiological recordings

Electrophysiology experiments were performed at room temperature (25 °C) using the whole-cell patch clamp configuration. Coverslips were bathed in external solution containing (concentrations expressed in mM): 150 NaCl, 1.0 $CaCl_2$, 2.8 KCl, 10 HEPES, 10 glycine, 25 tetrodotoxin (Calbiochem) and pH was adjusted to 7.2 with NaOH. Electrodes were pulled on a Sutter P-87 electrode puller (Sutter Instruments, Novato, CA) to a resistance of 1.5–3 MΩ when filled with internal solution containing (in mM): 140 CsF, 10 EGTA/CsOH, 1 $CaCl_2$, and 10 HEPES (pH adjusted to 7.2 with CsOH). Signals were amplified using an Axopatch 200B integrating patch clamp amplifier (Axon Instruments, Foster City, CA), filtered at 1 kHz, and digitised at 2 kHz with a DigiData 1200 (Axon Instruments) computer interface. Drugs were applied via a perfusion system with a stepper motor for fast solution changes (Warner Instruments, Hamden, CT). NMDA and SB-239063 were dissolved in external solution for recording. SB-239063 was diluted from a 1000× stock. Data were collected and analysed using commercially available software (pCLAMP 6.11, Axon Instruments).

2.5. Preparation of hippocampal cell cultures

Neuron-enriched hippocampal cultures were prepared from embryonic Sprague–Dawley rat fetuses (gestational age 17.5 days; Charles River) as described previously (Skaper et al., 2001). Hippocampi were incubated with 0.08% (w/v) trypsin, and dissociated in Neurobasal medium containing 10% heat-inactivated fetal calf serum (Skaper et al., 1990). Cells were pelleted by centrifugation (200 × *g*, 5 min) and resuspended in Neurobasal medium containing B27 supplement, 25 μM glutamate, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. The cell suspension was plated onto dishes previously coated with poly-D-lysine (10 μg/ml) and 10% heat-inactivated fetal calf serum, at a density of 4.5×10^4 cells/cm^2. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2/95% air. After 5 days, one-half the medium was replaced with an equal volume of maintenance medium (plating medium but containing B27 supplements without antioxidants, and lacking glutamate). Additional medium exchanges (0.5 volume) were performed every 3–4 days thereafter.

2.6. Magnesium withdrawal treatment to generate excitotoxicity

Toxicity experiments were performed on cells between 14 and 16 days in vitro. Cultures were washed once with

$Mg^{2+}Cl_2$-free Locke's solution (pH 7.0) containing 0.1 μM glycine and 30 μM histamine (Skaper et al., 2001). Control cultures were exposed to Locke's solution containing 1 mM $Mg^{2+}Cl_2$. Drug treatments were carried out for 15 min (22 °C) in a final volume of 0.5 ml. Thereafter, cells were washed with complete Locke's solution and returned to their original culture medium for 24 h. Cell survival was quantified 24 h after the insult by a colorimetric reaction with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Skaper et al., 1990). Absolute MTT values obtained were normalized and expressed as a percentage of sham-treated sister cultures (defined as 100%).

2.7. NMDA exposure in mixed cultures

Excitotoxicity generated by NMDA exposure was performed in 4-week-old mixed cultures of neurons and glia, prepared as described for electrophysiological recordings. Cell cultures were pretreated for 1 h with SB-239063 (20 μM), followed by a 5-min or 1-h exposure to NMDA in the presence of 10 μM glycine. SB-239063 was present throughout the treatment and over the subsequent 20–24 h until LDH readings were performed.

3. Results

3.1. SB-239063 protects against oxygen glucose deprivation

Based on the dose–response curve obtained in preliminary experiments (data not shown), the effects of SB-239063 (20 μM) on oxygen glucose deprivation toxicity were evaluated at various time points in neuron-enriched forebrain cultures. Cells were exposed for 45, 60, or 120 min to oxygen glucose deprivation in the presence or absence of the p38 inhibitor (Fig. 1). Forty-five minutes of oxygen glucose deprivation did not significantly increase the amount of LDH released (0.016 ± 0.0003 optical density units, $n = 3$) compared to control (0.010 ± 0.001 optical density units, $n = 3$). However, following 60 min of oxygen glucose deprivation, appreciable cell death was present and SB-239063 significantly ($P < 0.05$) decreased LDH release (0.023 ± 0.004 optical density units, $n = 3$) compared to vehicle treatment (0.041 ± 0.005 optical density units, $n = 3$). Although the extent of cell death after 60 min of oxygen glucose deprivation was similar to that seen after 120 min of oxygen glucose deprivation (0.038 ± 0.006 optical density units, $n = 3$), SB-239063 did not protect against the latter treatment (0.037 ± 0.007 optical density units, $n = 3$), suggesting that this duration of oxygen glucose deprivation was too severe to permit pharmacological intervention.

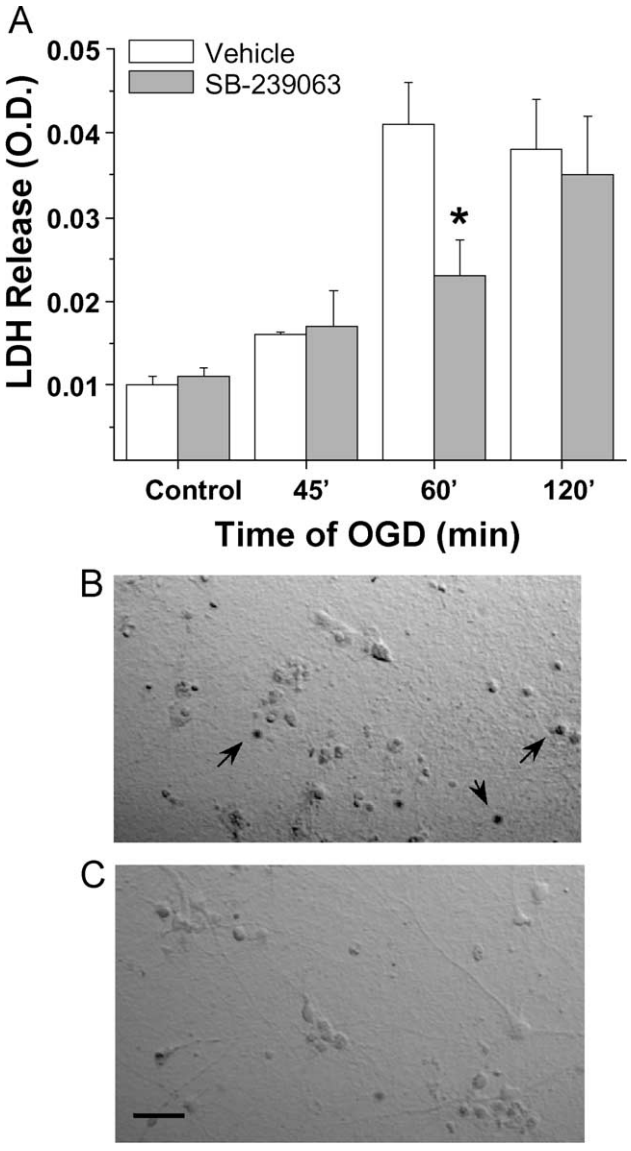

Fig. 1. (A) SB-239063 blocks excitotoxicity induced by oxygen glucose deprivation in neuron-enriched forebrain cultures. Neuronal cell cultures were exposed for various times to oxygen glucose deprivation. Addition of SB-239063 (20 μM) significantly decreased neuronal cell death following 60 min of oxygen glucose deprivation. Data are means ± S.E.M. and were analysed by two-tailed paired *t*-test (significance at * $P < 0.05$). Representative photomicrographs were taken of cell cultures 24 h after 60-min oxygen glucose deprivation treated with either vehicle (B) or 20 μM SB-239063 (C). Cells which had lost their phase-bright appearance become shrunken (pyknotic) and were dying following treatment are indicated by black arrowheads. Scale bar is 75 μm.

3.2. SB-239063 does not block NMDA-mediated currents

In order to insure that any neuroprotective action of SB-239063 was not due to a direct effect on NMDA receptor-mediated channel activity, whole cell electrophysiological recordings were performed. In these experiments, 30 μM NMDA was applied to mixed cultures of neurons and glia in the presence and absence of SB-239063 (Fig. 2). The p38 inhibitor did not alter NMDA elicited currents suggesting that any neuroprotective action of the MAP Kinase inhibitor was not a direct effect on NMDA receptors.

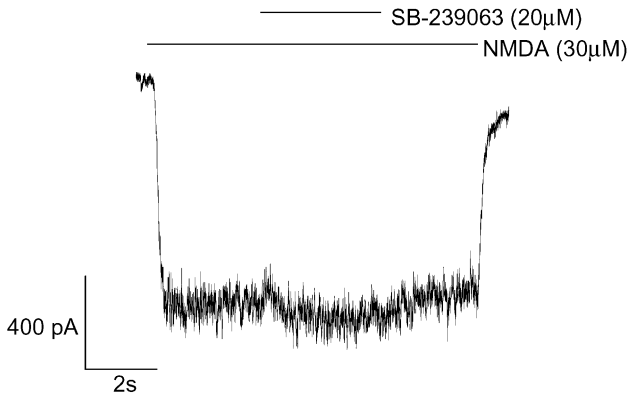

Fig. 2. SB-239063 does not alter NMDA-elicited electrophysiological responses. A representative trace of whole-cell responses to NMDA in the presence and absence of SB-239063 (20 μM). Application of SB-239063 was made for 3 s concomitantly during exposure to 30 μM NMDA. The p38 MAP Kinase inhibitor did not alter responses to agonist in any of the cells from which recordings were made ($n=4$).

3.3. Excitotoxicity generated by magnesium withdrawal can be attenuated with SB-239063

We used neuron-enriched hippocampal cell cultures to assess the effects of p38 inhibition on moderate excitotoxicity generated by removal of magnesium block from the NMDA receptor. Control experiments showed that the loss of viable neurons, as quantified by MTT assay, was proportional to the number of degenerating neurons, as estimated by trypan blue staining. By 24 h, approximately 50% of neurons treated with vehicle were no longer viable as assessed by MTT (52.0 ± 5.6%). Treatment with SB-239063 (1–30 μM) provided dose-related reduction in neuronal cell death (Fig. 3). SB-239063 was maximally neuroprotective at 10–30 μM with neuronal survival significantly ($P<0.05$) increasing by 25–43% (35.8 ± 2.4% and 27.7 ± 2.4% cell depth, respectively).

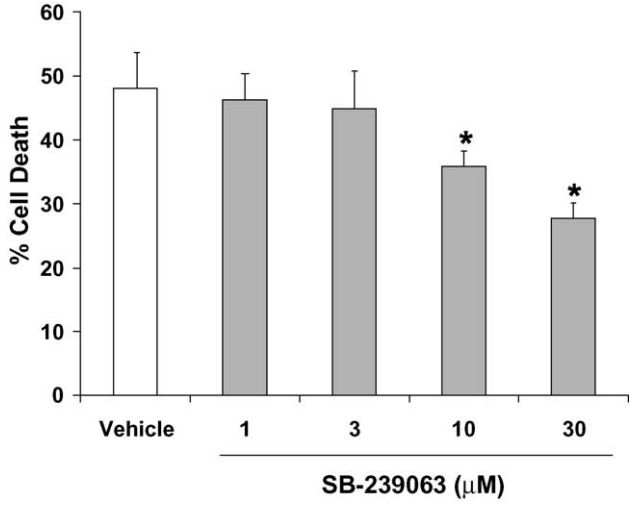

Fig. 3. SB-239063 reduces excitotoxic hippocampal neuronal cell death. In dissociated hippocampal cells, SB-239063 (10–30 μM) decreased neuronal cell death by 25–43% following Mg^{2+} withdrawal. Absolute MTT values obtained were normalized and expressed as a percentage of sham-treated sister cultures (defined as 100% survival). Percent cell death was calculated from these data. Values are means ± S.D. ($n=3$) and were analysed by ANOVA followed by Dunnett's post-hoc test (significance at $^*P<0.05$).

3.4. SB-239063 reduces cell death associated with mild, but not severe, NMDA exposure

As our earlier OGD experiments suggested that p38 inhibition may be more efficacious with mild excitotoxic insults, we next assessed the relative contribution of p38 to direct exposure to NMDA. In these studies, we assessed the efficacy of SB-239063 at attenuating mild (5 min) and severe (1 h) excitotoxicity in mixed cultures of neurons and glia. Cultures that were exposed for 1 h to either 30, 100 or 300 μM NMDA were not significantly protected by 20 μM SB-239063. However, when cells were exposed to a more mild insult (5 min), SB-239063 (20 μM) was capable of attenuating NMDA induced injury. Statistically significant neuro-

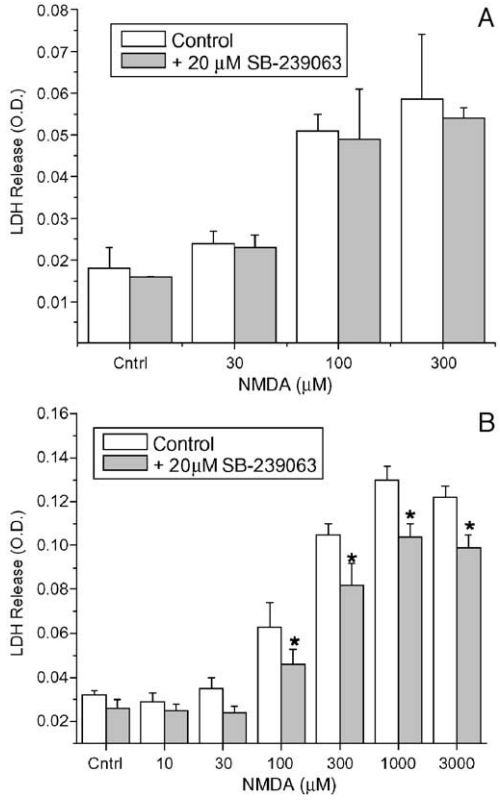

Fig. 4. SB-239063 is protective against mild, but not severe, NMDA exposure in mixed cortical cell cultures. (A) Four-week-old mixed cortical cell cultures were treated with vehicle or SB 239063 (20 μM) 1 h prior to exposure to NMDA (30, 100, and 300 μM). NMDA was removed after 1 h and cell death was measured 24 h later using an LDH assay. Data are means ± S.D. ($n=6$) and analysed by ANOVA. (B) Paired 4-week-old mixed cultures were exposed to various concentrations of NMDA (0.01, 0.03, 0.1, 0.3, 1, and 3 mM) in the presence or absence of SB-239063 as above except NMDA was removed after 5-min incubation. Data are means ± S.E.M. ($n=5–7$) and were analysed by paired Student's *t*-test (significance at $^*P<0.05$).

protection was observed at 100 μM, 300 μM, 1 mM and 3 mM NMDA with SB-239063 providing between 20% and 28% protection at these concentrations (Fig. 4).

4. Discussion

Several groups have reported that both glutamate and hypoxia activate p38 in neuronal cell cultures (Clerk et al., 1998; Kawasaki et al., 1997). However, in assessing a role for this MAP Kinase in the observed toxicity, these studies have relied on less specific MAP Kinase inhibitors such as SB-203580 (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole). The use of SB-239063 in the current study provides a number of advantages over SB-203580. We demonstrate in this work that SB-239063 does not directly alter NMDA induced currents, and we have previously shown that SB-239063 does not effect other MAP Kinases. SB-203580, however, has been shown to inhibit c-Jun N-terminal kinase (JNK) activity with an IC_{50} of 5 μM and cRaf with an IC_{50} of 0.4 μM (Barone et al., 2001a). Thus, SB-239063 provides a more precise means to assess the role of p38 in excitotoxicity. Indeed, this is the first study to unequivocally demonstrate a role for p38 in excitotoxicity. In this work, we demonstrates that activation of p38 MAP Kinase plays a critical role in neuronal cell death induced by mild oxygen glucose deprivation, magnesium withdrawal and glutamate receptor agonist exposure. These models share a requirement for NMDA receptor activation, (Kaku et al., 1991; Skaper et al., 2001; Speliotes et al., 1994) and are therefore considered excitotoxic in nature.

In previous studies, we and others have shown that several intracellular signals commonly associated with glutamate receptor stimulation and excitotoxicity may contribute to p38 activation. For instance, Koh et al. (1996) demonstrated that zinc is released during transient ischemia and 'free' zinc accumulates in dying neurons. We have recently shown that oxidant-induced zinc dysregulation is the most proximal events in an apoptotic cascade in which p38 activation leads to potassium efflux, and subsequently, energetic dysfunction and caspase activation (McLaughlin et al., 2001). Blockade of p38 with SB-239063 provided substantial neuroprotection against this oxidative and ionic dysfunction. Given the importance of oxidative stress and zinc dysregulation in excitotoxic insults, our current observations are perhaps not surprising as they closely parallel our previous observations.

p38 has been shown to be a critical mediator of the inflammatory response in CNS (Irving et al., 2000). Earlier work from our labs has shown SB-239063 can attenuate early neuronal injury (within 2 h) in an animal model of cerebral ischemia induced by electrocoagulation of the middle cerebral artery (Legos et al., 2001). This protection was maintained for at least 7 days suggesting a direct, long-lasting protective effect against the subsequent apoptotic cell death which is typical of this model. As cytokine production and neutrophil infiltration might be delayed up to 12 h (Legos et al., 2000), these initial studies demonstrating early neuroprotection by SB-239063 suggests that p38 MAP Kinase inhibitors can be beneficial via additional or alternative pathways which do not involve inflammation per se, such as blockade of excitotoxic signaling.

Taken together, these data demonstrate that in addition to its role in mediating the inflammatory response, p38 MAP Kinase may also contribute to early neuronal injury through excitotoxic pathways. It is, however, important to note that in excitotoxic models where significant protection was afforded by blocking p38, a substantial amount of cell death remained. This argues that even mild excitotoxicity clearly has additional p38-independent components which contribute to cell death. These experiments, in conjunction with others (Barone et al., 2001a,b; Irving et al., 2000; Walton et al., 1998), suggest that activation of p38 and other MAP Kinases and their functional importance may be specific to various cell types and/or duration and intensity of the stimulus. Low level excitotoxic insults may activate apoptotic signaling pathways in a manner similar to what we have previously shown in a subpopulation of cells which are responsive to p38 inhibition. However, more severe insults may not require apoptotic signaling cascades to cause death, are impervious to p38 blockade, and involve other signal transduction cascades. Taken together, these studies suggest that in instances of mild to moderate glutamatergic overactivation, p38 MAP Kinase inhibition may provide a means to prevent or minimize neuronal cell death. These results may be applicable to neurological disorders that have been associated with excitotoxic injury such as traumatic brain injury, stroke and epilepsy.

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References

Barone, F.C., Parsons, A.A., 2000. Therapeutic potential of anti-inflammatory drugs in focal stroke. Exp. Opin. Invest. Drugs 9, 2281–2306.

Barone, F.C., Irving, E.A., Ray, A.M., Lee, J.C., Kassis, S., Kumar, S., Badger, A.M., Legos, J.J., Erhardt, J.A., Ohlstein, E.H., Hunter, A.J., Harrison, D.C., Philpott, K., Smith, B.R., Adams, J.L., Parsons, A.A., 2001a. Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. Med. Res. Rev. 21, 129–145.

Barone, F.C., Irving, E.A., Ray, A.M., Lee, J.C., Kassis, S., Kumar, S., Badger, A.M., White, R.F., McVey, M.J., Legos, J.J., Erhardt, J.A., Nelson, A.H., Ohlstein, E.H., Hunter, A.J., Ward, K., Smith, B.R., Adams, J.L., Parsons, A.A., 2001b. SB 239063, a second-generation p38 mitogen-activated protein kinase inhibitor, reduces brain injury and neurological deficits in cerebral focal ischemia. J. Pharmacol. Exp. Ther. 296, 312–321.

Clerk, A., Fuller, S.J., Michael, A., Sugden, P.H., 1998. Stimulation of

''stress-regulated'' mitogen-activated protein kinases (stress-activated protein kinases/c-Jun N-terminal kinases and p38-mitogen-activated protein kinases) in perfused rat hearts by oxidative and other stresses. J. Biol. Chem. 273, 7228–7234.

Cobb, M.H., 1999. MAP kinase pathways. Prog. Biophys. Mol. Biol. 71, 479–500.

English, J., Pearson, G., Wilsbacher, J., Swantek, J., Karandikar, M., Xu, S.C., Cobb, M.H., 1999. New insights into the control of MAP kinase pathways. Exp. Cell Res. 253, 255–270.

Hartnett, K.A., Stout, A.K., Rajdev, S., Rosenberg, P.A., Reynolds, I.J., Aizenman, E., 1997. NMDA receptor-mediated neurotoxicity—a paradoxical requirement for extracellular Mg^{2+} in Na^{+}/Ca^{2+}-free solutions in rat cortical neurons in vitro. J. Neurochem. 68, 1836–1845.

Irving, E.A., Barone, F.C., Reith, A.D., Hadingham, S.J., Parsons, A.A., 2000. Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischaemia in the rat. Mol. Brain Res. 77, 65–75.

Kaku, D.A., Goldberg, M.P., Choi, D.W., 1991. Antagonism of non-NMDA receptors augments the neuroprotective effect of NMDA receptor blockade in cortical cultures subjected to prolonged deprivation of oxygen and glucose. Brain Res. 554, 344–347.

Kawasaki, H., Morooka, T., Shimohama, S., Kimura, J., Hirano, T., Gotoh, Y., Nishida, E., 1997. Activation and Involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells. J. Biol. Chem. 272, 18518–18521.

Koh, J.Y., Suh, S.W., Gwag, B.J., He, Y.Y., Hsu, C.Y., Choi, D.W., 1996. The role of zinc in selective neuronal death after transient global cerebral ischemia. Science 272 (5264), 1013–1016.

Lee, J.C., Young, P.R., 1996. Role of CSB/p38/RK stress response kinase in LPS and cytokine signaling mechanisms. J. Leukoc. Biol. 59, 152–157.

Legos, J.J., Whitmore, R.G., Erhardt, J.A., Parsons, A.A., Tuma, R.F., Barone, F.C., 2000. Quantitative changes in interleukin proteins following focal stroke in the rat. Neurosci. Lett. 282, 189–192.

Legos, J.J., Erhardt, J.A., White, R.F., Lenhard, S.C., Chandra, S., Parsons, A.A., Tuma, R.F., Barone, F.C., 2001. SB 239063, a novel p38 inhibitor, attenuates early neuronal injury following ischemia. Brain Res. 892, 70–77.

McLaughlin, B.A., Nelson, D., Silver, I.A., Erecinska, M., Chesselet, M.F., 1998. Methylmalonate toxicity in primary neuronal cultures. Neuroscience 86, 279–290.

McLaughlin, B., Pal, S., Tran, M.P., Parsons, A.A., Barone, F.C., Erhardt, J.A., Aizenman, E., 2001. p38 activation is required upstream of potassium current enhancement and caspase cleavage in thiol oxidant-induced neuronal apoptosis. J. Neurosci. 21, 3303–3311.

Rosenberg, P.A., 1991. Accumulation of extracellular glutamate and neuronal death in astrocyte-poor cortical cultures exposed to glutamine. Glia 4, 91–100.

Rosenberg, P.A., Aizenman, E., 1989. Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte-poor cultures of rat cerebral cortex. Neurosci. Lett. 103, 162–168.

Skaper, S.D., Facci, L., Milani, D., Leon, A., Toffano, G., 1990. Culture and Use of Primary and Clonal Neural Cells, vol. 2. Academic Press, San Diego, pp. 17–33.

Skaper, S.D., Facci, L., Kee, W.J., Strijbos, P., 2001. Potentiation by histamine of synaptically mediated excitotoxicity in cultured hippocampal neurones: a possible role for mast cells. J. Neurochem. 76, 47–55.

Speliotes, E.K., Hartnett, K.A., Blitzblau, R.C., Aizenman, E., Rosenberg, P.A., 1994. Comparison of the potency of competitive NMDA antagonists against the neurotoxicity of glutamate and NMDA. J. Neurochem. 63, 879–885.

Stanciu, M., Wang, Y., Kentor, R., Burke, N., Watkins, S., Kress, G., Reynolds, I., Klann, E., Angiolieri, M.R., Johnson, J.W., DeFranco, D.B., 2000. Persistent activation of ERK contributes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. J. Biol. Chem. 275, 12200–12206.

Walton, K.M., DiRocco, R., Bartlett, B.A., Koury, E., Marcy, V.R., Jarvis, B., Schaefer, E.M., Bhat, R.V., 1998. Activation of p38MAPK in microglia after ischemia. J. Neurochem. 70, 1764–1767.