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Cytokine and Trophic Biology as Cellular Transcriptional Susceptibility in Neuronal Ischemia

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Abstract: Cytokines and neurotrophins appear to constitute a series of self-amplifying systems in the development of essential features of ischemic neuronal injury that morphologically distinguish it in terms of either necrosis or apoptosis. An interaction of regional components at the tissue and organ level would involve vessels and particularly endothelium that are associated with proliferating glia and injured groups of neurons. It is in terms of a system of progression as applicable to cytokine action, trophic biology and gene transcription pathways that one may account for the development of ischemia as a selective susceptibility of neurons. A primary neuronal pathobiology would evolve developmentally in terms of oxidative stress, nitric oxide production and Calcium ion influx and would be reflected in lowered pH of regional tissues. Pinpointing the time of death of cells might be translated in terms of cytokine and trophic effects that promote cytoskeletal injury in the face of energy depletion. Reperfusion and no-reflow phenomena would further reflect involvement of cytokines and neurotrophins in neuronal ischemia. In fact, capillary bed loss would eventually result in central core necrosis of focal ischemic lesions that contrasts with the surrounding potentially viable penumbral zone.

Key words: Cellular transcriptional, tropic biology, cytokine, neuronal ischemia

INTRODUCTION

Inflammation characterizes bacterial and viral infections, multiple sclerosis, Alzheimer's disease and also cerebral ischemia[1].

Global or focal ischemic brain injury of tissues and of cells would range from vascular to glial to neuronal involvement. Such a range of set dynamics of injury would appear often primarily determined at fairly early stages of vascular occlusion. Cell component injury includes cytoskeletal involvement that changes dynamics of progression of damage to other cell components, plasmalemma and nuclear transcription pathways.

Progression of ischemia: Mechanisms of ischemic lesion expansion may include excitotoxicity, peri-infarct depolarization, lactic acidosis, microcirculatory disturbances and flow-metabolism uncoupling[2]. A loss of balance between inhibitory and excitatory stimulation may promote ischemic cell death as in cases of internalization of ionotropic GABA(A) receptors[3].

An integral approach to ischemic injury would account for core necrosis as evidenced involvement of focal tissue organ elements. Prolongation of a therapeutic window may be possibly achieved by suppression of neuroinflammation, as induced by MLN519, a proteasome inhibitor[4].

Endothelial cell injury appears to participate not only in deprivation of oxygen to tissues but to also promote progression of necrosis and apoptosis[5]. Ischemic CA1 pyramidal cell death may be initiated by activation of the c-Jun N-terminal kinase pathway and then involve apoptosis[6].

A clear delineation of necrosis pathways from apoptotic events might implicate a process originating from vascular endothelial cell damage with subsequent potential progression.

It might perhaps prove a reliable guide to possible outcomes in ischemia that apoptosis[7] arises in a context of cytokine action and reaction. Septic shock can induce diffuse cerebral damage with neuronal ischemia and apoptosis, and associated with increased expression of vascular inducible Nitric Oxide synthase[8]. Excitotoxic stimulation of superoxide and nitric oxide production leads to the formation of peroxynitrite and hydroxyl radical that damage lipids, proteins and DNA[9]. Necrosis as a deprivation system of effects, and apoptosis in progressive involvement of cell components, would implicate transcription factors that predetermine cell fate. Increased gene expression of somatostatin receptor 2 occurs after neuronal ischemia and mitochondria-mediated apoptotic pathways[10].

Ischemia as organ involvement: Realization of a true focus of organ involvement in neuronal ischemia mighthelp account for modes of interaction that deprive the cells of energy (as ATP) and of proteins, and also promote progression of active lesions as reflected in apoptosis as self-programmed cell death[11]. Gene transfer

of baculoviral p53 by adenoviral vector protects against neuronal apoptosis as occurs in cases of ischemia or neurodegeneration[12].

Deprived ATP production might define pathways of autophagocytosis intermediate in character and progression between necrosis and self-programmed apoptosis.

Reactive nitrogen oxide species may enhance ischemic injury via increased glutamate release and/or reduction of glutamate uptake[13]. The endoplasmic reticulum is susceptible to oxidative stress-induced injury after ischemia/reperfusion[14].

Endothelial cell participation: A lowered pH of the intracellular milieu would reflect fundamental changes in the extracellular microenvironment in defining ischemic injury.

Extracellular predetermination of cellular outcome in ischemic events would tend to reflect consequences of endothelial cell pathobiology.

Blood brain barrier breakdown within an ischemic lesion largely maps out the core of a focal ischemic lesion in the brain. Fundamental distinction between the core region and the penumbra might specifically implicate pathobiologic progressiveness in endothelial injury. Only insofar as it is necessary to distinguish cells involved in tissue injury is it also possible to implicate various cell components primarily targeted in cell death due to ischemia. Cyclin D1 expression appears implicated in exacerbated ischemic neuronal cell death that is induced by cyclooxygenase-2 activity[15].

Inhibition of mitochondrial respiration promotes accumulation of lactate and hydrogen ions and dramatically reduces ATP production with dysregulation of cellular ion metabolism and intracellular Ca^{+2} accumulation[16].

Endothelial protagonists in predetermined outcome of neuronal ischemic injury might contrast with a vascular occlusive phenomenon that alternates with reperfusion in further inducing necrosis or apoptosis.

Cytokine action: Ca^{+2} entry into cells would perhaps reflect consequences of activated gene transcription arising as a consequence of cytokines that include Interleukin 1β and Tumor Necrosis Factor α.

Neutrophils promote activation of endothelial cells and are participants in ongoing regional ischemia. They better define how core necrosis contrasts with an unstable penumbral zone with potential neuronal recovery. Gene transcription would allow or prevent consequences of involved cytokine action in neuronal susceptibility to further ischemic injury. Bone morphogenetic protein 7, a trophic factor in the Transforming Growth Factor β superfamily, reduces ischemic neuronal injury possibly via activation of p42, 44MAPK and PKC[17].

Estrogen attenuates Nuclear Factor K B activation induced by transient cerebral ischemia, implicating a critical role for suppression of postischemic inflammation in neuroprotection[18].

The no-reflow phenomenon would integrally compartmentalize focal ischemic injury that partly accounts for endothelial pathobiology.

Selective vulnerability of neurons: A vascular system supports cells and tissues in promoting functional adaptation to injury. A selective vulnerability to neuronal ischemia[19] might account for different modes of adaptation to cellular injury based principally on subcellular neuronal differentiation.

Astrocytic intercellular adhesion molecule-1 expression is involved in neuronal cell death, and is induced by the TNF-α dependent pathway[20].

Developmental pathways of subtype differentiation of neurons would go hand in hand with a propensity for adaptation or tolerance to ischemia inherently related topographically or physiologically to blood vessels, glia and other neurons as induced by Transforming Growth Factor β 1-mediated neuroprotection against excitotoxic injury[21]. Ionotropic glutamate receptor channels and L-type voltage-sensitive calcium channels are involved in early stages of the cellular cascade leading to neuronal death after hypoxia/ischemia[22].

Functional attributes of particular subtypes of neurons, as found in the hippocampal CA1 region, would implicate mechanisms of ischemic injury and of outcome that are developmentally predetermined. Caspase 3 is a key protease in the execution of apoptosis after hypoxia/ischemia of the immature brain[23]. Modes of involvement of the pontosubicular neurons in perinatal hypoxia/ischemia would attest to topographic determinants of selective neuronal vulnerability. Hippocampal and Layers 4-5 cortical neurons would not be susceptible to ischemia simply on the basis of specific vascular patterns of supply but rather to how ischemia itself evolves in relation to neurons that lack oxygen.

Structural and physiologic attributes of groups of neurons predetermine individual cellular injury manifested either as edematous cell change, ischemic cell change or as autophagocytosis. The Akt signaling pathway contributes to regulation of apoptosis and is modulated by growth factors[24]. Transient hypoxia triggers apoptosis in the CA1 hippocampal neurons of newborn rats and is followed by increased neurogenesis[25].

The actual realization of potential neuronal

ischemic injury would be an expression of how neurons undergo induced progression from point of initiation of the ischemic event to perpetuation of such injury as transforming events. Strategic suppression of neuroinflammation may range from transcriptional regulation of inflammatory gene expression of Nuclear Factor-κ B to proteasome, signaling pathways (ICE-cascade, MAPK/mKK/ERK cascade)[26].

It might be that ischemia constitutes an expression of how regions of supply somehow are developmentally predestined to progressive edematous or ischemic cell change and to cell necrosis or apoptosis. On the other hand, ischemic preconditioning may increase activity of several antioxidant enzymes[27]. Oxidative stress also plays a significant role in neurodegeneration of Parkinson's disease and of Huntington's disease[28].

Neuronal response: Perinatal systems of expressed neuronal injury arising from ischemia/hypoxia appear to constitute patterned modes of neuronal response to ischemic injury[29]. Neurotrophins appear effective in suppressing neuronal apoptosis but not necrosis[30]. One might view modes of involvement of striatum, thalamus, pontosubiculum and hippocampus in terms of developmental stages in evolving neuronal response to such injury. Vascular endothelial growth factor inhibits caspase-3 activity and modulates the phosphatidylinositol 3 kinase (PI3K)/Akt/nuclear factor-κB signaling pathway, inhibits outward delayed rectifier potassium channel currents and enhances migration and proliferation of neural progenitor cells[31]. Multicystic change of the brain in infants, for example, would attest to possible alternative but distinctive endresults in individual neuronal ischemic injury.

Adult neuronal injury as eosinophilic shrunken cells would involve the combined participation of injured cells operative at a regional tissue level.

Such regional pathology would implicate glial cells and the vascular supporting systems that integrally include endothelially lined capillaries, venules and arterioles.

The activation of poly(ADP-ribose) polymerase-1 (PARP-1) after exposure to nitric oxide or free oxygen radicals can lead to cell injury via irreversible depletion of NAD[32]. At a tissue and cellular level, ischemic and hypoxic injury would implicate an endresult response on the part not only of neurons but especially of regional capillary beds as well. A cell death mode switch from necrosis in the core region to apoptosis in the penumbra may constitute a self-protective mechanism[33]. The no-reflow phenomenon would implicate leukocytes and injured lining endothelium in a manner that permits global participation of neurons in operatively variable progression. Ischemic neuronal injury activates cyclin-dependent kinases, with aberrant entry of neurons into the cell cycle[34].

One might indeed view the morphologic expression of ischemic neuronal injury as transforming tissue change implicating in particular the regional endothelial and capillary beds. Human recombinant hepatocyte growth factor acts on the blood brain barrier and protects against apoptosis of cerebral endothelial cells at an early stage after the ischemia[35]. It is in this context that ventricular hemorrhage so prominently constitutes a singular component of perinatal ischemia/hypoxia pathology in the premature infant.

In the aged brain, an altered gene expression profile may further exacerbate neuronal ischemic injury and impair tissue reorganization and repair[36].

Subcellular involvement: Lowered pH constitutes an endresult of ischemia whereby the oxygen deficiency induces subsequent structural transformation of individual neurons and also of blood vasculature as well. A localized regional system of circulatory failure at the capillary level might entail modes of transforming potential recovery expressed as selective vulnerability of individual neurons in a given anatomic region. Vascular Endothelial Growth Factor protects neurons against experimental ischemia[37].

Further characterization at a subcellular level might implicate ionic exchange and membrane pumps that determine specific suborganelle injury. Cytochrome C-initiated activation of apoptosis protease factor-1 is a key step in the activation of apoptosis[38]. Changes in localization and complex formation by Bad, a pro-apoptotic member of the bcl-2 gene family, are probably involved in determining vulnerability to global ischemia[39].

Depolarization events of injured neurons implicate a regional participation in progression of ischemic injury that may functionally promote subsequent morphologic or structural cellular change.

Cytokine participation in ischemic cellular injury operatively bridges capillary bed pathology and neuronal ischemic injury and implicates predetermined pathways of necrosis or of apoptosis of regional neurons. Cathepsin B release triggers the activation of proinflammatory caspases in the absence of reperfusion[40]. In terms of such progression, necrosis might progress within a context of evolving tissue change that regionally characterizes that ischemic injury event. There is evidence that reactive gliosis and post-ischemic inflammation involving microglia contribute to ischemic change[41]. Astrocytes maintain glutamine synthesis and enable

neuronal formation of neurotoxic glutamate[42]. On the other hand, neuronal apoptosis might simply be a consequence of a strictly cellular event inducing active cellular participation. Erythropoietin prevents neuronal damage following ischemia by an anti-apoptotic mechanism[43]. It may play a significant role in the developing brain.

Self-programmed cell death pathways might be expressed forms of an induced involvement that transforms to active individual cell participation in lesion progression. Targeting the apoptotic cell death pathway may involve inhibition of tBrd-induced SMAC release and caspase-3 activation[44]. Transforming growth factor β1, a multifunctional cytokine, suppresses apoptosis of neurons in ischemia[45].

Subcellular organelles: Calcium ion influx[46] within cells might result from the eventual breakdown in energy supply to cell membranes involving plasmalemma and especially subcellular organelle units.

Reactive oxygen-nitrogen species play a critical role in the initiation of apoptosis. Mitochondrial permeability transition and poly(ADP-ribose) polymerase activation provide additional mechanisms for oxidative damage[9]. A combination of mitochondrial injury and accumulation of extracellular glutamate induces neuronal cell death[47].

Mitochondrial dysfunction in particular may play a central role in reperfusion injury following cerebral ischemia[48].

Lysosomal particles, endoplasmic reticulum stacks and sacs, polyribosomes and even nuclear chromatin would undergo injury that develops subsequent to onset of pathobiology of endothelially-lined capillaries and of neuronal subset groups at a regional level. Sustained calpain activation may lead to lysosomal rupture and ischemic neuronal necrosis rather than apoptosis[49]. Endoplasmic reticulum dysfunction is responsible for ischemia-induced shut down of translation[50]. The co-chaperone BAG-1 regulates 70 kDa shock proteins and protects cells from ischemic injury[51].

It is in this sense that the penumbra in focal ischemic lesions constitutes modes of interaction not only with the central ischemic core of necrosis but also with surrounding, less involved CNS tissues.

FOCI OF CENTRAL CORE NECROSIS

Acute ischemic injury is triggered by excitotoxic elevation of intraneuronal Ca^{+2} followed by reoxygenation-dependent oxidative stress, metabolic failure and cell death[52]. Glutamate uptake by astrocytes would critically provide neuroprotection in the ischemic penumbra. Polyamines appear implicated in the development of neuronal cell death[53].

Histiocytes also produce erythropoietin, and several angiogenic and neurotrophic factors in vascular and neuronal regeneration[54].

Realized pathways of central core necrosis in cerebral ischemia would entail loss of blood supply in subsequently evolving regions of tissue loss. Indeed, one might speak of a centrally predetermined pathway of regional tissue loss that evolves consequently as loss of integral capillary beds in cerebral ischemia. In such a context, lowered pH and neuronal depolarization would operatively accompany a depletion of ATP in further augmenting effects of ischemia in states of hyperglycemia.

Glucose but not lactate aggravate the effects of acidosis in neuronal ischemia[55]. Acute hyperglycemia activates the mitochondria-initiated cell death pathway after an intermediate period of ischemia[56]. Protein kinase C delta mediates reperfusion injury *in vivo* in multiple organs[57]. Glutamate exposure is less marked in the penumbral zone with delay in neuronal death. PKC activation is triggered by Ca^{+2} released from endogenous stores[58].

Gene transcription of cytokine action: The response to hypoxia is complex and involves the regulation of multiple signaling pathways and coordinated expression of hundreds of genes[46]. Inhibitors of p53 block p53-mediated induction of cell death and concomitantly enhance NF-κ B induced survival signaling[59]. Gene transcription events and cytokine action might constitute axial systems of induced injury that are regionally predetermined and that subsequently operate at the cellular level[60,61]. Nuclear Factor-κ B is involved in brain damage in ischemia and neurodegeneration[62].

Tumor Necrosis Factor α shows biphasic expression corresponding to ischemia and reperfusion[63], and is a proinflammatory cytokine[64], that may induce ischemic neuronal cell death[65]. Induction of the naip gene (that blocks apoptosis) may contribute to neuroprotection as exerted by Tumor Necrosis Factor α in transient focal ischemia. Inhibition of Tumor Necrosis Factor-α may induce neuroprotection by preventing its release from the inactive cell-bound precursor [28].

Leukocyte interactions with endothelial cells of regional capillary beds would implicate progression in the ischemic process as neuronal/endothelial interaction. Cross talk between nitric oxide and Zn^{+2} apoptotic signal transduction pathways may promote neuronal cell death or neurodegeneration[66]. Neutrophils contribute to vascular reperfusion injury[67]. One might view

endothelium a predisposing influence in any subsequent pattern of selective neuronal susceptibility. Vascular endothelial growth factor protects neurons from ischemic cell death directly but may worsen cerebral hemodynamics after stroke[68]. Indeed, primary neuronal pathobiology as cellular attributes of response to and consequence of ischemia would operate as transforming progression in cell necrosis or apoptosis. It is in such a context that cytokine participation would selectively individualize regional involvement in individual neuronal selective vulnerability.

Neuregulin-1 protects neurons from delayed ischemia-induced apoptosis by inhibiting proinflammatory responses[69]. Interleukin-1 participates in generating reactive oxygen molecules, and in further precipitating ischemic neuronal cell injury[11].

Cellular versus tissue injury: Perinatal hypoxia/ischemia implicates a propensity for evolving injury that is developmentally predetermined.

A lack of functional F as death receptors renders neurons resistant to hypoxia and ischemia[70].

One might view cerebral palsy arising from perinatal ischemia/hypoxia a varied constellation of events that renders neurons regional determinants of such injury. A hypoxic-ischemic insult of insufficient severity that results in rapid cell death and infarction can lead to prolonged evolution of tissue damage. Specific periods of increased vulnerability occur during development[71]. Aberrant CD40 expression on microglia as induced by gene transcription via cytokines such as Interferon gamma and Tumor Necrosis Factor α may implicate neuroimmunologic cascades[72].

The capillary bed is a participant in regional ischemia that determines attributes of a central core of necrosis and of a surrounding penumbra zonally evolving as change in blood supply.

Statins modify endothelial function and increase blood flow and also have anti-inflammatory action; they may improve sensorimotor recovery after intracerebral hemorrhage[73]. In terms of such change, a no-reflow phenomenon would arise in a context of reperfusion-related events.

Oxidative stress and nitric oxide production constitute fundamental consequences of endothelial and neuronal involvement and also of glial cell proliferation. Proliferation of cells of glial type might provoke the production of cytokines, free oxygen radicals and nitric oxide that precipitate ATP depletion affecting cellular outcome. Minocycline, a tetracycline derivative, inhibits the p38 mitogen-extracted protein kinase pathway, with reduced production of nitric oxide, Tumor Necrosis Factor α and Interleukin-1 β and of inducible nitric oxide synthase in microglia[74]. Tecrolimus targets astrocytes and microglia that modulate the neuroinflammatory response. Immunosuppressants in general are neuroprotective and neurotrophic in focal and global cerebral ischemia[75].

A sudden proliferation of glial cells would further deplete ATP stores as available to regional neurons and endothelial cells.

Intracellular ATP availability and ion homeostasis enhance neuronal survival after neuronal ischemia[76].

Glutathione protects against Zn^{+2} induced neural cell death in transient cerebral ischemia[77].

Mitochondria are central to neuronal response to ischemia with critical roles in ATP generation, free radical production and regulation of apoptosis[78]. Disrupted endoplasmic reticulum Ca^{+2} homeostasis is also implicated in neuronal cell death in ischemia[79].

Cytoskeletal injury: This appears a potential inducer of progression in neuronal necrosis that is distinct from apoptosis.

In terms of such necrosis there would evolve nuclear transcription events that promote cytokine production. Cytoskeletal injury is implicated in cytokine production involving central core necrosis. Foci of ischemia would implicate transformation in cell neuronal injury and in protein deprivation pathways[50].

Energy deprivation and protein breakdown are consequences of ischemia and also induce progressive cell necrosis affecting cell viability. Astrocytes secrete cytokines and neutrophilic factors to neurons, consistent with a neurosupportive role for astrocytes, and possibly implicating astrocytic gap junctions[80].

A large number of glial-glial gap junctions and glial glutamate release would perhaps induce progression of neuroinflammation via a syncytial switch participation of glial compartments and microglia[1]. p38/SAPK2 (p38/stress activated protein kinase 2) may be inhibited by a pyrimidyl imidazole with suppression of ischemic neuronal cell death, via control of astrocytic gap junction closure[81].

CONCLUSIONS

Considerations of how injury to cells does arise as an initial ischemia might implicate self-amplifying events that induce active cellular degeneration as primary neuronal pathobiology.

One might view energy deprivation as simply a powerful system pinpointing transformation of the injured cell and as induced by cytokines.

Irreversible cell injury would constitute consequence of transcription events associated with upregulated cytokine action and within a context of injury to both neurons and endothelium. Neurotrophin and vascular endothelial biology would further determine cyclical or acyclical imbalance states of progression that variably compromise neuronal recoverability. Neurons would act as a system mechanism of propagated target action in cytokine and trophic imbalance states that characterize potentially identifiable parameters of neuronal susceptibility patterns in ischemic injury implicating transcription pathways.

REFERENCES

1. Kielian, T. and N. Esen, 2004. Effects of neuroinflammation on glia-glia gap junctional intercellular communication: A perspective. Neurochem. Intl., 45: 429-36.
2. Back, T. and O.G. Schuler, 2004. The natural course of lesion development in brain ischemia. Acta Neurochir. Suppl., 89: 55-61.
3. Mielke, J.G. and Y.T. Wang, 2005. Insulin exerts neuroprotection by counteracting the decrease in cell-surface GABA receptors following oxygen-glucose deprivation in cultured cortical neurons. J. Neurochem., 92: 103-13.
4. Williams, A.J., R. Berti, J.R. Dave, P.J. Elliot, J. Adams and F.C. Tortella, 2004. Delayed treatment of ischemia/reperfusion brain injury: Extended therapeutic window with the proteosome inhibitor MLN519. Stroke, 35: 1186-91.
5. Wei, L., D.J. Ying, L. Cui, J. Langsdorf and S. Ping Yu, 2004. Necrosis, apoptosis and hybrid death in the cortex and thalamus after barrel cortex ischemia in rats. Brain Res., 1022: 54-61.
6. Muller, G.J., C. Stadelmann, L. Bastholm, F. Elling, H. Lassmann F.F. Johansen, 2004. Ischemia leads to apoptosis- and necrosis-like neuron death in the ischemic rat hippocampus. Brain Pathol., 14: 415-24.
7. Chan, P.H., 2004. Future targets and cascades for neuroprotective strategies. Stroke, pp: 000-000.
8. Sharshar, T., D. Annane, G.L. de la Grandmaison, J.P. Brouland, N.S. Hopkinson and G. Francoise, 2004. The neuropathology of septic shock. Brain Pathol., 14: 21-33.
9. Warner, D.S., H. Sheng and L. Batinic-Haberle, 2004. Oxidants, anti-oxidants and the ischemic brain. J. Exp. Biol., 207: 3221-31.
10. Stumm, R.K., C. Zhou, S. Schulz and M. Endres *et al.*, 2004. Somatostatin receptor 2 is activated in cortical neurons and contributes to neurodegeneration after focal ischemia. J. Neurosci., 24: 11404-15.
11. Zhu, H.C., X.Q. Gao, Y. Xing, S.G. Sun, H.G. Li and Y.F. Wang, 2004. Inhibition of caspase-3 activation and apoptosis is involved in 3-nitropropionic acid-induced ischemic tolerance to transient focal cerebral ischemia in rats. J. Mol. Neurosci., 24: 299-306.
12. Miagkov, A., J. Turchan, A. Nath and D.B. Drachman, 2004. Gene transfer of baculoviral p35 by adenoviral vector protects human cerebral neurons from apoptosis. DNA Cell Biol., 23: 496-501.
13. Vidwans, A.S. and S.J. Hewett, 2004. Enhanced release of synaptic glutamate underlies the potentiation of oxygen-glucose deprivation-induced neuronal injury after induction of NOS-2. Exp. Neurol., 190: 91-101.
14. Hayashi, T., A. Saito, S. Okuno, M. Ferrand-Drake, R.L. Dodd and P.H. Chan, 2004. Oxidative injury to the endoplasmic reticulum in mouse brains after transient focal ischemia. Neurobiol. Dis., 15: 229-39.
15. Wu Chen, R., Y. Zhang, M.E. Rose and S.H.Graham, Cyclooxygenase-2 activity contributes to neuronal expression of cyclin D1 after anoxia/ischemia *in vitro* and *in vivo*. Brain Res. Mol. Brain Res., 132: 31-7.
16. Kristian, T., 2004. Metabolic stages, mitochondria and calcium in hypoxic/ischemic brain damage. Cell Calcium, 36: 221-33.
17. Cox, S., B.K. Harvey, J.F. Sanchex, J.Y. Wang and Y. Wang, 2004. Mediation of BMP7 neuroprotection by MAPK and PKC in rat primary cortical cultures. Brain Res., 1010: 55-61.
18. Wen, Y., S. Yang, R. Liu, E. Perez, K.D. Yi, P. Koulen and J.W. Simpkins, 2004. Estrogen attenuates nuclear factor-κ B activation induced by transient cerebral ischemia. Brain Res., 1008: 147-54.
19. Jiang, X., D. Mu, C. Manabat, A.A. Kosby *et al.*, 2004. Differential vulnerability of immature murine neurons to oxygen-glucose deprivation. Exp. Neurol., 190: 224-32.
20. Yin, L., H. Ohtaki, T. Nakamachi, Y. Kudo, R. Makino and S. Shioda, 2004. Delayed expressed TNFR1 co-localize with ICAM-1 in astrocytes in mice brain after transient focal ischemia. Neurosci. Lett., 370: 30-5.
21. Boche, D., C. Cunningham, J. Gaulidie and V.H. Perry, 2003. Transforming growth factor-β1-mediated neuroprotection against excitotoxic injury *in vivo*. J. Cereb. Blood Flow Metab., 23: 1174-82.
22. Schwir, A., 2004. Neuroprotection against ischemic/hypoxic brain damage: Blockers of ionotropic glutamate receptor and voltage sensitive calcium channels. Curr. Drug Targets, 5: 603-18.
23. Joly, L.M., V. Muciguat, J. Mariani, M. Plotkine and C. Charrient-Marlangue, 2004. Caspase inhibition after neuronal ischemia in the rat brain. J. Cereb. Blood Flow Metab., 24: 124-31.

24. Saito, A., P. Narasimhan, T. Hayashi, S. Okuno, M. Ferrand-Drake and P.H. Chan, 2004. Neuroprotective role of a proline-rich Akt substrate in apoptotic neuronal cell death after stroke: Relationships with nerve growth factor. J. Neurosci., 24: 1584-93.
25. Daval, J.L., G. Pourie, S. Grojean, V. Lievre, C. Strazielle, S. Blaise and P. Vert, 2004. Neonatal hypoxia triggers transient apoptosis followed by neurogenesis in the rat CA1 hippocampus. Pediatr. Res., 55: 561-7.
26. Zhang, W. and D. Stanimirovic, 2002. Current and future therapeutic strategies to target inflammation in stroke. Curr. Drug Targets Inflamm. Allergy, 1: 151-66.
27. Arthur, P.G., S.C. Lim, B.P. Meloni, S.E. Munns, A. Chan and N.W. Knuckey, 2004. The protective effect of hypoxic preconditioning on cortical neuronal cultures is associated with increases in the activity of several antioxidant enzymes. Brain Res., 1017: 146-54.
28. Yang, L., N.Y. Calingasan, J. Chen, J.J. Ley, D.A. Becker and M.F. Beal, 2005. A novel azulenylnitrone antioxidant protects against MPTP and 3-nitropropionic acid neurotoxicities. Exp. Neurology, 191: 86-93.
29. Balduini, W., S. Carloni, E. Mazzoni and M. Cimino, 2004. New therapeutic strategies in perinatal stroke. Curr. Drug Targets. CNS Neurol. Disord., 3: 315-23.
30. Kim, D.H., X. Zhao, C.H. Tu, P. Casaccia-Bonnefil and M.V. Chao, 2004. Prevention of apoptotic but not necrotic cell death following neuronal injury by neurotrophins signaling through the tyrosine kinase receptor. J. Neurosurg., 100: 79-87.
31. Sun, F.Y. and X. Guo, 2005. Molecular and cellular mechanisms of neuroprotection by vascular endothelial growth factor. J. Neurosci. Res., 79: 180-4.
32. Iwashita, A., N. Tojo, S. Matsuura and S. Yamazaki *et al.,* 2004. A novel and potent Poly (ADP-Ribose) Polymerase-1 Inhibitor, FR247304 (5-chloro-2-[3-(4-phenyl-3, 6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone), attenuates neuronal damage in *in vitro* and *in vivo* models of cerebral ischemia. J. Pharmacol. Exp. Ther., 310: 425-36.
33. Lleda, H. and R. Fujita, 2004. Cell death mode switch from necrosis to apoptosis in brain. Biol. Pharm. Bull., 27: 950-5.
34. Love, S., 2003. Neuronal expression of cell cycle-related proteins after brain ischemia in man. Neurosci. Lett., 353: 29-32.
35. Date, I., N. Takagi, K. Takagi, T. Kago, K. Matsumoto, T. Nakamura and S. Takeo, 2004. Hepatocyte growth factor attenuates cerebral ischemia-induced learning dysfunction. Biochem. Biophys. Res. Commun., 319: 1152-8.
36. Carmichael, S.T., 2003. Gene expression changes after focal stroke, traumatic brain and spinal cord injuries. Curr. Opin. Neurol., 16: 699-704.
37. Han, M.K., M. Kim, S.Y. Bae and L. Kang *et al.*, 2004. VEGF protects human cerebral hybrid neurons from *in vitro* ischemia. Neuroreport,15: 847-50.
38. Cao, G., M. Xiao, F. Sim F and X. Xiao *et al.*, 2004. Cloning of a novel Apaf-1 interacting protein: A potent suppressor of apoptosis and ischemic neuronal cell death. J. Neurosci., 24: 6189-21.
39. Abe, T., N. Takagi, M. Nakano, M. Furuya and S. Takeo, 2004. Altered Bad localization and interaction between Bad and Bcl-xL in the hippocampus after transient global ischemia. Brain Res., 1009: 159-68.
40. Beuchona, A., J. Braudeau, A. Reis, C. Couriaud and B. Onteniente, 2004. Activation of proinflammatory caspases by cathepsin B in focal cerebral ischemia. J. Cereb. Blood Flow Metab., 24: 1272-9.
41. Kaminska, B., K. Gaweda-Walerych and M. Zawadzka, 2004. Molecular mechanisms of neuroprotective action of immunosuppressants facts and hypotheses. J. Cell Mol. Med., 8: 45-58.
42. Hertz, L. and H.R. Zielke, 2004. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. Trends Neurosci., 27: 735-43.
43. Spandon, E., V. Soubasi, S. Papoutsopoulou, G. Karkavelas, C. Sineonidou, A. Kaiki-Astara and O. Giuba-Tziampiri, 2004. Erythropoietin prevents hypoxia/ischemia-induced DNA fragmentation in an experimental model of perinatal asphyxia. Neurosci. Lett., 366: 24-8.
44. Becattini, B., S. Sareth, D. Zhai, K.J. Crowell, M. Leone, J.C. Reed and M. Pelecchia, 2004. Targeting apoptosis via chemical design: inhibition of bid-induced cell death by small organic molecules. Chem. Biol., 11: 1107-17.
45. Zhu, Y., C. Culmsee, S. Klumpp and J. Krieglstein, 2004. Neuroprotection by transforming growth factor-β1 involves activation of nuclear factor-κ B through phosphatidylinositol-3-OH kinase/Akt and mitogen-activated protein kinase-extracellular-signal regulated kinase 1,2-signaling pathways. Neuroscience, 123: 897-906.
46. Seta, K.A., Y. Yuen, Z. Spicer and G. Lu *et al.*, 2004. The role of calcium in hypoxia-induced signal transduction and gene expression. Cell Calcium, 36: 331-40.
47. Garcia, O., L. Almeida Am Messieu and J.P. Bolanos, 2005. Increased mitochondrial respiration maintains the mitochondrial membrane potential and promotes survival of cerebellar neuronsin an endogenous model of glutamate receptor activation. J. Neurochem., 92: 183-90.

48. Perez-Pinzon, M.A., 2004. Neuroprotective effects of ischemic preconditioning in brain mitochondria following cerebral ischemia. J. Bioenerg Biomembs., 36: 323-7.
49. Yamashima, T., A.B. Toncheo, T. Tsukada, T.C. Saido, S. Imajoh-Ohmi, T. Momoi and E. Kominaini, 2003. Sustained calpain activation associated with lysosomal rupture executes necrosis of the postischemic CA1 neurons in primates. Hippocampus, 13: 791-800.
50. Paschen, W., T. Hayashi, A. Saito and P.H. Chan, 2004. GADD34 protein levels increase after transient ischemia in the cortex but not in the CA1 subfield: Implications for post-ischemic recovery of protein synthesis in ischemia-resistant cells. J. Neurochem., 90: 694-701.
51. Kermer, P., M.H. Digicaylioglu, M. Kaul and J.M. Zapata *et al*., 2003. BAG-1 overexpression in brain protects against stroke. Brain Pathol., 13: 495-506.
52. Starkov, A.A., C. Chinopoulos and G. Fiskum, 2004. Mitochondrial calcium and oxidative stress as mediators of ischemic brain injury. Cell Calcium, 36: 257-64.
53. Babu, G.N., K.A. Sailor, J. Beck, D. Sun and R.J. Dempsey, 2003. Ornithine decarboxylase activity in *in vivo* and *in vitro* models of cerebral ischemia. Neurochem. Res., 28: 1851-7.
54. Swanson, R.A., W. Ying and T.M. Kauppinen, 2004. Astrocyte influences on ischemic neuronal death. Curr. Mol. Med., 4: 193-205.
55. Crossberg, T., A. Rytter, F. Asztely, A. Soder and T. Wieloch, 2004. Glucose but not lactate in combination with acidosis aggravates ischemic neuronal death *in vitro*. Stroke, 35: 753-7.
56. Ding, C., Q. He and P.A. Li, 2004. Activation of cell death pathway after a brief period of global ischemia in diabetic and non-diabetic animals. Exp. Neurol., 188: 421-9.
57. Bright, R., A.P. Raval, J.M. Dembner, M.A. Perez-Puizon, G.K. Sternberg, M.A. Yenari and D. Mochly-Rosen, 2004. Protein kinase C delta mediates cerebral reperfusion injury *in vivo*. J. Neurosci., 24: 6880-8.
58. Larsen, G.A., J. Beig-Johnsen, M.C. Moe and M.L. Vinje, 2004. Calcium-dependent protein kinase C activation in acutely isolated neurons during oxygen and glucose deprivation. Neurochem. Res., 29: 1931-7.
59. Culmsee, J. Siewe., V. Junker and M. Retiorinskara *et al.,* 2003. Reciprocal inhibition of p53 and nuclear factor-κ B transcriptional activities determines cell survival or death in neurons. J. Neurosci., 23: 8586-95.
60. Allan, S.M., 2004. Pragmatic target discovery from novel gene to functionally defined drug target: The interleukin-1 story. Methods Mol. Med., 104: 333-46.
61. Kim, J.B., C.S. Piao, K.W. Lee, P.L. Han, J.L. Ahn, Y.S. Lee and J.K. Lee, 2004. Delayed genomic responses to transient middle cerebral artery occlusion in the rat. J. Neurochem., 89: 1271-82.
62. Juttler, E., I. Potrovita, V. Tarabin and S. Prinz *et al*., 2004. The cannabinoid discanabinol is an inhibitor of the nuclear factor-κ B (NF-κB). Neuropharmacology, 47: 580-92.
63. Wang, X., G.Z. Feuerstein, L. Xu and H. Wang *et al*., 2004. Inhibition of tumor necrosis factor-α-converting enzyme by a selective antagonist protects brain from focal ischemic injury in rats. Mol. Pharmacol., 65: 890-6.
64. Yin, L., H. Ohtaki, T. Nakamachi and K. Dohi *et al*., 2003. Expression of tumor necrosis factor α (TNF α) following transient cerebral ischemia. Acta Neurochir Suppl., 86: 93-6.
65. Thompson, C., D. Gary, M. Mattson, A. Mackenzie and G.S. Robertson, 2004. Kainic acid-induced naip expression in the hippocampus is blocked in mice lacking TNF receptors. Brain Res. Mol. Brain Res., 123: 126-31.
66. Bossy-Wetzel, E., M.V. Talantova, W.D. Lea and M.N. Scholzke *et al*., 2004. Crosstalk between nitric oxide and zinc pathways to neuronal cell death involving mitochondrial dysfunction and p38-activated K^+ channels. Neuron, 41: 351-65.
67. Schaller, B. and R. Graf, 2004. Cerebral ischemia and reperfusion: The pathophysiologic concept as a basis for chemical therapy. J. Cereb. Blood Flow Metab., 24: 351-71.
68. Wang, Y., E. Kilie, U. Kilic, B. Weber, C.L. Bassetti, H.H. Marti and D.M. Hermann, 2005. VEGF overexpression induces post-ischemic neuroprotection, but facilitates hemodynamic steal phenomena. Brain, 128: 52-63.
69. Xu, Z., J. Jiang, G. Ford and B.D. Ford, 2004. Neuregulin-1 is neuroprotective and attentuates inflammatory responses induced by ischemic stroke. Biochem. Biophys. Res. Commun., 322: 440-6.
70. Graham E.M., R.A. Sheddon, D.L. Flock, D.M. Ferreiro, L.J. Martin, D.P. O'Riordan and F.J. Northington, 2004. Neonatal mice lacking functional Fas death receptors are resistant to hypoxic-ischemic brain injury. Neurobiol. Dis., 17: 89-98.
71. Vannucci, S.J. and H. Hagberg, 2004. Hypoxia-ischemia in the immature brain. J. Exp. Biol., 207: 3149-54.
72. Benveniste, E.N., V.T. Nguyeu and D.R. Wesemann, 2004. Molecular regulation of CD40 gene expression in macrophages and microglia. Brain Behav. Immun., 18: 7-12.

73. Jung, K.H., K. Chu, S.W. Jeong and S.Y. Hin *et al.*, 2004. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. Stroke, 35: 1744-9.
74. Suk, K., 2004. Minocycline suppresses hypoxic activation of rodent microglia and culture. Neurosci. Lett., 366: 167-71.
75. Kaminska, B., K. Gawede-Walerych and M. Zawadzka, 2004. Molecular mechanisms of neuroprotective action of immunosuppressants facts and hypotheses. J. Cell Mol. Med., 8: 45-58.
76. Martinez-Sanchez, M., F. Striggow, U.H. Schroder, S. Kahlert, K.G. Reymann and G. Reiser, 2004. Na^{+} and Ca^{+2} homeostasis pathways, cell death and protection after oxygen-glucose-deprivation in organotypic hippocampal slice cultures. Neuroscience, 128: 729-40.
77. Cho, I.H., J.Y. Im, D. Kim, K.S. Kim, J.K. Lee and P.L. Han, 2003. Protective effects of extracellular glutathione against Zn^{+2} induced cell death *in vitro* and *in vivo*. J. Neurosci. Res., 74: 736-43.
78. Ouyang, Y.B. and R.G. Giffard, 2004. Cellular neuroprotective mechanisms in cerebral ischemia: Bcl-2 family proteins and protection of mitochondrial function. Cell Calcium, 36: 303-11.
79. Verkhratosky, A. and E.C. Toeseu, 2003. Endoplasmic reticulum Ca^{+2} homeostasis and neuronal death. J. Cell Mol. Med. 7: 351-61.
80. Nakase, T., G. Sohl, M. Theis, K. Willecke and C.C. Naus, 2004. Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin 43 in astrocytes. Am. J. Pathol., 164: 2067-75.
81. Zvalova, D., J. Cordier, M. Mesnie, M.P. Junier and H. Chneiweiss, 2004. p38/SAPK2 controls gap junction closure in astrocytes. Glia, 46: 323-33.