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EXPLORING PATHOGENIC MECHANISMS IN ISCHEMIC CEREBRAL DEATH: A RAY OF HOPE FOR DESIGNING NEW THERAPEUTIC STRATEGIES FOR STROKE

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ABSTRACT

In ischemic stroke, the blood supply to the brain is disrupted by cerebrovascular disease. After coronary heart disease (CHD) and cancer, stroke is the third commonest cause of death worldwide which necessitates exploring key targets that can treat the disease. Long duration or severe ischemia leads to necrotic cell death. However, if ischemia is transient or incomplete, the genes executing programmed cell death may be activated that depends upon the duration and severity of the ischemic insult which further is dependent on the exact location of the arterial occlusion and whether or not reperfusion occurs. The finely tuned balance of proapoptotic and antiapoptotic is not only a key point in regulating cell death or survival but also serves as a switch between the two forms of cell death, apoptosis and necrosis. Compelling evidences from more recent studies strongly suggest the involvement of cell death/survival signaling pathways. A greater understanding and future studies of the various signaling pathways involved in the pathophysiology of stroke such as Glutaminergic excitotoxic cascade, marked increase in intracellular calcium, PPAR (peroxisome proliferator activated receptor) pathways and Hsp (Heat shock protein) pathways, role for leukocytes in propagating tissue damage after ischemia and reperfusion may provide novel therapeutic strategies in clinical stroke.

KEYWORDS: ischemia, stroke, cell death, signaling pathways

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INTRODUCTION

Stroke is the leading cause of adult disability and remains the third most common cause of death worldwide[1]. Limited therapies for protection and recovery of neurons from ischemic insults have made physicians helpless. However, the success of rt-PA has revolutionized acute stroke management and proved that stroke is a treatable disease. This has also fueled the interest in the development of newer therapies for treatment[2,3]. Stroke is a disease that affecting the blood vessels that supply blood to the brain and occurs when a blood vessel that brings oxygen and nutrients to the brain either bursts (hemorrhagic stroke) or is clogged by a blood clot or some other mass (ischemic stroke). The devastating effects of a severe stroke are often permanent due to the fact that dead brain cells are not replaced[4,5]. Ischemic stroke being the most common type accounts for about 87 percent of all strokes[4]. It occurs due to formation of a blood clot (thrombus) and blockage of blood flow in an artery bringing blood to part of the brain[6,7].

Cerebral ischemia as the initial step to stroke

Cerebral ischemia occurs when the amount of oxygen and other nutrients is insufficient to meet the metabolic demands of brain tissue. The blood supply to the brain is disrupted by cerebrovascular disease in ischemic stroke. For decades, far-reaching research and clinical advances have been focused on the vascular aspects of cerebral ischemia to combat stroke. Carotid endarterectomy, thrombolytic therapy, anticoagulation for cardiogenic stroke, antiplatelet agents like therapeutic interventions, with the treatment of risk factors such as hypertension and hyperlipemia, have led to significant effects on the morbidity and mortality of stroke. The final event in cerebral ischemia is the death of neurons, resulting in irreversible loss of neurologic function. However, many secondary biochemical changes that aggravate injury may occur in response to the initial insult. In cerebral ischemic rodent models, it has been demonstrated that as

much as 50% or more of ischemic brain may be spared from infarction by preventing as well as attenuating these secondary biochemical events. Understanding the mechanisms by which neuronal cell death takes place has resulted in a number of therapeutic strategies aiming to prevent secondary biochemical changes and thus decrease the damage that results from cerebral ischemia. Thus the advent of animal and tissue culture models of ischemia has led to many new insights into the mechanisms by which ischemic neurons die.

Necrotic Cell Death

Although the primary pathologic mechanism in ischemic stroke is the depletion of energy stores; many compelling evidences indicates the involvement of excitatory amino acids (EAAs) in exacerbation of ischemic injury. Under physiologic conditions, EAAs such as glutamate released by approximately 40% of all synapses in the central nervous system participates in many neurologic functions, including memory, movement, sensation, cognition, and most importantly synaptic plasticity. Choi et.al demonstrated that micromolar extracellular glutamate and other EAAs produce rapid increases in intraneuronal cytosolic Ca^{2+} concentrations which is rapidly lethal to primary neuronal cultures (Fig 1).

The increase in intraneuronal Ca^{2+} in response to extracellular EAAs *in vitro* is mediated by the opening of a receptor gated ion channel, the N methyl-D-aspartate (NMDA) channel primarily gating calcium entry into the neuron. Glycine is a co-agonist required in addition to glutamate to open the NMDA Ca^{2+} channel. Treatment with antagonists that compete with glutamate and other EAAs for the receptor (competitive NMDA antagonists) or antagonists that bind to the ion channel itself (noncompetitive antagonists) and antagonists binding to the glycine site can block calcium entry into neurons, block excitotoxicity *in vitro* and prevent cell death induced by glutamate. A possible way to rescue neurons in culture from EAA toxicity is by removal of extracellular calcium and sodium from the culture media following glutamate exposure. Conversely, inhibition of the sodium–calcium exchanger that normally facilitates extrusion of calcium results in an increase in neuronal death. Dantrolene, an agent attenuating decompartmentalization of intracellular stores of calcium, reduces glutamate neurotoxicity in cortical neurons. Neurons containing high concentrations of calcium binding proteins, such as calbindin or parvalbumin, are relatively resistant to excitotoxic injury. Although NMDA antagonists are not effective in temperature controlled global ischemia models, a large number of studies have found decreased infarction volume in both permanent and temporary middle cerebral artery occlusion models in rodents. Moreover, blocking the translation of a gene encoding a subunit of the NMDA receptor with intraventricular injection of antisense oligonucleotides also decreases infarction volume after middle cerebral artery occlusion in the rat. Many other studies also support the hypothesis that excitotoxicity contributes to ischemic injury *in vivo*.

Several calcium-dependent or calcium-induced enzymes mediate the toxic effects of increased intracellular calcium (Fig 1) which includes nitric oxide synthase, cyclooxygenase, phospholipase A2, and calpain 1. Therefore, EAA-mediated elevation of intracellular calcium concentrations activates both cyclooxygenase and nitric oxide synthase, which then synergistically contribute to ischemic brain injury. EAAs also activate other receptors besides the NMDA channel like ionotropic receptors coupled directly to membrane ion channels and metabotropic receptors increasing intracellular calcium by mobilizing calcium from stores in the endoplasmic reticulum. Studies show that antagonists of the metabotropic receptor, depending on their subunit specificity are neuroprotective in models of focal ischemia. Moreover, a number of complex interactions and positive feedback loops may augment the contribution of EAAs to ischemic brain injury in addition to the direct downstream effects of enzymes that are activated by elevation of intracellular calcium. Thus free arachidonic acid can potentiate NMDA evoked currents in neurons and inhibit reuptake of glutamate by astrocytes. Although non-NMDA antagonists have shown limited effects in primary neuronal tissue culture models, they are very effective in both global and focal ischemia models in rodents with a longer time window of efficacy than do NMDA antagonists when administered after the onset of ischemia. Likewise, voltage-dependent calcium channel antagonists are ineffective *in vitro* but the nimodipine is effective in reducing infarction volume in temporary focal ischemia in rats. These data provide compelling evidence that EAA-induced increases in intracellular Ca^{2+} are toxic to neurons in culture with concrete hold up to indicate that excitotoxicity mediated by the NMDA receptor contributes to injury from cerebral ischemia *in vivo* with substantial involvement of non-NMDA receptors as well.

Programmed Cell Death

Many of the key molecular events in programmed cell death have now been determined (Fig.2) with the release of cytochrome c from the mitochondria as a key event in initiating apoptosis in many cell types. Cytosolic cytochrome c complexes with APAF-1 and procaspase 9 this in turn leads to cleavage of procaspase 9 into its active form, caspase 9. Caspase 9 then cleaves and

activates other caspases, including caspase 3. Caspases were first identified by their homology with CED3, the key gene that irreversibly commits neurons in C. elegans to programmed cell death are a family of proteases that play a key role in executing programmed cell death. A dozen mammalian caspases have been identified that have a multiplicity of roles in executing programmed cell death and other cellular functions. Among these, caspase 3 has the closest homology with CED3 and appears to play a key role as the final committed step in programmed cell death. Caspase 3 executes programmed cell death via cleavage of many other proteins such as cytoskeletal protein(s), DNA repair proteins such as PARP, and other proteins as well as cleaves ICAD, an inhibitor of CAD (caspase-activated DNase), an endonuclease that cleaves DNA between histosomes. The result is cleavage of DNA between histosomes, a hallmark of programmed cell death. Genes of the bcl-2 family play an important role in controlling exit of cytochrome c from the mitochondria into the cytosol. Anti-apoptotic bcl-2 family members, such as bcl-2 itself and bcl-x-long, inhibit the egress of cytochrome c. Pro-apoptotic members of the bcl-2 family, such as bcl-x-short and bax, form dimers with themselves or with anti-apoptotic bcl-2 family members. The balance between the pro-apoptotic (bcl-x-short and bax) and anti-apoptotic (bcl-2 and bcl-x-long) bcl-2 family proteins in the mitochondrial membrane determines whether permeability of the membrane will increase to allow egress of cytochrome c into the cytoplasm. Under some circumstances, cytochrome c exits the mitochondria via the mitochondrial permeability transition pore which can open in response to prolonged depolarization, produced by such stimuli as an increase in intracellular calcium. However, bcl-2 family members themselves may form pores in membranes, and some evidence also indicates that bax induces egress of cytochrome c from the mitochondria independent of the mitochondrial permeability transition pore. Initiation of the mitochondrial apoptosis is also controlled by expression and translocation of bax from the cytosol to the mitochondria initiates programmed cell death. More than 20 additional proteins are found in the bcl-2 family, including many that are also involved in mitochondrial homeostasis. Thus, a key event in apoptosis, egress of cytochrome c from the mitochondria, is controlled by bcl-2 family proteins. The molecular mechanisms by which programmed cell death is initiated are numerous and complex. Programmed cell death is activated via cell surface receptors, including the Fas receptor and tumor necrosis factor-α (TNF- α) which triggers activation of caspase 8, which in turn cleaves the bcl-2 family protein bid and translocates from the cytoplasm to the mitochondria, where it initiates cytochrome c egress. Other mechanisms controlling the initiation of programmed cell death includes the ERK (externally regulated kinase) and JNK protein kinase cascades. Finally, DNA base oxidation and other DNA damage may initiate programmed cell death via expression of the p53 transcription factor. These and other mechanisms have been involved in the initiation of programmed cell death in ischemic neurons.

Compelling evidences show that many of the mechanisms that initiate programmed cell death are activated in ischemic neurons under certain conditions. TNF-α mRNA transcription is induced as an early response after cerebral ischemia. However, ischemic injury has shown to exacerbate in TNF- α -receptor null mice, which suggests that TNF signaling pathways may instead have beneficial effects in ischemic injury under some circumstances. Caspase 8, which is activated by both the Fas and TNF receptors, is expressed and activated after cerebral ischemia. Expression and activity of both the ERK and JNK kinase pathways changes following cerebral ischemia. The increased expression of ERK after focal ischemia and inhibitor of NEK-1, another kinase in the ERK pathway, protect the brain against focal cerebral ischemia. Single-stranded DNA damage trigger expression of p53 in an early event in cerebral ischemia reperfusion injury. A number of studies in cerebral ischemia support a role for bcl-2 family genes in controlling ischemic neuronal death. In rodent models of ischemia, anti-apoptotic members of the bcl-2 family, including bcl-2 and bcl-x long, are expressed in neurons that are ischemic yet survive. Abundant *in vivo* evidences suggests that caspase activity exacerbates ischemic injury. Transgenic mice that express a dominant negative mutation of caspase 1 had smaller infarctions as compared to their wild-type litter mates. Intraventricular infusion of peptide inhibitors of caspases decreased infarction volume in rats blocked damage in response to injection of excitotoxins subjected to temporary middle cerebral artery occlusion. Caspase 3 is activated, and treatment with a specific peptide inhibitor of caspase 3 ameliorated neuronal death in the global ischemia model. These and other studies support a role for caspases in ischemic neuronal injury[8]. Thus blockade of excitotoxicity via all these pharmacologic strategies has proved effective in temporary focal ischemia models in rodents, the model that most closely resembles human stroke. Unfortunately, results with these agents in human trials have to date been very disappointing, for several possible reasons.

CELL SURFACE SIGNALLING

Neuronal excitability is the result of an imbalance of ions across a cell's membrane. During ischemia the balance of ions across a cell's membrane is disrupted and the altered Ca^{2+} homeostasis mediates an excitotoxic cascade culminating over hours to days. The cascade of events leading to this death can be divided into 3 stages[9,10].

Potassium Channels

K^+ channels are a major contributor to a cell's resting potential as their activation helps to maintain a hyperpolarized resting membrane potential. K^+ efflux occurs much sooner after the onset of ischemia than either the Na^+ or Ca^{2+} influx, which occurs after ATP levels have fallen by more than 50%[11]. There are a number of different types of K^+ channels. The metabolic nature of an ischemic insult suggests that ATPsensitive K^+ channels, activated by a decrease in ATP are one of the first channels to respond during ischemia. In hypoxic rats, the early K^+ efflux in the dorsal hippocampus can be blocked by pretreatment with 4-aminopyridine (4-AP) a blocker of voltage-activated K^+ channels which is found predominantly in the dendritic portion of hippocampal pyramidal neurons[10]. At later phases after ischemia, other K^+ channels like the Ca^{2+}-activated K^+ channels are activated[11], by an increase in intracellular Ca^{2+} and the ATP-sensitive K^+ channels[12-15]. Astrocytes attempts to buffer this increase in $[K^+]e$ by switching to anaerobic glycolysis and swell 5 to 10 times their normal size. Eventually, astrocytes no longer able to cope with the increase in $[K^+]$ e lyse[10]. These are the events that occur during the first stage of ischemia. There is little knowledge of the behavior or expression of K^+ channels in the second stage but their role in *in vitro* neuronal apoptosis[16], suggests warranty of a closer examination of these channels. However, the development of therapeutic agents targeting K^+ channels in the brain is not easy due to the fact that K^+ channels are ubiquitously expressed throughout the body thus specificity will be a problem[17].

Sodium Channels

Na^+channels play an important role in neuronal excitability and are as widely expressed as K^+ channels. Na^+ channels have been studied less in ischemic gray matter, but have been extensively studied in ischemic white matter[18-20]. In cerebral ischemia there is a pronounced Na^+ influx at the end of the first stage of ischemia (Fig 3), coincident with the Ca^{2+} influx and the anoxic depolarization associated with energy failure[21,22]. Although problems of specificity do exist for therapeutic agents targeting Na^+ channels as, there are evidences using local anesthetics suggesting inhibition of Na^+ channels as a neuroprotective. Some anticonvulsants that inhibit Na^+ channels are also neuroprotective *in vivo*[23]. Lamotrigine and its derivatives BW1003C87 and BW619C89 are protective in models of focal and global ischemia whereas, the Na^+ channel blockers riluzole and its derivative RP66055 are also protective in both focal and global models of cerebral ischemia. However, the role of Na^+ channels in cerebral ischemic death should not be over emphasized because these agents are not selective[24]. Riluzole antagonizes N-methyl- D-aspartate (NMDA) receptors and lamotrigine and its derivatives inhibit Ca^{2+} channels as well. Furthermore, many of the Na^+ channel antagonists exhibit severe cardiovascular effects that eliminating them as therapeutic agents for the treatment of stroke[25].

Calcium Channels

As Ca^{2+} influx and the disruption of Ca^{2+} homeostasis play an important role in ischemic cell death, Ca^{2+} channels have received a lot of attention in studies of cerebral ischemia (Fig 3). In addition to the Ca^{2+} influx through voltage-gated Ca^{2+} channels, a much larger portion of the influx occurs through ligand-gated ion channels[26]. The voltage-gated Ca^{2+} channels there are primarily of 5 types, L, T, N, R, and P/Q, which are defined by their subunit molecular biology and pharmacology. L-type channels are located mainly on the cell bodies of neurons and to a lesser degree on dendrites and have thought to activate gene responses. Much of the insight on the role of the various Ca^{2+} channel subtypes in ischemic neuronal injury comes from studies using pharmacological agents[27]. Dihydropyridines have shown limited success in animal models of cerebral ischemia. Nimodipine, which has high blood brain barrier permeability, is neuroprotective in some models of focal and global ischemia but isradipine66 and AT-22767 failed to reduce lesion volume in focal models of ischemia[24]. But several clinical trials have demonstated no neuroprotective efficacy using Ca^{2+} channel antagonists. However, due to the importance of glutamate in ischemia, the Ca^{2+} channels involved in glutamate release provides an attractive therapeutic target. The conotoxin, SNX-111, which specifically blocks N-type channels, is neuroprotective in both focal and global models of ischemia whereas antagonists of Q-type (SNX-230), and P-type (daurisoline), Ca^{2+} channels have failed to provide neuroprotection against ischemia although the efficacy of SNX-111 was impressive in animal models, this efficacy was not translated into success in clinical trials for the treatment of stroke. Along with K^+ and Na^+ channel antagonists, agents targeting voltage-gated Ca^{2+} channels will face the problem of specificity because of the ubiquitous expression of these channels[28].

COX-2 EXPRESSION FOLLOWING EXCITOTOXICITY

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most used drugs worldwide. The two enzymes COX-1 and COX-2share similarities in terms of catalytic mechanisms and kinetics, but there are two major structural differences that have important significance from the pharmacological and biological viewpoint. COX-2 has a larger and more accommodating cyclooxygenase active site as compared with COX-1[29] and COX-1exhibits negative allosterism at low AA (arachadonic acid) concentrations. Thus, when both isoforms are expressed in the same cell, COX-2 competes more effectively for newly released AA[30]. COX-1 is widely expressed in neurons and microglia in several animal species[31], microglia in both rat[30] and human[29] and relative high levels are also found in hippocampal and cortical neurons in control human brain[32]. Moreover, the brain is one of the few tissues that constitutively express COX-2. However, a dramatic increase in COX-2 mRNA and protein levels occurs following *in vivo* excitotoxic injury. Upregulation of COX-2 mRNA and protein has been found in neuronal cultures exposed to excitotoxic insults *in vitro*. Studies have shown that the increased COX-2 expression following kainate excitotoxicity can be prevented by blockade of glutamate receptors[31] and by an antagonist of platelet-activating factor (PAF) receptor[30]. Substantial COX-2 overexpression was found in vulnerable neurons following kainic acid injection. On the contrary, there is no elevation in the COX-1 expression by excitotoxic damage[31].

Several dynamics are responsible for the cytotoxicity of COX-2 in the setting of cerebral ischemia, which result in neuronal injury, increased production of free radicals and PGE2 amongst the most recognized mechanisms of toxicity linked to increased COX-2 activity. However, other processes which could potentially lead to neuronal death are found to be modulated by COX-2 like: 1) promotion of cell cycle activity by increasing cyclin D1 expression, and 2) metabolism of endocannabinoids. Oxidative stress is one of the major determinants of ischemic neuronal death[32]. Cerebral ischemia results in a substantial increase in the availability of arachidonic acid, the substrate for the COX enzymatic pathway. Experimental evidences support the theory that COX catalytic activity is linked to the production of free radicals[29]. Detailed biochemical investigations have demonstrated that free radicals are produced by the peroxidase step of the COX reaction in which PGG2 is converted to PGH2. The two major types of radicals so far known to be involved in COX activity are tyrosyl radicals on proteins and carbon-centered radicals on the substrate arachidonic acid[32]. However, there is still debate on the chemical nature of the free radical(s) involved in COX-2-mediated oxidative stress during inflammation and cerebral ischemia due to the characteristically short half-life of free radicals, and the technical difficulties associated with their direct measurement in biological systems[33].But, there is an prodigious line of evidence indicating that augmented COX activity following cerebral ischemia and excitotoxicity is associated with oxidative damage. Pharmacological inhibition of COX-2 with either nimesulide or rofecoxib has shown to result in a significant reduction in measures of oxidative injury in the hippocampus following global cerebral ischemia in gerbils. These COX-2 inhibitors prevented ischemia-induced glutathione depletion and the increase in lipid peroxidation, as was assessed by the levels of lipid hydroperoxides, malondialdehyde (MDA) and 4-hydroxy-alkenals. Nimesulide treatment reduced lipid peroxidation and prevented the depletion of reduced glutathione following reperfusion in a rat model of global forebrain ischemia. Treatment with nimesulide significantly reduced oxidative injury in the rat hippocampus after systemic kainate injection. A microdialysis study in the hippocampus of freely moving rats showed that the COX inhibitors flurbiprofen and indomethacin, or the COX-2 selective inhibitor NS-398 effectively reduced 8-epi- PGF2α (15-F2t-IsoP), a reliable marker of free radical-mediated lipid peroxidation, following infusion of NMDA. It has been reported that the COX-1 inhibitor valeryl salicylate reduced measures of oxidative stress in the gerbil hippocampus following temporary global ischemia which supports the previous study. This suggests that COX-1 may also contribute to oxidative injury following excitotoxicity and brain ischemia[31].

INFLAMMATION AND CELL ADHESION MOLECULES

A number of recent investigations have recognized role of leukocytes in propagating tissue damage after ischemia and reperfusion in stroke[34]. Experimental data obtained from animal models of middle cerebral artery occlusion implicate inflammatory cell adhesion molecules, chemokines, and cytokines in the pathogenesis of ischemic damage.Three structural classes of cell adhesion molecules influences leukocyte migration, homing, and cell-cell interactions during the inflammatory response: (1) selectins, (2) integrins, and (3) proteins of the immunoglobulin superfamily.

Selectins

The selectins are glycoproteins and mediate low-affinity endothelial-leukocyte interactions, thereby promotes the margination and rolling of leukocytes via interactions of carbohydrate residues. This family of cell adhesion molecules includes P-, E-, and L-selectin. P-selectin found on platelets as well as endothelial cells, and its counterreceptor on leukocytes contains the oligosaccharide sialyl Lewis X. Pre-existing cytoplasmic stores of P-selectin in endothelial cell Weibel-Palade bodies permit its rapid mobilization to the cell surface within minutes of endothelial cell activation by thrombin[34], complement, and histamine this early relocalization in turn aids in the preliminary adhesion of leukocytes. However, there is evidence of its increased expression in post-ischemic cerebral vasculature. This suggests a role for P-selectin in ischemic cerebral injury via the promotion of leukocyte adhesion and the development of the postischemic no-reflow state. The role of E-selectin in the pathogenesis of ischemic stroke is less well established as there is no preformed pool of E-selectin. But E-selectin is found in endothelial cells and leukocytes and is important in the development of the inflammatory response as *in vivo* study supports the hypothesis that leukocyte adhesion molecules contribute to neutrophil accumulation and the ensuing reperfusion injury. However, there are conflicting data from studies of human serum, in which soluble isoforms of adhesion molecules can be quantified, presumably after they are shed from activated cell surfaces[35]. These conflicting results make the relevance of E-selectin in human cerebral ischemia unclear.

Leukocytes and the acute inflammatory response

The leukocytes originating from the myeloid stem cell include the monocytes and neutrophils. Leukocytes circulate in the bloodstream and enter the tissues when recruited to sites of infection or inflammation. The acute inflammatory response is initiated by the adherence of neutrophils to the ischemic endothelium and if reperfusion is established and circulating blood returns through the vessels, it carries the additional neutrophils underlying reperfusion injury to the sites of tissue ischemia. Once adhered to the microvasculature, the neutrophils cross the blood-brain barrier, tissue damage is incited through their release of oxygen-free radicals and proteolytic enzymes. Thus, recently much interest has been shown in cerebroprotective strategies targeted specifically at neutrophils[34]. Experimental models of stroke with neutrophil depletion, inhibition of neutrophil adhesion, and inhibition of neutrophil function have shown to reduce infarct sizes and improved outcomes. Specifically, protein kinase C has been shown to play a significant role in neutrophil adhesion, degranulation, and superoxide generation. Knockout mice with deletionally mutant for protein kinase C demonstrated diminished infarct volumes when subjected to transient cerebral ischemia. Neutrophils have also been concerned in ischemic injury as a source of MMP-9, which is a protease that degrades the basal lamina and mediates breakdown of the blood-brain barrier after cerebral parenchymal injury. Although the exact mechanism is not known, it has been shown that MMP-9 is released into ischemic brain concurrently with neutrophil accumulation within ischemic microvessels[35].

HEAT SHOCK PROTEINS (HSP)

The major antiapoptotic pathway, the phosphatidylinositol- 3-kinase (PI-3-kinase)/Akt pathway, mainly responds to growth factor withdrawal. The Akt kinase is activated via the PI-3-kinase, Akt phosphorylates the proapoptotic factors, like Bcl-2-associated death protein (BAD), and enables them to bind to the protein, which sequesters and inhibits them and thus stalls apoptosis. Akt enhances the production of NO by phosphorylating eNOS at Ser-1179, as another mechanism to inhibit apoptosis. Once activated, endothelial cell survival pathways involve Akt, and it remains to be seen whether NO plays a role in this process. Hsp27 has shown to be associated with Akt and protects its kinase activity from heat stress and serum deprivation in PC12 embryonal carcinoma cells. Moreover, in neutrophils Hsp27 association is necessary for Akt activation. Akt induced phosphorylation of Hsp27 results in its dissociation from Akt and enhanced neutrophil apoptosis. Hsp90 interacts with both the Akt-activator 3-phosphoinositide-dependent protein kinase-1 (PDK1) and Akt itself and is a necessary chaperone for the Akt kinase thus Hsp90 serves as a molecular scaffold to promote the Akt-induced phosphorylation and activation of eNOS. Heat shock proteins in tumor cells such as Hsp70, Hsp27, and Hsp90 can inhibit apoptosis by direct physical interaction with apoptotic molecules, are over expressed in several tumor Moreover, because of Hsp-induced simultaneous stabilization of various proteins, Hsp inhibitors target not only a specific molecule, but makes them potentially more effective in the induction of tumor cell apoptosis. A recent report has shown an important element of tumor specificity of Hsp90 inhibitors. Activated Hsp90 forms a large complex with various co-chaperones in tumor cells; whereas it is found in a latent, uncomplexed state in normal cells. The geldanamycin-derivative, 17-AAG, binds to the tumor specific, complexed form of Hsp90, with a 100-fold higher affinity than the latent form in nontransformed cells raising a possibility of Hsp90

behaving as a tumor selective catalyst in converting geldanamycin derivatives to their active conformation. Hsp90 inhibition leads to the dissociation of various Hsp90 client proteins from the chaperone complex and to their consecutive degradation by the proteasome as well as induces apoptosis of various tumor cells. Hsp90 inhibition also results in defect in a number of proliferative signals including the Akt-dependent survival pathway. Inhibition of Hsp90 was shown to reduce chemoresistant tumors with poor prognosis as well[36]. The most important Hsp90 inhibitors are geldanamycin, its less toxic analogue, 17-allylamino-17-demethoxy- geldanamycin (17-AAG, radicicol, and its more stable oxime derivatives, having higher affinity for Hsp90 than geldanamycin. Recently, new geldanamycin analogues and a third class of inhibitors, the purine scaffold inhibitors are being developed, and there are ongoing efforts to synthesize even more Hsp90-interacting drug candidates.

CEREBRAL MECHANISMS OF PPAR IN THE BRAIN

PPARs are expressed in cerebral or spinal blood vessels[37], as well as in neurons and astrocytes, whereas oligodendrocytes exclusively show PPARβ/δ expression. PPARβ/δ has been found in numerous brain areas, while PPARα and PPARγ have been localized to more restricted brains areas thus the extent of expression depends on the isoform of PPAR involved. Regardless of the aetiology, neuronal death is induced by inflammatory and oxidative processes including both necrotic and apoptotic neuronal death. Fibrates, lipid-lowering agents, contributes to secondary prevention of stroke, thus it has been supposed that these PPARα activators could also preventively protect the brain against noxious biological reactions induced by cerebral ischaemia, such as oxidative stress and inflammation[38]. Recently, it has been established that PPARα agonists induces an acute neuroprotection if administered just before cerebral ischaemia or during the reperfusion period. A study showed that administration of the PPARγ agonist troglitazone or pioglitazone 24 or 72 h before and at the time of cerebral infarction dramatically reduced infarction volume and improved neurological function following transient middle cerebral artery occlusion in rats which was exerted in a dose-dependent manner. Intracerebroventricular administration of pioglitazone reproduced this neuroprotection, demonstrating that the activation of intracerebral PPARγ confers neuroprotection and neurological improvement following ischaemic injury. Moreover, a non-thiazolidinedione PPARγ agonist (L-796449) has also shown to exert neuroprotective effect in experimental stroke.

The neuroprotection observed with PPAR agonists is due to the virtue of several mechanisms including oxidative stress modulation and anti-inflammatory effect. Study demonstrated that PPARα agonist-induced neuroprotective effect is associated with an increase in activity of numerous antioxidant enzymes; in particular Cu/Zn superoxide dismutase (PPREs (PPAR-response elements) have been found in the gene of Cu/Zn superoxide dismutase) and glutathione peroxidase thus in turn decrease in cerebral oxidative stress. This modulation of antioxidant enzymes is accountable for a decrease in ischaemia-induced reactive oxygen species production and lipid peroxidation[37]. The neuroprotective effects of PPAR agonists are also associated with inhibition of ischaemia-induced inflammatory markers (interleukin-1β, COX-2 and inducible nitric oxide synthase). However, different PPAR isoforms do not modulate the inflammatory pathways involved in neuroprotection in a similar manner. For example, PPARγ agonists prevent ischaemia-induced COX-2 over expression but it is not prevented by PPARα agonists. Moreover, PPAR induced modulation of oxidative stress and inflammation are associated, since prevention of COX-2 induction results from oxidative stress inhibition. PPARγ agonists, such pioglitazone, are able to decrease microglial activation when administered intracerebrally thus the cellular target of these anti-inflammatory effects may probably on the microglial cells with the key target being NF-κB, which plays a crucial role in neuronal death[38]. However, ahead of this direct effect on ischaemia-induced deleterious pathways explaining neuroprotection, the challenge is to demonstrate that PPAR activators may lead to neurological improvement by neurorepair, since PPARγ s are also involved in the regulation of neural stem cell proliferation and differentiation

CONCLUSION

Currently the management of stroke varies greatly from center to center and from country to country. For the last two decades, the search for the neuroprotective therapies for acute ischemic stroke has experienced a roller coaster ride. Moreover, approval of rt-PA (Alteplase) for acute stroke management in many countries has transformed the management of ischemic stroke as it is beyond doubt effective and safe when administered properly. Initial preclinical studies demonstrated many drugs effective for treating acute stroke in animal models but subsequent clinical trials were frustrating, and none of the agents has proven effective. The various outcomes of preclinical and clinical trials have been the subject of much discussion. However, much extensive research needs to be envisaged. Learning from past failures, thus may give

reasons to believe that at least some of the neuroprotective agents will be proven to be valuable. From several results accumulated over the past decade and more recent studies strongly suggest the involvement of cell death/survival signaling pathways. Substantial progress in understanding the mechanisms of apoptotic cell death and survival signaling pathways has been provided by genetic manipulation of factors in these signaling pathways. Thus future studies of these pathways may provide novel therapeutic strategies in clinical stroke.

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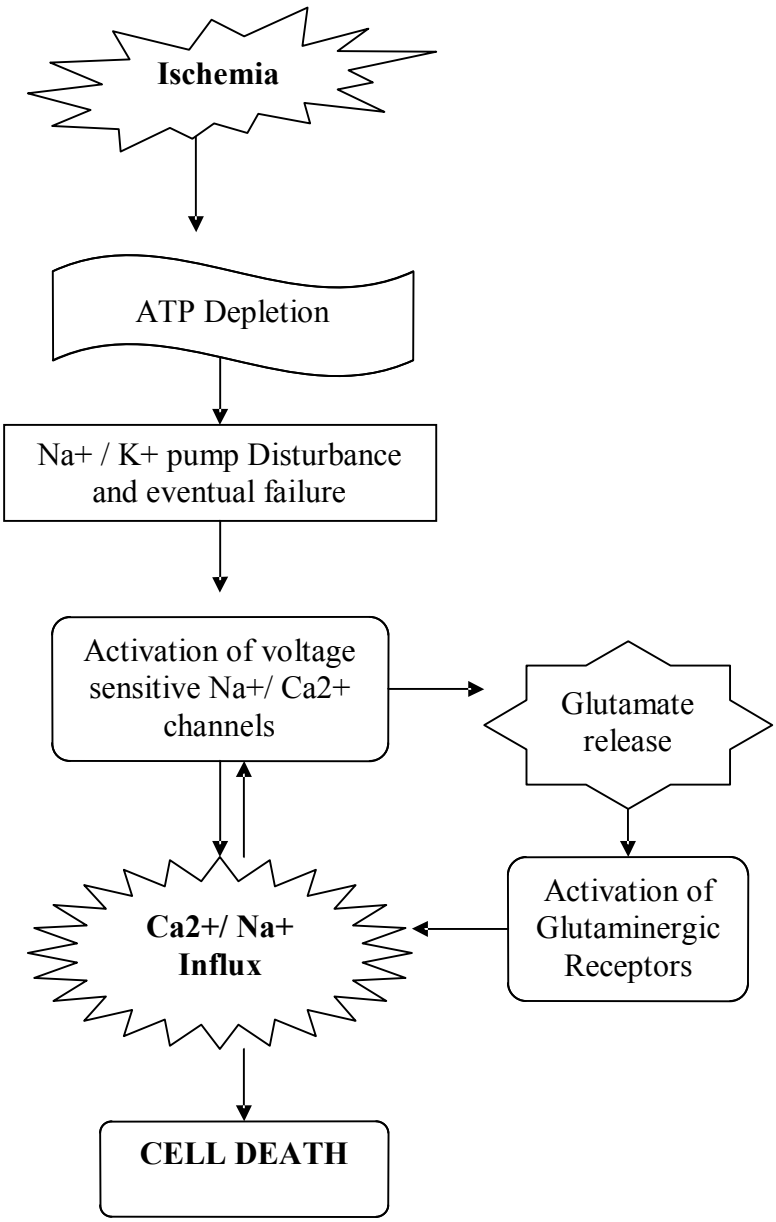

Fig 1: Initial ischemic cascade

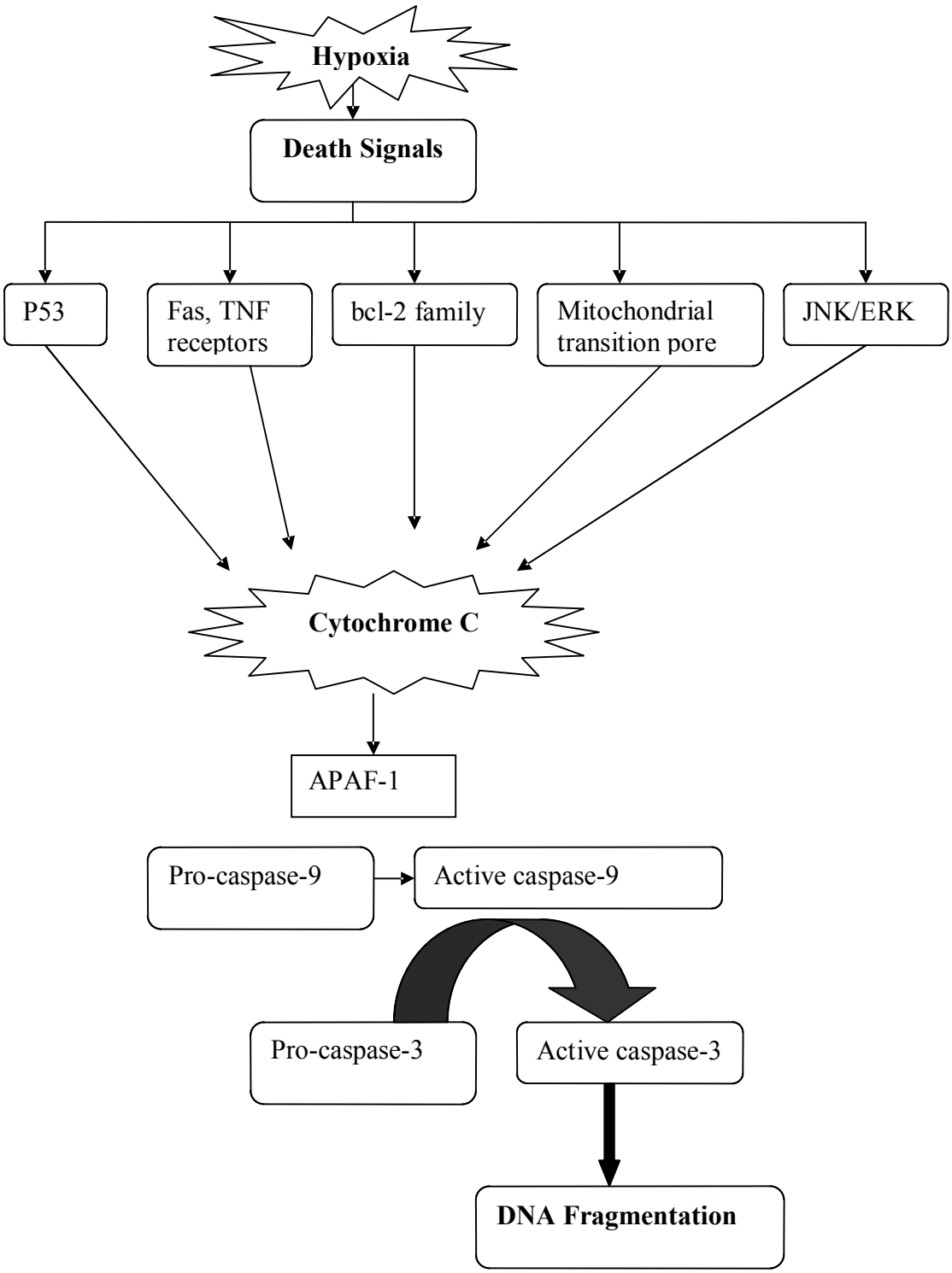

Figure: 2 The molecular mechanisms that control programmed cell death.

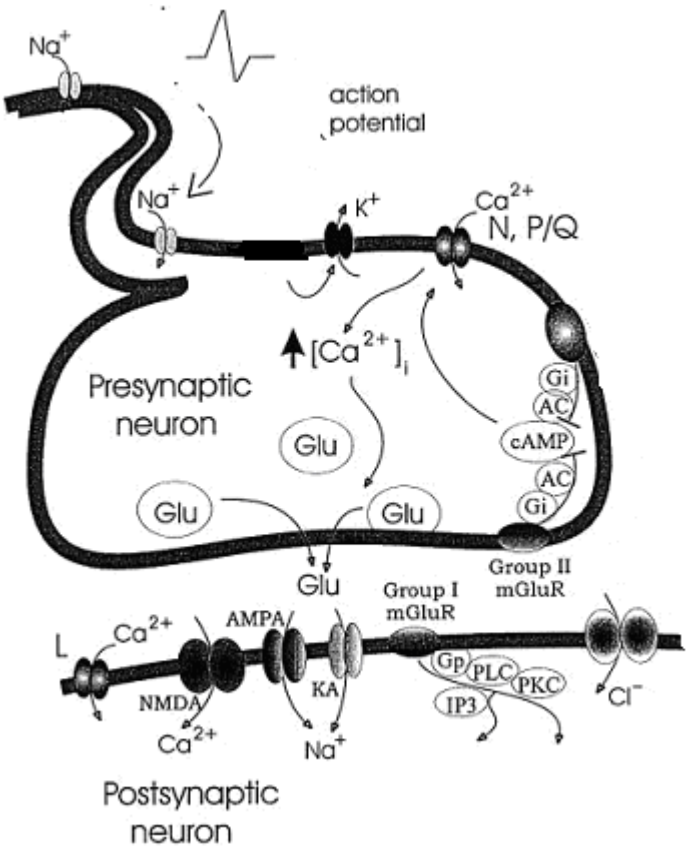

Figure: 3 Cell surface signaling in excitotoxic cascade of ischemic damage.

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