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REVIEW

Some new prospects in the understanding of the molecular basis of the pathogenesis of stroke

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Abstract Stroke is one of the leading causes of mortality and morbidity in advanced countries of the world. Despite the fact that reactive oxygen and nitrogen species (ROS and RNS) are the by-products of normal metabolic processes and mediate important physiological processes, they can inflict damage to the cell if produced in excess due to oxidative stress. In the present review, we focus on the cellular and molecular aspects of ROS and RNS generation and its role in the pathogenesis of stroke produced by hypoxia-reperfusion (H-R) phenomena that elicit oxidative stress. We outline the reasons for the vulnerability of the brain to ischaemic insult, chronic infection and inflammation as well as the natural defence mechanisms against radical mediated injury. We deal with the effect of ROS and RNS on intracellular signaling pathways together with the phenomena of apoptosis, mitochondrial injury and survival associated with these pathways. The intracellular signaling mechanisms influenced by reactive species can have significant effects on the outcome of the condition. Future studies should focus on understanding the molecular mechanisms involved in the action of anti-radicals agents, and their mode of action.

Keywords Stroke · Reactive oxygen species · Chronic inflammation/infection · Oxidative stress · Ischaemia reperfusion injury · Signaling pathways · Apoptosis

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Introduction

Stroke or "cerebral infarction" is the third leading cause of death in Western countries (Adibhatla and Hatcher 2003). Stroke is a complex condition arising from interplay of factors, such as genetic predisposition and others, that impinge on the integrity of blood vessels, interfering with the blood supply to the central nervous system (CNS) (Adibhatla and Hatcher 2005; Adibhatla et al. 2002). The risk factors for stroke include high blood cholesterol, hypertension, low physical activity, hyperhomocysteinemia (resulting from disturbed methionine metabolism) and smoking (Alexandrova and Bochev 2005; Asahi et al. 2000; Bolander-Gouaille 2000). Basically two types of stroke have been demonstrated in humans: (1) that induced by a total loss of blood flow to the brain, such as during a cardiac arrest, (2) cerebral ischaemia arising from a focal loss of blood flow to the brain due to arterial blockage (Carden and Granger 2000) (Fig. 1).

Ischemic injury to neurons is mainly caused by the interruption of blood flow, hypoxia, ATP depletion and subsequent re-oxygenation of the brain in ischaemia-reperfusion (Chan 2001). It has been observed that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are predominantly involved in the pathogenesis of stroke. They also play an important role in the exacerbation of the phase following stroke by triggering off pro-apoptotic pathways that reduce the survival chances of the neurons (Chen et al. 2005; Osta et al. 2005; Crack and Taylor 2005).

In cerebral ischemia there is an ischaemic gradient that can be divided into a core, which is the central ischaemic zone, and the penumbra, which is located in more peripheral zones. The core of the cerebral infarct is not recoverable but the penumbra may recover and is identified as a target for the development of therapeutic strategies. In the

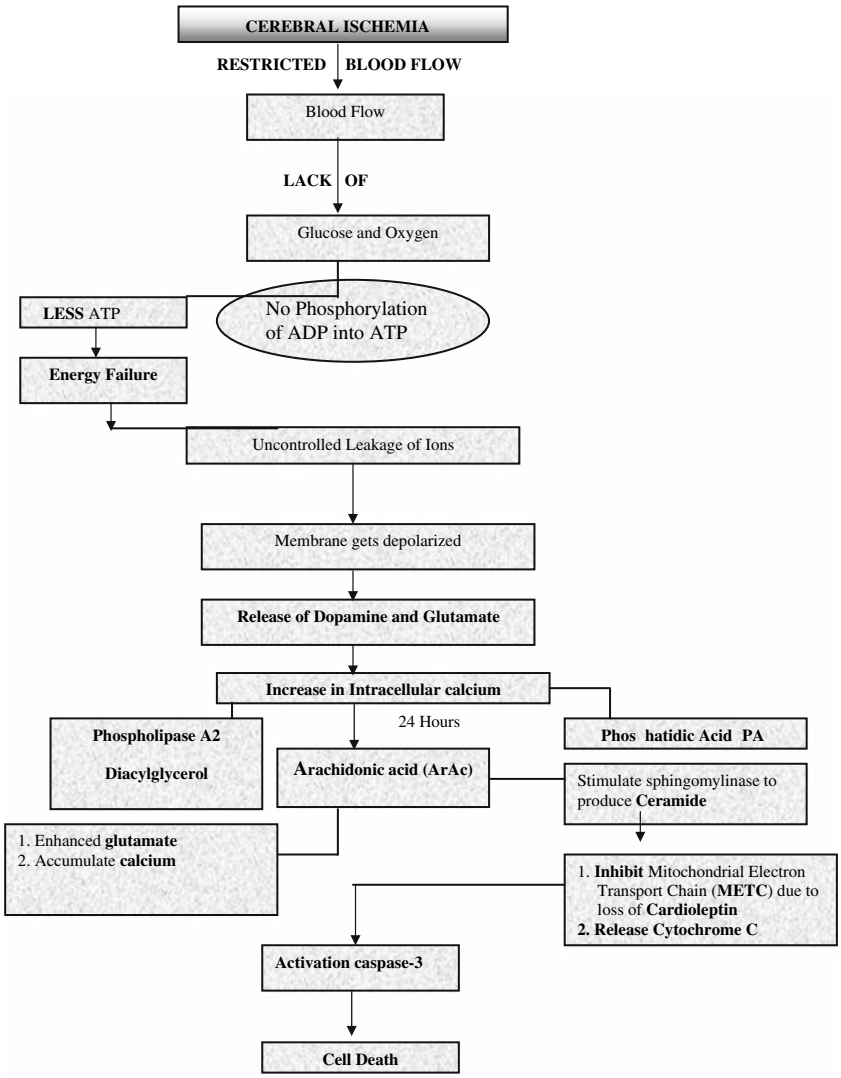

Fig. 1 The inter relationship of cerebral ischemia and resultant cell death

penumbra, functional alterations occur in the neurons and glial cells. The principal pathological processes in acute CNS injury (like stroke, mechanical trauma, or subarachnoid haemorrhage) involve pathological permeability of the blood brain barrier (BBB), energy failure, loss of cell ion homeostasis, acidosis, increased intracellular calcium, excitotoxicity and free radical-mediated toxicity. This can lead to ischaemic necrosis or apoptosis with associated loss of calcium and glutamate homeostasis (Adibhatla et al. 2002; Crack et al. 2001). Experimental models of stroke have been developed in animals in an attempt to mimic the events of human cerebral ischaemia. The focal model involves the transient or permanent occlusion of the middle cerebral artery (MCA) to be used as a model of cerebral ischaemia (Dhar-Mascareno et al. 2005). A global model has also been developed to mimic human cardiac arrest and involves the bilateral occlusion of the carotid and vertebral arteries leading to the development of stroke (Carden and Granger 2000). Cellular models have provided useful tools for the study of ROS-mediated mechanisms of cellular dysfunction (Endo et al. 2006a, b) (Fig. 2).

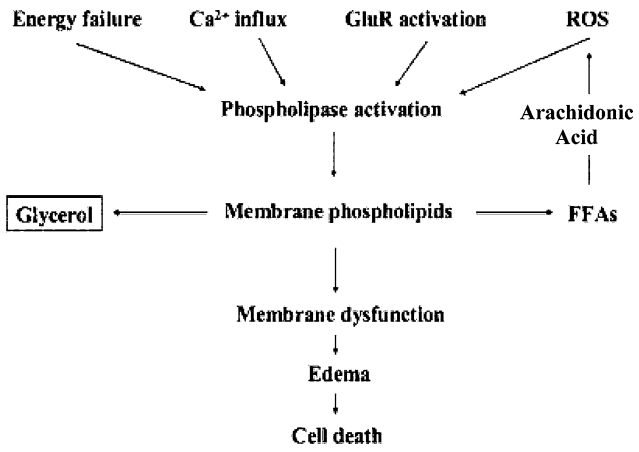

Fig. 2 Effect of ROS on arachidonic acid metabolism pathway

Cellular defenses against reactive species

In order to preserve the integrity of cells from oxidative damage, cells have evolved different mechanisms to scavenge various potentially damaging species.

These antioxidant defences include radical scavengers like α-tocopherol (vitamin E), β-carotene and ascorbate (vitamin C) and enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Endo et al. 2006a, b). Ascorbate is the most effective plasma antioxidant and can prevent lipid peroxide formation resulting from leucocyte activation (Adibhatla and Hatcher 2005). Ascorbic acid (AA) cannot penetrate the BBB but the oxidized form of ascorbic acid, dehydroascorbic acid (DHA), can enter the brain by facilitative transport (Fang et al. 2002). The non-enzymatic antioxidant mechanism involves reduced glutathione (GSH), which is a tripeptide (γ-glutamylcysteinylglycine) that can donate electrons to oxidized species by virtue of its sulfhydryl moiety. NADPH is the source of reducing equivalents needed to replenish GSH stores (Figueroa et al. 2006).

Oxidative stress causes damage in both acute and chronic neurodegenerative diseases like Parkinson disease (PD), amyotrophic lateral sclerosis (ALS) and stroke (Fujimura et al. 1998, 2000). Antioxidant enzymes like glutathione peroxidase (GPx), catalase and SOD have proved useful pharmacological agents in models of neurodegeneration. Recently, SOD mimetics, are being explored as a possible treatment option and have emerged in antioxidant therapeutics (Figueroa et al. 2006). Three isoforms of SOD, the dimeric copper/zinc SOD (CuZn-SOD, SOD1), manganese SOD (MnSOD, SOD2) and tetrameric, proteoglycan-bound Cu/Zn extra cellular SOD (ecSOD) are found, each with a specific distribution. SOD1 is cytosolic and nuclear; SOD2 is a mitochondrial enzyme while ecSOD is localized in cerebrospinal fluid, cerebral vessels and extracellular space (Fujimura et al. 2000; Gilgun-Sheriki et al. 2002).

Role of RNS species

Nitric oxide (NO) scavenging mechanisms include the following:

(a) Rapid oxidation of NO by oxyhemoglobin to form NO_3^- (nitrate), a stable end oxidation product of NO.
(b) NO may react with GSH to form nitrosothiol or with heme to produce heme-NO. Nitrosothiol can serve as a carrier of NO in the plasma.
(c) NO or its derivative peroxynitrite ($ONOO^-$) can nitrosylate tyrosine residues of proteins and hence can be removed.
(d) GSH can interact with $ONOO^-$, forming oxidized glutathione (GSSG). This oxidized glutathione can be reconverted to GSH by the NADPH-dependent glutathione reductase (Herrmann 2001). However, the activity of GSSG reductase declines after transient ischaemia, probably because of free radical-mediated inactivation of the enzyme. But, surprisingly, changes in GSSG reductase activity are not accompanied by concomitant changes in GSSG levels, indicative of the involvement of some other compensatory mechanism that tends to keep GSSG levels normal or near normal (Herrmann and Knapp et al. 2002).

Vulnerability of brain to oxidative stress

The brain is particularly susceptible to ROS damage for the following reasons:

(a) It contains more easily peroxidisable, polyunsaturated fatty acids.
(b) It is not particularly enriched with antioxidants (Catalase, GPx).
(c) It has high oxygen consumption.
(d) It has high levels of iron and ascorbate, which can be deleterious because of their capability to act as pro-oxidants under pathological conditions (Figueroa et al. 2006; Hewett et al. 2000). Iron is the most abundant trace element in the body and an increase in extra cellular or intracellular iron concentrations promotes ROS production and lipid peroxidation. Increased levels of extracellular non-heme iron promote inducible nitric oxide synthase (iNOS) protein expression and this can lead to increase NO synthesis, contributing to oxidative stress because of the associated peroxynitrite generation (Herrmann 2001; Hillered et al. 2002; Hou and MacManus et al. 2002).
(e) Reactions involving dopamine and glutamate oxidation occur in the brain (Chen et al. 2005).

Pro-oxidant effect of cerebral ischaemia

Lactic acid accumulation in the milieu of neurons as a consequence of ischaemia leads to acidosis. The acidic environment itself has a pro-oxidant effect for the following three reasons:

(a) Increase in H^+ concentration enhances rate of conversion of superoxide anion to hydrogen peroxide (H_2O_2)
(b) Increase in H^+ concentration causes conversion of superoxide anion to a more reactive species, the hydroperoxyl radical (HO_2)

(c) Acidosis also increases iron availability for free radical formation by promoting the dissociation of iron from protein-bound iron (Fe^{2+}) (Hewett et al. 2000; Costa et al. 2005).

Events of cerebral ischaemia with reference to ROS and RNS

Early events during cerebral ischaemia include phospholipid metabolism, release of free fatty acids from phospholipids, production of lipid peroxides and ROS from polyunsaturated fatty acids, and increased release of excitatory amino acids (EAA) like glutamate. These also happen to be major factors implicated in neuronal injury in stroke and other neurodegenerative diseases (Figueroa et al. 2006; Huang et al. 2001). One feature of stroke is that hypoxia causes increase in intracellular calcium ($[Ca^{2+}]_i$) in almost all cells. These changes stem from the activation of various plasma membrane calcium conductances including voltage operated calcium channels and ligand-operated channels (Ishibashi et al. 2002).

Glutamate is the major EAA in the brain and acts through its ionotropic receptors. Ischaemia causes glutamate accumulation in the interstitial space because of enhanced efflux of glutamate and reduction of glutamate uptake. Hypoxia leads to depletion of ATP reserves and this causes an uncontrolled leakage of ions across the plasma membrane, leading to membrane depolarisation and neurotransmitter release, for example glutamate and dopamine. The excessive glutamate can lead to brain damage through membrane depolarization, with subsequent calcium influx via glutamate receptor operated ion channels (Adibhatla et al. 2002; Hillered et al. 2002; Ishibashi et al. 2002). The neurotoxic effects of glutamate can be potentiated by the presence of free radicals generated by a mixture of xanthine and xanthine oxidase, a well-recognised superoxide and hydroxylradical generating system (Iwashita et al. 2002). Glutamate interaction with its receptors causes phospholipase activation (there is considerable evidence implicating phospholipase A_2 (PLA_2) activation in transient ischaemia and contributing to neuronal damage) (Kamada et al. 2006). Activated phospholipases act on the phospholipids in the cell membrane and release arachidonic acid, which undergoes further metabolism by cyclooxygenase/lipoxygenase (COX/LOP) to produce arachidonic acid metabolites together with ROS; the end result of this chain of events is cell death (Huang et al. 2001; Kim 2005). Quinolinic acid is an endogenous metabolite of tryptophan and is a selective agonist at N-methyl-D-aspartate (NMDA) receptors (Liu and Rosenberg 2005). Homocysteine may have an excitotoxic effect on different NMDA receptors subtypes and may increase hydroxyl radical formation (Alexandrova et al. 2005). Ishige et al in an experimental stroke model system using oxidative stress (mouse hippocampal cell line HT-22) showed that the loss in cellular GSH up to 85% of the control level caused a 5 to 10-fold increase in levels of ROS. However, a greater GSH loss was found to stimulate the mitochondria to produce a 100-fold increase in ROS (Ishige et al 2001). Another free fatty acid, docosahexaenoic acid (DHA) is also an important source of ROS and lipid peroxidation (Kim et al. 2005). Membrane lipids are prone to oxidative damage because they not only have a high percentage of polyunsaturated fatty acid but also because of the localisation of systems producing ROS in the membrane (MacGrego et al. 2003).

It has become increasingly evident that ROS play a significant role in reoxygenation injury. During hypoxia and reperfusion (H-R) vascular endothelium is a primary site of ROS generation that can lead to cell death. Since oxidative stress is known to induce loss of mitochondrial membrane potential dilated cardiomyopathy, in addition to cytochrome *c* release from the mitochondria (Manabe et al. 2004).

The reperfusion that follows cerebral ischaemia uncouples oxidative phosphorylation and increases ROS generation and lipid peroxidation (Figueroa et al. 2006). Reperfusion after ischaemia causes reoxygenation and provides an excessive substrate supply for oxidation reactions, hence causing excessive production of ROS in mitochondria and this can lead to depletion of endogenous antioxidants (Fujimura et al. 2000; Metodiewa and Koska 2000). The reperfusion period required for cerebrovascular ROS generation is much shorter than that required for brain damage and oedema formation. Therefore, it has been suggested that oxidative injury plays a role in cerebrovascular damage after ischaemia reperfusion, thus predisposing the brain tissue to damage (Paterno et al. 2004). One study employed in vivo erythrocyte sedimentation rate (ESR) spectroscopy/spin probe techniques with three nitroxyl probes of different membrane permeabilities in rats after transient middle cerebral artery occlusion (MCAO) (Pong 2003). It showed that ROS are generated at the interface of the cerebrovascular cell membrane after reperfusion and that ROS produced in the initial stages of transient MCAO cause brain injury. The authors observed BBB impairment and oedema formation, but visible histological damage did not occur until 30 min after reperfusion following MCAO (Table 1).

Source of ROS and RNS in stroke

Cyclooxygenase-2 (COX-2), a rate limiting enzyme involved in the synthesis of prostaglandins and normally expressed in brain in glutamatergic neurons, has been implicated in the pathogenesis of brain diseases like stroke,

Table 1 Temporal aspects of stroke and the time course of major events

Time	Condition	Increase levels	Decrease levels
0 hr	Ischemia	PtdCho, PLC, ArAc, LTC_4	–
0.5 h	Reperfusion	–	ArAc
1–3 h	Ischemia	PLA_2/PLD_2/LTC_4/PGE_2/MDA/ TNF-α/IL-1β	CCTα, GSSG reductase
6 h	Ischemia	PLA_2/PLD_2/MDA/TNFα	–
24 h	Ischemia	PLA_2/PLD_2/ArAc//LTC_4/PGE_2/MDA-OH, HNE/TNFα	CCTα/Phospholipids GSSG, GSH
2 days	Ischemia	PLA_2/PLD_2 MDA, OH, HNE	–
3 days	Ischemia	PLA_2/PLD_2 MDA, OH, HNE	GSSG
6 days	Ischemia	PLA_2/PLD_2 MDA, OH, HNE	–

PtdCho phosphotidylcholine, *PLC* phospholipase C, *ArAc* arachidonic acid, *LTC_4* leukotriene C_4, *PLA* phospholipase A_2, *PLD_2* phospholipase D2, *MDA* malondialdehyde, *TNF-α* tumor necrosis factor α, *IL-1β* Interleukin 1β, *PGE_2* prostaglandin E_2, *CCTα* cytidylyltransferase-α, *GSSG reductase* oxidized glutathione, *OH* hydroxyl radical, *HNE* 4-hydroxynonenal, *GSH* glutathione.

which involves activation of N-methyl-D-aspartic acid (NMDA) receptors. However, it is unclear whether COX-2 derived prostanoids or ROS are involved in the production of these effects in neurodegenerative states.

It has been found that direct injection of NMDA into the somatosensory cortex causes brain damage and that the damage can be attenuated by COX-2 inhibitor NS-398 but the concomitant production of free radicals is not attenuated by the use of this inhibitor (Schneider et al. 1999). This study suggested that prostanoids, and not ROS, are involved in COX-2 mediated component of the damage arising from activation of NMDA receptors associated with glutamate neurotoxicity (Schwartz-Bloom and Sah 2001).

Agonists at A1 receptors and antagonists at A2A receptors are known to protect acutely against neuronal damage caused by toxins or ischaemia-reperfusion, and these compounds can also protect against the cell damage inflicted by ROS. Endogenous adenosine may be neuroprotective, since its levels rise substantially in association with a period of ischaemia-reperfusion. There is also growing evidence that the efficacy of adenosine receptor activation can be reduced by the concomitant activation of glutamate receptors responding to NMDA, probably acting via the release of NO. A1 receptors can also reduce calcium influx in neuronal and cardiac tissues, possibly secondary to the modulation of potassium conductances including the adenosine triphosphate (ATP)-sensitive potassium channels in heart and hippocampal neurons (Simonyi et al. 2005).

A study (Stone 2005) found that ischaemia-induced oedema was prevented by using a combination of glutamate-receptor antagonists (AP5/CNQX), Cyclosporin A (CsA) blockade of mitochondrial permeability transition) and Tempol (ROS scavengers). ROS is a major factor that has been implicated in the causation of ischaemia-induced injury during reperfusion and could be originating from glutamate receptor activation, mitochondrial dysfunction or increased intracellular calcium. In vivo as well as in vitro studies have shown that oxidative stress can to lead to cerebral oedema. The observation that use of a combination therapy of Tempol, AP5/CNQX and CsA afforded more benefits than use of AP5/CNQX alone suggests that ROS also originates from sources other than glutamate receptor-dependent sources.

Evidence from both in vivo and in vitro models indicates that a rise in intracellular calcium is a key mediator of neuronal injury produced by ischaemia/reperfusion. The elevation in intracellular calcium triggers a series of downstream events that include: (1) the activation of enzymes such as phospholipase A_2, proteases, and endonucleases; (2) the accumulation of eicosanoids (unsaturated free fatty acids); (3) the generation of ROS; and (4) disruption of mitochondrial function. A major route for ischaemia-induced calcium entry into the postsynaptic neuron is through NMDA receptors. Generations of superoxide radicals have been found to inhibit gamma-aminobutyric acid (GABA) responses in cerebral cortical synaptoneurosomes in calcium-dependent manner (Stone 2001).

Effect of ROS and RNS on intracellular signaling pathways

Oxidative stress caused by ROS may either be through effects on proteins, lipids and DNA or intracellular signaling pathways that involve changes in regulation of gene expression (Hewett et al. 2000; Sugawara and Chan 2003; Sugawara et al. 1999). So, ROS and RNS can either alter the redox state of cells to affect specific pathways or oxidatively modify proteins (Sugawara et al. 2004). A few examples of the pathways affected by ROS and RNS are as follows:

ROS and matrix metalloproteinases activation pathways

Cerebral ischaemia activates different pathways like signaling mechanisms, gene transcription and enzyme formation

by instituting changes in the redox state of cells. Matrix metalloproteinases (MMPs) and serine proteases, so induced and activated, attack the integrity of the BBB by the breakdown of the extracellular matrix around cerebral blood vessels and neurons. ROS and RNS can regulate the redox state in the cells and hence affect signaling pathways that transcribe, induce and activate these enzymes. Important MMPs found in the brain include gelatinases, stromelysins, and membrane-type metalloproteinases. These MMPs can exacerbate the phase following an acute stroke. Previous studies have shown (Taylor and Crack 2004)(47) that the activation of MMPs is mediated by the S-nitrosylation by NO, an ischaemic attack with subsequent reperfusion induced MMP-9 in brain cells. Vesicles containing preformed inactive MMP-9 are seen in neutrophils and can be released during the inflammatory process. Paradoxically, high nitric oxide (NO) concentration was also found to inhibit MMP-9 activation at the endothelial cell/tumour cell interface (Taylor and Crack 2004).

ROS and effects on apoptotic pathways

Damage induced by ROS can trigger cell death by influencing pathways that reduce the survival potential of cells. It has been suggested (Hewett et al. 2000) NO and O_2^- may contribute to damage of nuclear genetic material through the formation of peroxynitrite. Low levels of these species trigger apoptosis but higher levels cause the cell to undergo necrosis. Possible mechanisms by which the apoptotic effects are mediated could involve mitochondria, DNA repair enzymes and death membrane receptors. A large body of evidence has collected regarding the role of mitochondrial apoptotic pathway after ischaemia (Toescu 2004).

ROS and effect on mitochondrial apoptotic pathway

In global ischaemia, SOD1 over-expression is associated with a 50% decrease in hippocampal CA1 cell death and it has been suggested that this protection may be afforded through interference of the mitochondrial apoptotic pathway (Umemoto et al. 2004). In SOD_2 knockout mice, the findings include larger infarct volume after permanent MCAO, increased mitochondrial cytochrome *c* release, and DNA fragmentation. Similarly, studies have shown the neuroprotective role of extracellular superoxide dismutase (ecSOD) (Fujimura et al. 2000), despite the fact that the brain has lower levels of this protein compared to other organs. Studies suggest that an increase in mitochondrial superoxide (O_2^-) levels after an episode of ischaemia causes not only cytosolic release of cytochrome *c* but also results in (mitochondrial) DNA fragmentation and this can lead to unfavourable gene expression (Hewett et al. 2000).

Oxidative stress is also known to induce loss of mitochondrial membrane potential (DCm) and this can lead to cytochrome *c* (Cyt *c*) release into cytoplasm (Manabe et al. 2004). As mentioned earlier, ROS signaling in mitochondria leads to Cyt *c* translocation from mitochondria to cytosol after transient focal cerebral ischaemia in rats (Weber et al. 2005), in brain slices that have been subjected to hypoxia-ischaemia (Williams and Henkart 1996) and in vulnerable hippocampal CA1 neurons after transient global cerebral ischaemia (Won et al. 2002). Cytochrome *c* is a water soluble peripheral membrane protein of mitochondria and also participates in the mitochondrial respiratory chain. Hypoxia and reperfusion leads to ROS production in mitochondria, which are involved in the release of Cyt *c*, possibly through mechanisms that involve Bcl-2 family proteins, Bcl-2, Bcl-XL, Bax, or Bid. It is believed that the Bcl-2 family exerts regulatory effects on permeability transition pores (PTP) in the mitochondrial membrane and controls its permeability. Proapoptotic proteins of the Bcl-2 family (Bax, Bcl-XS, Bak, Bid) facilitate release of cytochrome c by affecting PTP (Fig. 3). After release from mitochondria, cytochrome *c* binds with CED-4 homolog, Apaf-1 and deoxyadenosine triphosphate, forming the "apoptosome" and causes activation of caspase-9. Caspase-9 then activates a battery of caspases including caspase-3,-2,-6,-8,-10 in a downstream fashion. Second mitochondria-derived activator of caspases (Smac) is also released from mitochondria in response to apoptotic stimuli and binds Inhibitor of Apoptosis Proteins (IAPs), enhancing activation of caspase-3. IAP normally has an anti-apoptotic role because they prevent procaspase activation but they are no longer active after binding to Smac. Activated caspase-3 exerts cytotoxic effects by cleaving nuclear DNA repair enzymes, ultimately culminating in apoptosis because of a significant elevation in oxidative DNA lesions (ODLs) and oxidative RNA lesions (ORLs) in the brain. Caspase-3 also causes activation of caspase-activated DNase (CAD), which can cleave DNA. The downstream caspases cleave proteins like poly ADP-ribose polymerase (PARP). Excessive activation of PARP causes exhaustion of cellular stores of nicotinamide-adenine dinucleotide and ATP, depriving the cell of the "energy currency" it requires to maintain its functions, leading to cell death as a result of energy depletion. PARP is also implicated in the caspase-independent apoptosis pathway. The translocation of apoptosis-inducing factor from the mitochondria to the nucleus depends on PARP activation in neurons experiencing DNA-damaging stimuli like oxidative stress. NADPH oxidase and nNOS contribute to increased oxidative stress with subsequent activation of PARP, and NADPH oxidase and nNOS inhibitors attenuate neuronal death without direct inhibition of PARP. The activation of N-methyl-D-aspartate receptor (NMDAR) and production of superoxide anion and NO by nNOS are possibly

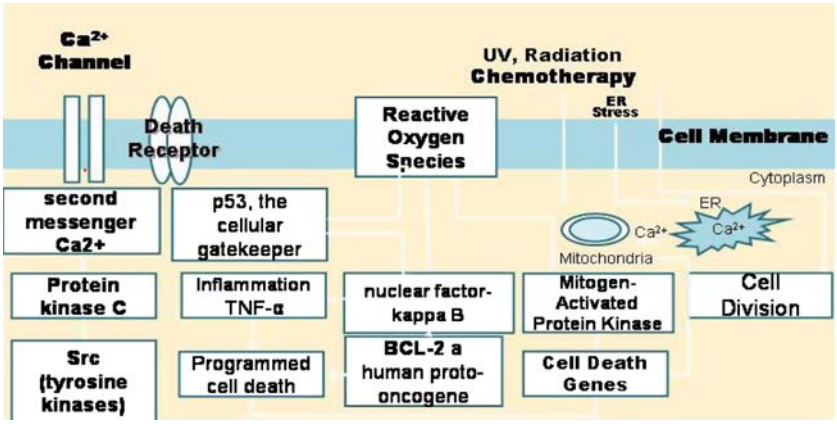

Fig. 3 Mitochondrial apoptotic pathway in response to ER stress

involved in signaling the release of cytochrome c from mitochondria. Alternatively, these free radicals can cause formation of peroxynitrite (ONOO) and hydroxyl ions and inflict oxidative damage on lipids, proteins and DNA. This sequence of events also results in cell death (Herrmann and Knapp 2002; Hillered et al. 2002). Cytosolic antioxidant copper/zinc-superoxide dismutase (SOD) prevents the early release of mitochondrial cytochrome *c* in ischaemic brain after transient focal cerebral ischaemia in mice by decreasing the levels of superoxide (Yamato et al. 2003).

ROS and regulation of PI3K/Akt pathway

Bcl-2-associated death promoter (Bad), a proapoptotic member of the B-cell lymphoma 2 (Bcl-2) family, is normally present in phosphorylated form in an inert complex with chaperone molecule 14-3-3. Bad is dephosphorylated in response to apoptotic stimuli and detaches from 14-3-3. Next, it is translocated to the outer membrane of mitochondria, where its dimerisation with Bcl-X_L ultimately contributes to mitochondrial cytochrome *c* release. Cerebral ischaemia has been known to cause the essential dephosphorylation and translocation of Bad from the cytosol to the mitochondria and this signaling is believed to involve ROS and RNS. However, Bad can be prevented from exerting its proapoptotic functions via a number of mechanisms and research is focused on up-regulating these survival signaling pathways after cerebral ischaemia (Kamada et al. 2006). Ras protein causes activation of phosphatidylinositol-3 kinase, an upstream effector for activation of Akt. Akt then activates antiapoptotic pathways (Metodiewa and Koska 2000). The PI3-K/Akt pathway is activated by a variety of growth factors in cells ranging from fibroblasts to neurons (Chen et al. 2005) Akt exerts antiapoptotic effects by phosphorylating Bad, preventing its homodimerization (and from forming heterodimers with Bcl-X_L). As a result, Bad is no longer able to affect Bcl-X_L. Consequently, cytochrome *c* is not released from the mitochondria because the channel formation on the mitochondrial membrane by Bax is blocked (Sugawara et al. 2004; Metodiewa and Koska 2000). Akt also causes phosphorylation of caspase-9 and inactivates it. Akt also moves into the nucleus where it leads to the phosphorylation and inactivation of a pro-apoptotic member of the pro-apoptotic Forkhead family of transcription factors (e.g., forkhead transcription factor (FKHR)-L1). It thus modulates Bcl-2 family members like antiapoptotic Mcl-1 and pro-apoptotic Bim. This causes inhibition of the Ferrous Ammonium Sulfa (Fas) pathway. Protein kinase B (PKB or Akt) also causes phosphorylation of caspase-9 at Ser^{196} but the details of this inactivation remain unclear. According to a growing body of evidence, Akt also modulates p53 by counter-regulating its regulator murine double minute 2 (Mdm2) (Chen et al. 2005; Metodiewa and Koska 2000). The PI3K/Akt pathway, essential for cell survival and insulin signaling, a regulator of cell cycles through involvement with cyclin D and p21, is governed by the redox state of the cell. It undergoes inactivation under oxidative stress, leading to cell death (Sugawara et al. 2004; Metodiewa and Koska 2000).

ROS and nuclear factor-κB

ROS has been shown to affect genetic expression. NF-κB affects genes encoding pro-inflammatory cytokines, adhesion molecules, anti-oxidant enzymes and growth factors (Chen et al. 2005). Activation of NF-κB by H_2O_2 or hydroperoxides in neurons has been shown to have anti-apoptotic effects and is protective against glutamate exposure, glucose deprivation, hypoxia and low K^+. Conversely, in microglial cells and astrocytes, activation of the same transcription factor leads to production of neurotoxic oxyradicals and excitotoxins as well as increase in nitric oxide synthase (NOS) and NO production, promoting neuronal cell death after ischaemia. After cerebral ischaemia, both NF-κB and Akt have been observed to undergo activation (Adibhatla et al. 2002; Chen et al. 2005; Zipfel et al. 2000). It is also known that Akt is involved in the activation of NF-κB, which may be involved in inducing apoptosis (Chen et al. 2005; Sugawara et al. 2004).

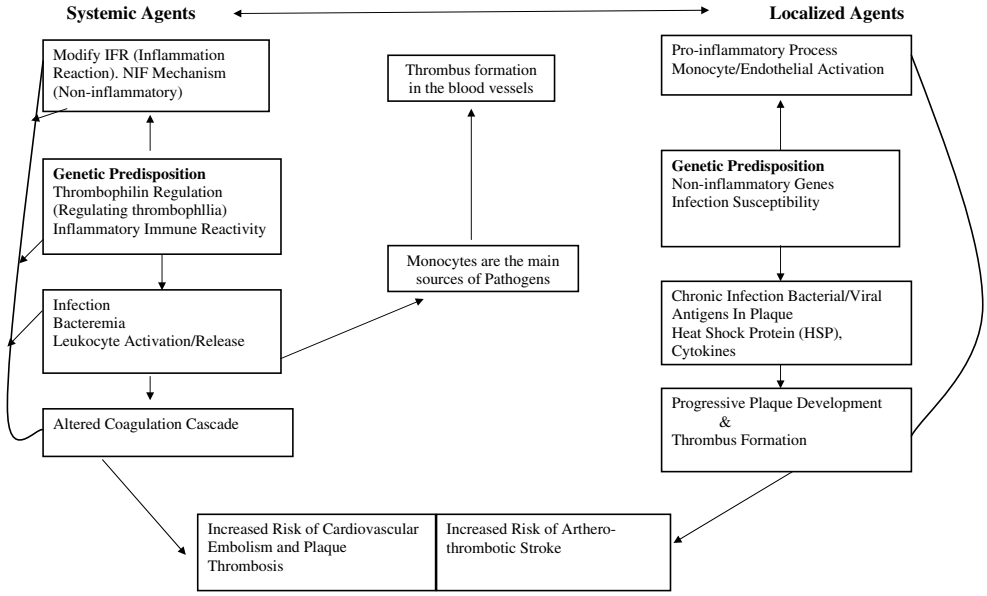

Fig. 4 A schematic diagram showing the interrelationship of systemic and localized agents, which play important roles in cardiovascular embolism and plaque thrombosis and the increased risk of athero-thrombotic stroke

ROS and regulation of other pathways

Oxidative stress can thus affect a variety of pathways in the cell and these pathways determine the survival status of the cell. Pro-apoptotic pathways involve the p38, c-Jun N-terminal kinase (JNK), Janus kinase (JAK)/signal transducer and activator of transcription (STAT), ataxia telangiectasia mutated (ATM) and p53 while anti apoptotic pathways include extracellular signal regulated kinase (ERK1/2), PI (3)K/Akt and heat shock factor-1 (HSF-1) (Chen et al. 2005; Sugawara et al. 2004).

Chronic infection and inflammation

Chronic and acute infectious diseases have been considered to modify stroke risk independent of usual risk factors. Stroke is an etiologically diverse disease, but atherosclerosis contributes to a large proportion of cases either directly or indirectly. Atherosclerosis is a chronic vascular inflammatory condition and infectious diseases are believed to contribute to its pathophysiology (Ross 1999). It is now hypothesized that infection/inflammation, specific genetic predispositions and traditional risk factors interact with each other and may cooperatively enhance the risk of stroke (Perttu and Armin Grau 2003) (Fig. 4).

Conclusion

In the foregoing discussion, we conclude that the reactive species (ROS and RNS) along with chronic infection and inflammation are important players in the neuronal injury associated with stroke. The free radicals themselves or the molecules that they influence or interact with, as well as the intracellular signaling pathways leading to the development of apoptosis or necrosis in a temporal way following H-R represents potential therapeutic targets for the treatment of neuronal damage associated with stroke. Thus, the prevention of stroke should logically involve the augmentation of natural antioxidant reserves of the brain, and the therapeutic agents can be the molecules mimicking natural radical scavengers of the body. By prevention of the major triggers for the progression of stroke, we can reduce a major health burden as well as decrease the mortality and morbidity associated with it.

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