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·Minireview·

The mechanisms of brain ischemic insult and potential protective interventions

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Abstract: The mechanisms of brain ischemic insult include glutamate excitoxicity, calcium toxicity, free radicals, nitric oxide, inflammatory reactions, as well as dysfunctions of endoplasmic reticulum and mitochondrion. These injury cascades are interconnected in complex ways, thus it is hard to compare their pathogenic importances in ischemia models. And the research in cellular and molecular pathways has spurred the studies in potential neuroprotections mainly in pharmacological fields, such as anti-excitotoxic treatment, calcium-channel antagonism, approaches for inhibition of oxidation, inflammation and apoptosis, *etc*. Besides, other protective interventions including thrombolysis, arteriogenesis, regeneration therapy, and ischemia preconditioning or postconditioning, are also under investigations. Despite the present difficulties, we are quite optimistic towards future clinical applications of neuroprotective agents, by optimizing experimental approaches and clinical trials.

Key words: brain ischemia; glutamate receptors; calcium toxicity; endoplasmic reticulum stress; neuroprotection

1 Brain ischemia

1.1 Definition What's brain ischemia? Is it the reduction of cerebral blood flow (CBF)? The answer is "No". The analysis of the correlation between early local blood flow and histological infarct frequency showed that the likelihood of infarction was at zero[1] or low (probably less than 5%)[2] in animals or patients, respectively, when early CBF remained above 50% of that in control.

During the initial hours of vascular occlusion, the metabolic and ionic disturbances in the periphery of focal ischemia proceed at widely varying flow thresholds (Fig.1) in the following order: firstly protein synthesis is inhibited at a threshold of about 0.55 mL/(g·min) followed by a stimulation of anaerobic glycolysis below 0.35 mL/(g·min), a breakdown of energy state at about 0.20 mL/(g·min) and anoxic depolarizations of the cell membranes below 0.15 mL/(g·min)[3].

The two different thresholds of hypoxia for the preservations of functional and structural integrities were identified by Symon *et al*.[4]. The higher threshold is at approximately 35% of normal CBF, and the lower one is at approximately 25-20% of normal (Fig.1).

1.2 The ischemic penumbra Like a half-shaded zone surrounding a solar eclipse, the penumbra lies peripherally to the core zone of a focal ischemic lesion, and possesses important features: summarily, the substantial initial size, which amounts to about one half of the entire early ischemic lesion; the narrow range of perfusion, which makes the penumbra precariously sensitive to small changes of perfusion pressure; the electrophysiologically dynamic property, which enables penumbra to undergo recurrent energy-consuming depolarizations; and finally the metabolical instability with severe metabolism/flow dissociation.

As a result of these factors, the penumbra clearly has a limited life span and appears to undergo irreversible injury within a few hours unless reperfusion is initiated and/or

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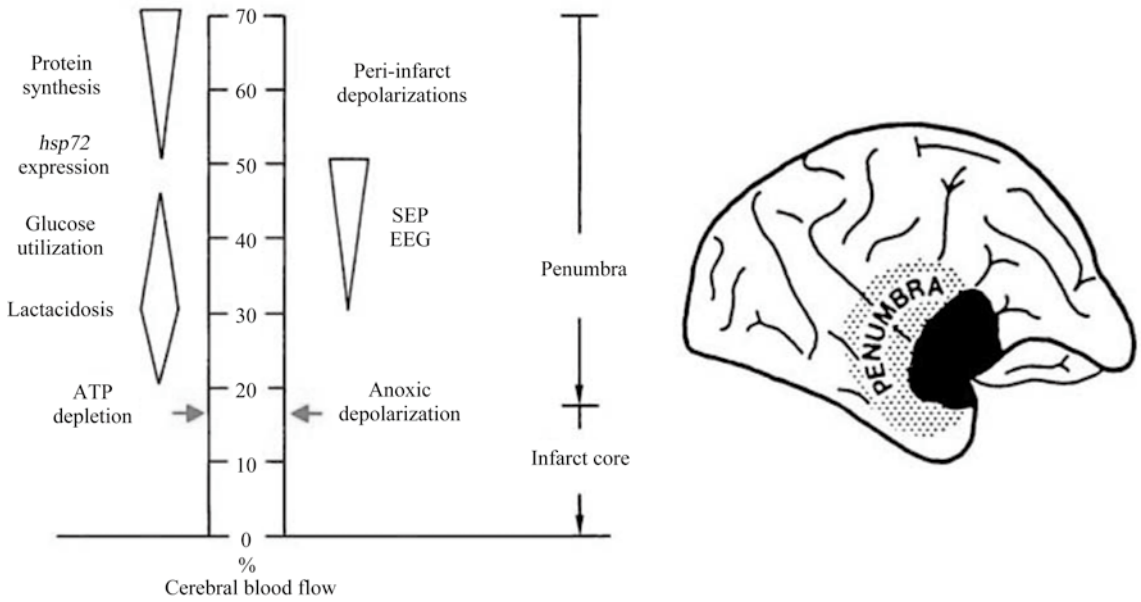

Fig. 1 Thresholds of metabolic (left) and electrophysiological (right) disturbances during graded reduction of cortical blood flow. The infarct core is the region in which blood flow decreases below the threshold of energy failure, and the penumbra is the region where energy state is preserved, with constrained blood supply. SEP: somatically evoked potentials; EEG: electroencephalogram (adapted from Hossmann, Cell Mol Neurobiol 2006)[3].

neuroprotective therapy is administered[1].

The penumbra is of considerable importance in understanding the stroke pathophysiology because it is the conceptual basis not only of the progressive evolution of ischemic injury, but also of the therapeutic ameliorations of the acute neurological symptoms from stroke[3].

1.3 Progression of ischemic injury With unequivocal evidences from non-invasive imaging techniques, brain infarcts have been proved to be growing, which is not due to the progression of ischemia, based on the finding that the activation of collateral blood supply and spontaneous thrombolysis tended to improve blood flow over time.

The fates of brain cells following cerebral ischemia depend on the severity and duration of the ischemic insult, the vulnerabilities of the neurons, and reperfusion. Moreover, the brain's intrinsic cell-cell and intracellular signaling mechanisms that are normally responsible for information processing, can induce harmful effects under ischemic conditions and have significant impacts on outcome.

Infarct progression can be divided into three phases. During the acute phase, tissue injury is the direct consequence of the ischemia-induced energy failure, and terminal depolarization of cell membrane occurs as a result. This injury is established within a few minutes after the onset of ischemia. During the subsequent subacute phase, the infarct core expands into the peri-infarct penumbra and becomes congruent with it after 4-6 h. Also, there occurs the largest increment of infarct volume during this period[3,5]. The main mechanisms of this subacute infarct expansion include peri-infarct spreading depressions and multiple biological disturbances, collectively referred to as molecular cell injury. Finally, a delayed phase of injury evolves and may last for several days or even weeks. During this phase, secondary phenomena such as vasogenic edema, inflammation and possibly programmed cell death may co-contribute to further injury progression.

1.4 Molecular mechanisms of injury progression In the border zone of permanent focal ischemia or the central part of transient vascular occlusion, cellular disturbances cannot be explained by the lasting impairment of blood flow or energy metabolism. In fact, these disturbances are referred to as molecular injury, cascades of which are interconnected in complex ways (Fig. 2), making it difficult to predict their relative pathogenic importances in different ischemia models[3].

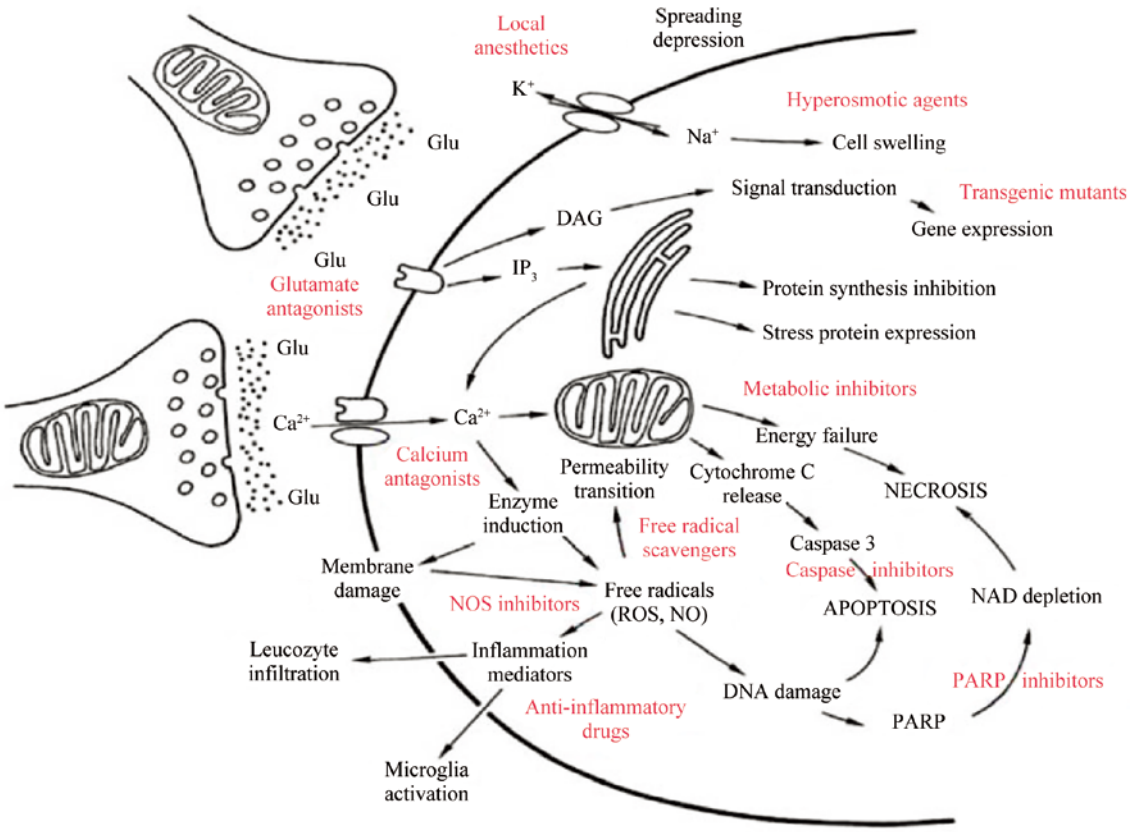

Fig. 2 Schematic representation of molecular injury pathways leading to ischemic cell death. Injury pathways can be blocked at numerous sites, providing multiple approaches for the ameliorations of both necrotic and apoptotic tissue injuries (adapted from Hossmann, Cell Mol Neurobiol 2006)[3].

1.4.1 Excitoxicity Shortly after the onset of ischemia, both excitatory and inhibitory neurotransmitters are released, resulting in the activations of their specific receptors. However, the release is probably unspecific because of the co-release of other intracellular metabolites. Besides, the release thresholds for glycine, adenosine and γ-aminobutyric acid (GABA) are possibly slightly higher than that for glutamate.

Among these factors, particular attention has been paid to glutamate, which would induce excitotoxicity at high concentrations. Glutamate is the major excitatory neurotransmitter in mammalian brain and acts as a key mediator of intracellular communication, plasticity, cell growth and differentiation. Under normal physiological conditions, the extracellular concentration of glutamate is maintained in the micromolar level, when glutamate is responsible for initiating postsynaptic signaling through distinct ionotropic and metabotropic glutamate receptors.

The ionotropic glutamate receptors include N-methyl-D-aspartate (NMDA) receptor, which performs in the predominant route of Ca^{2+} flux during glutamatergic transmission. The other two receptors are α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor and kainate receptor, both of which are generally involved in Na^+ conductance.

Since the AMPA receptor-gated channel is permeable for monovalent cations, its activation results in the inflow of Na^+ along its electrochemical gradient, resulting in the depolarization of the postsynaptic membrane. The depolarization relieves the Mg^{2+} block of the Ca^{2+} permeable channel gated by the NMDA receptor, and opens voltage-sensitive Ca^{2+} channel (VSCCs), including N, P, L and T types. Among the four types, the first two may be mainly localized at presynaptic endings, while L and T types are abundant at postsynaptic membranes. Calcium thus enters the cell in multiple

pathways. However, debate still exists at present on whether changes in subunit composition of the AMPA receptor can render the channel it gates permeable to calcium[6].

The activations of ionotropic glutamate receptors result in the inflow of calcium from the extracellular into the intracellular compartment, leading to calcium overload in mitochondria and activations of calcium-dependent catabolic enzymes. It is believed that activation of NMDA receptor is required for most of the neuronal degenerations caused by intense glutamate exposure. Metabotropic receptors mediate their actions through GTP-binding protein-dependent mechanisms that cause mobilization of Ca^{2+} from internal stores. The activations of metabotropic glutamate receptors induce the IP_3-dependent signal transduction pathway, leading to the stress response of endoplasmic reticulum and adaptive genomic expression via the induction of immediate-early-genes(IEG)[3,5].

It is widely believed that glutamate antagonists may inhibit the spread of the peri-infarct depolarization and ameliorate the resulting injury. However, Xiong ZQ and his colleagues recently found that NR2A- and NR2B-containing NMDA receptor subtypes play differential roles in ischemic neuronal death and ischemic tolerance. NR2A subtype-specific antagonist NVP-AAM077 enhanced neuronal death after transient global ischemia and prevented the induction of ischemic tolerance. In contrast, NR2B subtype-specific antagonist ifenprodil attenuated ischemic cell death and enhanced the preconditioning-induced neuroprotection[7].

1.4.2 Calcium toxicity Calcium ions are regulators of several cellular processes, including enzyme function, cell growth and differentiation. Besides, Ca^{2+} is also responsible for neurotransmitter release, modulation of membrane excitability and regulation of synaptic plasticity in the nervous system.

The influx, buffering, storage and extrusion of calcium ensure the intraneuronal Ca^{2+} homeostasis. The highly efficient calcium transport systems are critical in maintaining a steep gradient of calcium concentration both between extra- and intracellular compartments, and between cytosol and endoplasmic reticulum (ER)[5].

The extracellular calcium can enter neurons through voltage- and transmitter-gated Ca^{2+} channels, and through other unspecific channels, such as those opened by ROS, while Ca^{2+} can also be extruded from the cells by Ca^{2+}-$3Na^+$ exchange or by an ATP-driven Ca^{2+}-$2H^+$ exchanger.

Another determinant of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is the calcium traffic across the membranes of the ER. Calcium sequestered by the ER can be released through a series of events regularly, starting with activations of surface receptors coupled to phospholipase C, followed by the formation of IP_3 and the activations of IP_3 receptors on ER membranes, and finally Ca^{2+} is released from the ER[8,9].

The third determinant of intracellular calcium movement, and thereby of $[Ca^{2+}]_i$, is the mitochondrion. When there occurs a substantial or excessive rise in $[Ca^{2+}]_i$, large amounts of calcium may accumulate in the mitochondrion. This is mainly attributed to the uniporter, which carries calcium into the mitochondrion along the electrochemical gradient, and has a much higher total activity than the export pathways that encompass $2Na^+$-Ca^{2+} exchange. In other words, if the net influx of Ca^{2+} exceeds the extrusion capacity of $2Na^+$-Ca^{2+} exchange, intramitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$) increases, and Ca^{2+} will be sequestered within the mitochondrion[6].

Initial ischemia transient gives rise to sustained perturbations in Ca^{2+} handling of both plasma membrane and ER membrane, resulting in a gradual rise in $[Ca^{2+}]_i$ and the decline of calcium concentration in the ER. When $[Ca^{2+}]_i$ exceeds the "set point" for calcium sequestration in the mitochondrion, there occurs the accumulation of Ca^{2+} in mitochondrion until it's "overloaded", leading to the activations of catabolic enzymes, mitochondrial disturbances, free radical production and immediate early gene responses. The fall of calcium concentration in the ER is also pathogenic. It evokes an ER stress response, which mediates a great number of ER-dependent functional disturbances[6].

Enzymes relative to the elevation of Ca^{2+} concentration include proteases, lipases and endonucleases, consequently leading to the breakdown of cell membrane, cytoskeleton and genomic DNA. Besides, they affect protein phosphorylation by altering the activities of protein kinases and phosphatases.

An influx of Ca^{2+} through NMDA receptors may lead to

the activation of neuronal nitric oxide synthase (nNOS) and the release of NO, the latter of which further leads to the formations of superoxide (O_2^-), peroxynitrite ($ONOO^-$) and hydroxyl (OH) radicals. Mitochondrion is an important target of $ONOO^-$ induced by NO^-, and mitochondrial dysfunction during severe hypoxia-ischemia results in increased generations of oxygen free radicals, leading to prompt dysfunction of cellular membrane, and finally the necrosis[5].

Immediate early gene responses, mitochondrial disturbances and ER stress response will be discussed in detail in the corresponding paragraphs.

1.4.3 Free radicals Free radicals (oxidizing molecules) are generally reactive oxygen or nitrogen species, such as hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, singlet oxygen, superoxide anion and peroxyl radical. These free radicals are highly reactive and unstable, and can react with various cellular components, due to the unpaired electrons in their outer shells.

Free radicals such as reactive oxygen species are produced through a variety of biochemical reactions and cellular functions (such as mitochondria metabolism). Under normal conditions, free radicals are counteracted by antioxidants produced at a similar rate. During the cerebral ischemia and reperfusion, rapid overproductions of free radicals exceed the detoxification and scavenging capacities of cellular antioxidant enzymes viz superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non-enzymatic antioxidants[vitamin E, vitamin C and glutathione(GSH)], resulting in severe and immediate damage to cellular proteins, DNA and lipids, and eventually in cell death. Free radical formations and the effects of these toxic molecules on cell functions are collectively called "oxidative stress".

1.4.4 Nitric oxide toxicity Nitric oxide (NO) is produced when NO synthase (NOS) acts on arginine. There are at least three isoforms of NOS: eNOS, which is constitutively expressed in endothelial cells, nNOS in neurons and the inducible isoform iNOS mainly in macrophages.

Pathophysiologically, NO has two opposing effects. In endothelial cells, NO leads to vascular dilation, together with an increase of blood flow and the alleviation of hypoxic injury, whereas in neurons, NO contributes to glutamate excitotoxicity and free radical-induced injury (by formation of peroxynitrite)[3].

1.4.5 Inflammatory reactions Inflammatory events are initiated at the interfaces of blood microvessels within few hours following the ischemia initiation, and are responsible for the transition from ischemic to inflammatory injury. Many factors are involved in the inflammatory injury, mainly including cytokines (IL-1, IL-6, TNF-α and TGF-β), adhesion molecules (selectins, integrins and immunoglobulins), eicasanoids and inducible neuronal nitric oxide synthase (iNOS). These molecules are produced by endothelial cells, astrocytes, microglial cells and leukocytes (granulocytes, monocyte/macrophages, lymphocytes).

Cytokines such as IL-1, IL-6 and TNF-α, are important mediators of inflammatory reactions in cerebral ischemia. IL-1 expression was increased in microglia, astrocytes and neurons after transient of permanent cerebral ischemia[5]. IL-1, which exists in two isoforms (IL-1α and IL-1β), acts with type I receptor on a variety types of cells, and with type II receptor on neutrophils, type B lymphocytes and macrophages. Some possible mechanisms include release of arachidonic acid, enhancement of NMDA excitotoxicity and stimulation of nitric oxide synthase. Besides, TNF-α and other cytokines will induce the expressions of adhesion molecules that promote the adherence, accumulations and emigrations of leukocytes.

In addition, iNOS and cyclooxygenase-2 (COX-2) have also been suggested to play important roles in inflammation development. Previous reports have demonstrated the attenuations of ischemic brain injury and neurological deficits by the interference of COX-2 inhibitor[10], COX-2 genetic flaw[11] and iNOS inhibitor[12].

Furthermore, when microglial cells are phagocytically active, they can produce a multitude of inflammatory mediators and undergo several changes in the following order: proliferation, chemotaxis, morphological alterations and generations of immuno-modulatory molecules. By inhibiting activations and proliferations of microglia, both minocycline[13] and isoflurane[12] show protective effects in stroke models. This protection may be due to the improvements of blood-brain barrier viability and integrity.

These findings therefore highlight the important roles of inflammatory mediators in cerebral ischemia, and targeting one or all of these events may present great potential for preventing ischemic brain tissue damage.

1.4.6 Astrocyte Astrocyte plays an important role in stroke-induced brain injury. As primary mediators of neuronal death during ischemia and reperfusion, glutamate excitotoxicity, oxidative stress and acidosis are influenced by astrocytes in several ways.

Firstly, glutamate uptake by astrocytes normally prevents the accumulation of excitotoxic glutamate in extracellular spaces in brain, which appears to be critical for neuronal survival in the ischemic penumbra. Conversely, glutamate efflux from astrocytes volume sensitive organic ion channels and other routes may contribute to extracellular glutamate elevation. Moreover, glutamate activation by neuronal NMDA receptors is modulated by glycine and D-serine, both of which are transported by astrocytes, and D-serine production is localized mainly to astrocytes.

Secondly, astrocytes influence neuronal antioxidant status through ascorbate release and dehydroascorbate (oxidized form) uptake, and through indirect support for neuronal glutathione metabolism. In addition, glutathione in astrocytes serves as a sink for nitric oxide and thereby reduces neuronal oxidant stress during ischemia.

Astrocytes probably also influence neuronal survival during the post-ischemic period. On one hand, activated astrocytes secrete nitric oxide, TNF-α, matrix metalloproteinases and other factors, contributing to delayed neuronal death, and facilitating brain edema via aquaporin-4 channels localized to the astrocyte endfoot-endothelial interface. On the other hand, the production of erythropoietin (a paracrine messenger in brain) from astrocytes is upregulated after ischemia. Erythropoietin stimulates the Janus kinase-2 (JAK-2) and nuclear factor-kappaB (NF-κB) signaling pathways in neurons to prevent programmed cell death after ischemic or excitotoxic stress. Astrocytes also secrete several angiogenic and neurotrophic factors that are important for vascular and neuronal regenerations after stroke[14].

1.4.7 Endoplasmic reticulum dysfunction The ER plays a pivotal role in the physiology and biochemistry of cells[15]. Besides calcium storage and signaling[16,17], protein synthesis is another central function of ER. Newly synthesized membrane-bound and secretory proteins are all folded and processed in the ER lumen, where carbohydrate side chains are attached to the peptide backbone to form the glycoproteins. These reactions are so important that any blockade in this process is potentially lethal for cells[18].

Unfolded proteins accumulate in the ER lumen as part of the cellular response to cerebral hypoxia/ischemia, which results from an imbalance between the load of newly synthesized proteins and the ER folding/processing capacity. As a result, stress responses are activated, including unfolded protein response (UPR) and ER-associated degradation (ERAD), to resist such potentially lethal conditions. UPR includes the shutdown of translation to ameliorate the ER work load and the activations of protein expressions that are involved in the folding and processing reactions. And ERAD is induced to degrade unfolded proteins in the Ubiquitin-Proteasome pathway[19].

UPR is characterized with 2 signal transduction pathways, triggered by the activations of PERK and the inositol-requiring enzyme (IRE1)[20-23]. In the physiological state, the ER chaperone glucose-regulated protein (GRP)78 is bound to both kinases, thus blocks the activations of the enzymes. Under conditions when unfolded proteins accumulate in the ER lumen as a result of ER dysfunction, GRP78 is required to refold these proteins. It dissociates from PERK and IRE1, and binds to the unfolded proteins, triggering the oligomerizations, autophosphorylations and activations of PERK and IRE1.

Activated PERK phosphorylates the α subunit of the eukaryotic initiation factor 2 (eIF2a), resulting in the shutdown of the initiation step of translation[20], while activated IRE1 is turned into an endonuclease that cuts out a sequence of 26 bases from the coding region of xbp1 mRNA[21-23]. The resulting xbp1 mRNA is translated into a new protein that translocates to the nucleus and functions as a transcription factor, specifically activating expressions of proteins involved in the folding and processing reactions for ER resident proteins.

Although global protein synthesis is severely sup-

pressed due to the phosphorylations of PERK and eIF2a, some messages can escape this blockade of translation, including the message coding for the activating transcription factor (ATF) 4[24,25]. Various ATF4-dependent genes have been identified, such as CHOP (gadd153), Herp, grp78 and gadd34[26-29]. GRP78 is crucial in the survival program[18], whereas CHOP (GADD153) is a major player in ER stress-induced apoptosis[30]. In a word, when the ER is stressed, the activated genetic programs may induce either a survival program that will make cells resistant to the stressful conditions, or a death program that induces apoptosis.

So ER dysfunction may induce a state of tolerance, impaired cellular functions, or apoptosis, depending on the severity, duration and the cell type affected. In transient cerebral ischemia, ER stress-induced suppression of protein synthesis is believed to be so strong that it inhibits sufficient activation of the genetic arm of the ER stress response, thus apoptosis is induced. CHOP is the major player in ER stress-induced apoptosis. And the ER resident protease caspase-12 plays a significant role in the execution of ER stress-induced apoptosis[31]. Caspase-12 induces the activation of caspase-3, and this activation may be triggered through the apoptotic cross-talk that has been shown to be set off by the stressed ER and may result in mitochondrial dysfunction[32-35]. Besides the direct ER stress-induced apoptotic pathways, apoptotic crosstalk between the ER and mitochondria has also been identified and has been shown to activate the traditional mitochondria-triggered apoptotic pathway, including the mitochondrial release of cytochrome C, which in turn, activates caspase-3[15].

1.4.8 Mitochondrial disturbances The concurrence of increased cytosolic calcium activity and the generations of reactive oxygen species leads to the increase in permeability of the inner mitochondrial membrane (mitochondrial permeability transition, MPT) with two important pathophysiological consequences.

The breakdown of the electrochemical gradient interferes with mitochondrial respiration and the consequent oxidative phosphorylations of adenine nucleotides. Furthermore, the equilibration of mitochondrial ion gradients causes swelling of the mitochondrial matrix, which will eventually cause disruption of the outer mitochondrial membrane and the release of pro-apoptotic mitochondrial proteins, including cytochrome C and caspase-9, both of which are particularly important, since they activate the cysteine protease caspase-3, a direct component in apoptotic pathways. Ischemia induced mitochondrial disturbances thus contribute to delayed cell death both by impairment of the energy state and by the activations of apoptotic injury pathways[3].

1.4.9 Gene expression As reported by Ginsberg MD and his colleagues, they used DNA microarray technology to specifically compare gene expressions between brains with 2-hour middle cerebral artery occlusion (MACO) and a later 3-hour recirculation, and brains in sham controls. Genes with differential expression patterns were divided into 2 broad categories: those already known to be regulated by ischemia-hypoxia and those not well recognized previously, termed newly connected genes. Twenty-eight genes were up-regulated and six were down-regulated as a response to ischemia-hypoxia, including immediate early genes, heat shock proteins, antioxidative enzymes, trophic factors and genes involved in RNA metabolism, inflammation and cell signaling. In addition, thirty-five newly connected genes were also up-regulated and fourty-one were down-regulated[1].

1.5 Cell death in cerebral ischemia There appear to be at least three distinct modes of cell death in ischemia: apoptosis, autophagy and necrosis[36-38].

Apoptosis and autophagocytotic cell death clearly involve ordered physiological processes, during which newly formed structures are kept intact and the progress toward cell elimination is stereotyped.

Necrosis occurs in a haphazard and indiscriminate fashion due to loss of osmotic homeostasis, and it typically involves large numbers of cells within a tissue. The cells swell as they take up water and ultimately the plasma membrane is ruptured, with subsequent leakage of cellular contents into surrounding tissues and invariably an inflammatory response.

Despite the effort to define individual form of cell death based on the appearance, it is clear that in all circumstances, cell disassembly involves nuclear fragmentation/dissolution, organelle disruption (sooner or later) and eventually membrane lysis and phagocytosis. And they are all evolutionarily

conserved and require the activations of one or more families of proteases and possibly other degradative enzymes[39].

The cell death undergoes four major stages. The first stage is the induction stage, which includes several changes initiated by ischemia and reperfusion, including inhibition (and subsequent reactivation) of electron transport, decrease of ATP and PH, increase of cell Ca^{2+} and arachidonic acid, release of glutamate, gene activation for cytokine synthesis, synthesis of enzymes involved in free radical production, and accumulation of leukocytes. These changes lead to the activations of five damaging events, termed perpetrators, including the damaging actions of free radicals and their product peroxynitrite, the actions of the Ca^{2+}-dependent protease calpain, the activities of phospholipases, the activity of poly-ADPribose polymerase (PARP), and the activation of the apoptotic pathway.

The second stage of cell death involves the long-term changes in macromolecules or key metabolites caused by the perpetrators.

The third stage of cell death involves long-term damaging effects of these macromolecular and metabolite changes, and of some induction processes, on critical cell functions and structures , leading to the defined end stages of cell damage. These targeted structures and functions include the plasmalemma, the mitochondria, the cytoskeleton, protein synthesis and kinase activities.

The fourth stage is the progression to the morphological and biochemical end stages of cell death. Of these four stages, the last two are the least well understood. Quite little is known on how the perpetrators affect the structures and functions, and whether and how each of these changes contribute to cell death[36].

Qin AP *et al.* presented possible molecular mechanisms underlying the participations of the lysosomal enzymes in three different types of cell death in ischemic brain damage. Moreover, their research on the selective cathepsin inhibitors may provide a novel therapeutic target for treating stroke and promoting recovery[40].

2 Neuroprotection

In a wider sense, neuroprotection is the preservation of structural and functional integrities of the brain by any kind of intervention with the deleterious effects of ischemia. However, neuroprotection generally refers to pharmacological treatment that may alleviate the molecular injury cascades leading to neuronal death.

2.1 Recovery of blood flow Rapid initiation of reperfusion is the most effective treatment to reduce infarct area and ameliorate the behavioral deficits caused by ischemia. Recirculation after transient clip or filament occlusion of the middle cerebral artery restores blood flow almost instantaneously, indicating that occlusion does not cause structural damage of the vessel. It's worthy noting that reversal of mechanical occlusion does not replicate the naturally occurring reperfusion patterns and that this experimental paradigm is not readily applicable to clinical stroke.

In most stroke cases, collateral blood supply and spontaneous thrombolysis result in reperfusion to some degree. The speed and rate of reperfusion are greatly accelerated by applications of thrombolytic agents. Studies have shown that reperfusion therapy should be initiated within at most 3–4 hours for successful effect. And a large European trial in patients showed that thrombolysis treatment within 4–6 hours failed overall to show a positive effect[4].

Arteriogenesis, the adaptive growth of pre-existing collateral arteries, was recently shown to occur in the brain under conditions of reduced arterial blood supply[41]. And subcutaneous injection of granulocyte-macrophage colony stimulating factor (GM-CSF) resulted in the marked acceleration of this process[42]. So therapeutically induced arteriogenesis may be of considerable interest for preventing infarction in patients with uncompensated cerebrovascular disease[3,43].

2.2 Pharmacological treatments to alleviate the molecular injury cascades It's normally accepted that a brain region where blood flow has declined below the threshold of energy failure is immune to neuroprotection. However, neuroprotection may contribute to the temporary preservation of the penumbra, bridging the interval between the onset of ischemia and the restitution of blood flow, and/or preventing secondary neuronal death during reperfusion. These interventions include approaches of anti-excitotoxicity, anti-

Table 1. Promising strategies for neuroprotection in cerebral ischemia

Antagonism of excitatory amino acids
N-methyl-D-aspartate (NMDA) antagonists:
Competitive[44,45]
Noncompetitive[44,46,47]
Glycine-site [48]
Non-NMDA (AMPA/Kainic acid receptor) antagonists[49]
Cannabinoids[50] (antagonism of NMDA, AMPA and kainic acid receptor)
Agents affecting nonglutamatergic neurotransmission
Monoaminergic system
Dopamine receptor agonists[51,52]
Inhibition of ischemia-induced norepinephrine release[53]
5-HT receptors agonists[54]
γ-GABA[55,56]
Adenosine agonists[57]
Therapeutic hypothermia[1]
Calcium-channel antagonism[58]
N-voltage sensitivity calcium channel (VSCC) antagonists[59]
L- voltage sensitivity calcium channel (VSCC) antagonists[60]
Ionotropic glutamate receptor (IGRC)[58]
Calcium-permeable acid-sensing ion channels (ASICs) antagonists[61]
Sodium-channel antagonism/blockade of glutamate release[44]
Potassium channel opener[62]
Antagonism of free radicals
Superoxide dismutase, catalase[63]
21-aminosteroids[64,65]
Free radical scavengers:[66,67]
Spin-trap agents[68]
Agents affecting nitric oxide: statins[69], aminoguanidine[12], minocycline[70]
Inhibition of cytoskeletal (spectrin) proteolysis[71]
Antagonism of neutrophil activation or binding[72]
Anti-CD11b or CD18 monoclonal antibody[73]
Immunosuppressive agents[74-78]
Inhibition of astrocyte activity
A2A adenosine receptor antagonisms[79,80]
Cytokine receptor antagonists[4]
Neurotrophic factors[81]
Human albumin[1]
Agents of anti-apoptosis[82]
Ovarian hormones[83]
Anesthetic agents: isoflurane[12], propofol[84], xenon[85], sevoflurane[85]
Combinations of neuroprotectants[86]
Traditional Chinese drugs: Ginkgo Biloba Extract[87], PTS[88], puerarin[89], Shuxuetong[56]

Modified from Ginsberg MD, Am J Neuroradiol 1997[4].

apoptosis, anti-inflammation, anti-oxidation and free-radical inhibition. And protective effects have also been observed by interfering with calcium homeostasis and the erythropoietin receptor (Table 1)[3,4].

2.3 Ischemic preconditioning The molecular signaling cascades initiated by brain ischemia are not solely destructive, but may also exert neuroprotective effects[90]. In fact, most of the injury pathways described above, including ischemia itself, induce a transient state of increased ischemic tolerance, based on the observation that the initial injury remains subliminal for tissue destruction[91,92]. This effect is called "ischemic preconditioning" and can be differentiated into three phases: the induction phase, during which molecular sensors that respond to the preconditioning stimulus are activated by transcription factors; the transduction phase, which results in the amplification of the signal, and the effector phase, when proteins with protective roles are switched on.

It has become clear that multiple effectors contribute to ischemic tolerance, including: (1) activations of fundamental cellular defense mechanisms such as antioxidant systems, heat shock proteins[93] and cell death/survival determinants; (2) responses at tissue level, especially reduced inflammatory responsiveness; (3) a disruption of the neuronal excitatory/inhibitory balance, shifting toward the inhibition[94].

Ischemic tolerance increases 2-3 days after the preconditioning stimulus and disappears slowly after 1 week. Such a long effect provides the unique opportunity to protect patients from injury caused by reduced hemodynamic reserve capacity until blood supply can be restored[3]. However, clinically, it is only possible in cases that the occurrence of stroke is predictable and controllable.

2.4 Ischemic postconditioning Since ischemia often happens suddenly and cannot be predicted, anther endogenous protective strategy, termed "ischemic postconditioning" attracts considerable attentions. Ischemic postconditioning is defined as a series of intermittent interruptions of blood flow (several repeated cycles of brief reperfusion and reocclusion) in the early phase of reperfusion that would mechanically alter the hydrodynamics of reperfusion. Studies have shown that ischemic postconditioning protects against both focal[95] and global[96] cerebral ischemia/reperfusion-induced injury in rats.

2.5 Regeneration With the discovery of functionally active stem cells in the hippocampus and the subventricular zone of the adult brain, possible endogenous regenerations of brain infarcts began to be investigated. In fact, despite the documentation of neurogenesis in several focal ischemia models, the number of spontaneously regenerating neurons is low. And it is conceivable that functional relevant neurogenesis could be promoted by trophic factors, such as brain derived neurotrophic factor (BDNF) and other growth factors.

Regeneration therapy has also been tried by transplanting immortal neuroepithelial cells, neural stem cells and stem cells derived from fetal brain tissue, bone marrow or umbilical cord blood. Although studies are not consummate, they may help understand the mechanisms underlying these processes and help find targets of future therapeutic interventions[3].

3 Challenges and opportunities

The abilities of pharmacological agents to limit secondary biochemical damage and cell death have been well established in numerous animal models of stroke, yet the results of such neuroprotective treatments in human are disappointing.

It should be addressed that the rodent genome—now largely elucidated—is strikingly similar with human genome, and data from animal models are also strikingly similar with published results in patients with acute stroke[1], which belies the assertion that data from lower species may not be relevant to human stroke.

The failure can be mainly explained as follows. Firstly, the data of pharmacological evaluation are inadequate, including therapeutic windows, therapeutic index and pharmacokinetic, pharmacodynamic and penetration data. Had such data been available, certain negative clinical trials might have been prevented at the beginning, possibly resulting in better designed studies. Secondly, human trial design and statistical analysis differ considerably from those used in animal trials. Modifications in designing clinical trials are needed, including enlarging sample size, targeting moderate injury levels, improving statistical methodology and identifying more appropriate subgroups[97].

In a word, the failure has underscored the difficulties in developing such research from bench to bedside. However, the careful study in rodent ischemia models and refined clinical trial methodologies have provided renewed, albeit cautious optimism regarding future clinical applications of neuroprotective agents.

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脑缺血的损伤机制及其保护性干预

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摘要：脑缺血的损伤机制包括谷氨酸兴奋毒性、钙毒性、自由基、一氧化氮、炎性反应以及内质网和线粒体功能障碍等。这些损伤性级联反应相互联系，错综复杂，很难比较它们在不同模型中的主次作用。越来越多的对细胞及分子损伤途径的基础研究，推动了对脑保护治疗的研究。迄今为止，脑保护治疗仍以药物治疗为主，例如，抗兴奋毒性治疗、钙通道阻滞、抗氧化、抗炎、抗凋亡治疗等。此外一些研究还包括溶栓、动脉生成和神经元再生，以及缺血前适应和缺血后适应等。虽然将这些研究成果应用于临床还存在许多困难，但是通过改进动物实验和临床实验方法，我们有理由对脑保护治疗持乐观的态度。

关键词：脑缺血；谷氨酸受体；钙毒性；内质网应激；脑保护