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Stroke: Just a Chemistry

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Abstract

Stroke is a significant health issue with its neuropathological mechanisms requiring better understanding. Within the course of ischemic stroke, obstruction of a major brain artery results in an abrupt blood flow limitation below a critical threshold and focal ischemic brain insult arises. Chemical shift imaging using magnetic resonance spectroscopy and more advanced imaging methods have recently provided some novel insights into the chemistry of stroke. Given the fact that stroke disturbs the normal brain chemistry, assessing and perhaps targeting the metabolic pathways ought to be a practical and useful approach to better understand the neurobiochemical aspects of stroke. This perspective paper focuses on the evolving dimensions of neurochemical research in stroke.**[GMJ.2016;5(2):49-55]**

Keywords: Stroke; Neurochemistry; Ions; Chemical Shift Imaging; Neuropathophysiology

Introduction

Well-supported theories and evolving evidence have spotlighted the significance of neurochemical changes following stroke. The diminished oxygen and glucose delivery disrupt ATP build-up and the resultant energy failure triggers ischemia-induced and neuroinflammatory phenomena such as acidosis, production of reactive oxygen species, apoptosis, necrosis and eventually neuronal cell death. The disturbance in metabolic pathways and events such as excessive extracellular glutamate release, increased anaerobic glycolysis and disconcertion in glutamate–glutamine cycle are shown to play important roles in protein synthesis turnover and brain ischemic injury [1-4].

Following ischemic stroke and depending on the duration and severity of ischemic insult, rapid necrotic cell death or expression of apoptotic genes may occur. While depletion of energy stores is recognized as the primary pathologic mechanism in stroke, a considerable body of evidence suggests that excitatory amino acids (EAAs) potentially contribute to ischemic injury. EAAs, including glutamate, are found in almost 30% of synapses in the central nervous system [5]. EAAs are known to take part in various cognitive and neurological functions such as memory, sensation, movement and synaptic plasticity. Meanwhile, they can exert pathologic effects as well. Early neuroscience research demonstrated EAA-mediated toxicity when administered in the arcuate nucleus of the hypothalamus and further studies confirmed that increased extracellular glutamate and other EAAs in-

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duce a notable rise in cytosolic calcium concentrations [6, 7].

Such an increase in intracellular calcium is lethal to primary neuronal cultures and the direct link between extracellular calcium stimulation and induced cell death in neurons when exposed to glutamate highlights the importance of calcium entry in the process of excitotoxicity [8].

In fact, the increased intraneuronal calcium in response to extracellular EAAs in vitro has resulted from opening of N-methyl-D-aspartate (NMDA)-gated ion channel. As such, NMDA antagonists would compete with glutamate and other EAAs for the receptor and prevents calcium entry into the neurons thus hindering glutamate-induced cell death. In addition to glutamate, glycine is also required to open NMDA calcium channels, hence antagonists which bind to glycine site on the NMDA receptor counteract excitotoxicity in vitro [9, 10]. Many key molecular events in programmed cell death have now been determined. Just as calcium entry into the neuron is a key step in excitotoxicity, the release of cytochrome c from the mitochondria is a key event in initiating apoptosis in many cell types. Cytosolic cytochrome c complexes with APAF-1 and procaspase 9. As a result, procaspase 9 is cleaved into its active form, caspase 9. Caspase 9 then cleaves and activates other caspases including caspase 3. The molecular mechanisms by which programmed cell death is initiated are numerous and complex. Programmed cell death may be activated via cell surface receptors including Fas receptor and tumor necrosis factor-alpha (TNF-a). Activation of these receptors triggers activation of caspase 8, which in turn cleaves bcl-2 family protein [9, 10]. A schematic outline of such molecular mechanisms is illustrated in Figure-1.

In line with the above, compelling in vivo evidence has postulated that NMDA-mediated excitotoxicity contributes to injury from cerebral ischemia. An abrupt substantial increase in extracellular amino acids concentration has been monitored by microdialysis in animal models of acute cerebral ischemia. Despite the lack of efficacy of NMDA antagonists in global ischemia models, many studies have

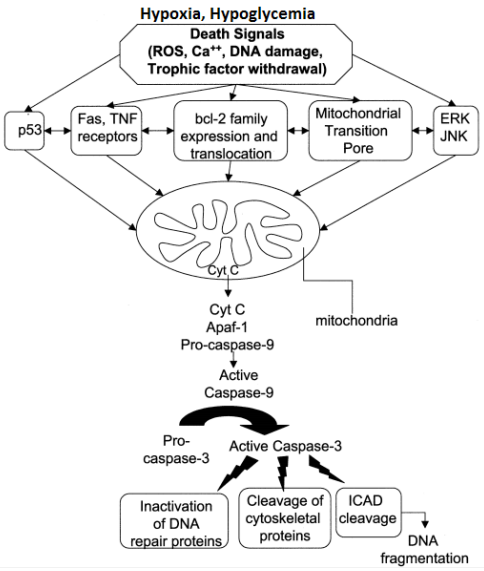

Figure 1. A schematic diagram illustrating the molecular mechanisms that control programmed cell death. ROS: Reactive Oxygen Species, Fas: Apoptosis Stimulating Fragment, TNF-a: Tumor Necrosis Factor-alpha, bcl2: B-cell lymphoma 2, ERK: Extracellular-regulated Kinase, JNK: c-Jun N-terminal kinase, Cyt C: Cytochrome C, Apaf-1: Apoptotic protease activating factor-1, ICAD: Intracellular Caspase Activated DNase.

shown that they reduce infarct volume in both permanent and temporary middle cerebral artery occlusion (MCAo) models in rodents. In addition, when translation of a gene which encodes a subunit of the NMDA receptor is blocked via intraventricular injection of antisense oligonucleotides, the infarct volume in rat model of MCAo is notably decreased [9, 11].

Cascade of Neurochemical Reactions in Stroke

Many calcium-dependent or -induced enzymes such as phospholipase A2, nitric oxide synthase, Calpain 1, andcyclooxygenase regulate the process of excitotoxicity (Figure-2). Calpain 1 is a calcium-activated protease specifically linked to glutamate receptors in the hippocampus. This enzyme contributes to the conversion of xanthine dehydrogenase to xanthine oxidase. The latter metabolizes xanthine to its reactive oxygen species, superoxide. Likewise, calcium activates the phospholipase A2 which facilitates the release of arachidonic

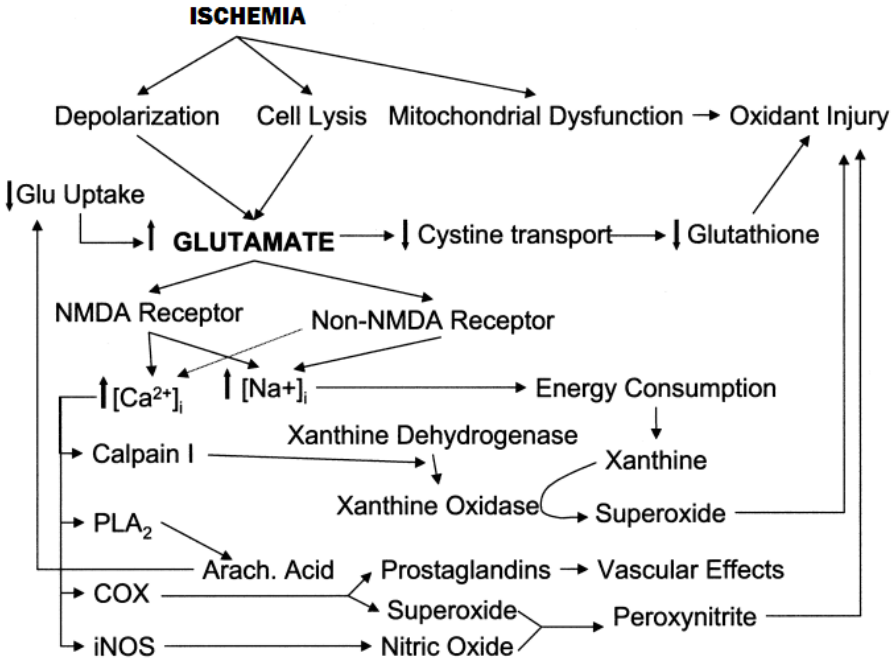

Figure 2. Schematic diagram showing the core mechanisms through which neurons are injured by ischemia and excitotoxicity

acid from injured cell membranes. The above stream of chemical events collectively result in the oxidant injury following stroke [12-16]. Neurons which have not faced immediate necrosis in stroke often die within subsequent days and weeks. These cells might survive when we know about, and intervene, their cascade of chemical reactions leading to their death. Upon hemorrhagic stroke, the breakdown of hemoglobin from the blood cells flooding into the brain tissue may increase the tissue iron. Recent imaging investigations have employed SSRL's XRF imaging beam lines (Stanford Synchrotron Radiation Lightsource) to examine the possible role of iron in distorting the required fine chemical balance serving brain cells survival. While other imaging methods suggest that the tissue iron concentration is raised in the lesioned hemisphere of the brain; XRF imaging has shown that iron is much accumulated in the peri-hematoma region. Referring to the above evidence, Iron chelators were recently tested as adjunct stroke therapy in experimental models. Documented by quantitative XRF mapping, although a ferric iron chelator resulted in diminished iron levels in the brain, it failed to recover the stroke-related disabilities in the experimental rodent model [17-19].

Investigating Stroke Damage Markers

Ischemic stroke occurs secondary to narrowing of the brain vessels or an entrapped clot in them. This limits the blood flow through a vessel and disturbs normal brain chemistry. Although it is well known that specific neurons in the brain are more susceptible to ischemia, the chemistry behind their vulnerability is yet to be better defined. Integrating XRF mapping and sulfur K-edge X-ray absorption spectroscopy (XAS) along with traditional biochemical and histological methods has recently been shown to offer a deep understanding of the neurochemical image of ischemic stroke as well as the underlying biochemical pathways by which treatments tend to reduce the expected damage. Applying the beam lines 10-2 and 2-3 in SSRL's XRF imaging method in healthy rats' brain and stroke models at var-

ious time points, the distributions of ions such as Zn, K, Cl and Ca were determined before and after gentle brain cooling which is considered as a promising stroke therapy [17, 18, 20].

In the same vein, neurobiochemical markers in stroke patients have attracted much attention over the recent decade [21, 22]. The cellular activation and disintegration which occur after cell injury in the central nervous system leads to the release of proteins which belong to specific cell types. These include neuron-specific enolase (NSE), glial fibrillary astrocytic protein (GFAP), myelin basic protein (MBP) and the calcium-binding protein S100B. These damage markers can be measured both in cerebrospinal fluid (CSF) and blood. The poor relation between CSF and serum levels of these damage markers are often not strong and this prevents us from making comments about the surrogacy of brain markers in the blood. Therefore, since CSF concentrations more accurately represent cerebral pathological changes, brain damage markers in ischemic stroke seem to be better measured in CSF in future studies [23-25].

The unique biochemical background of the above damaged markers roots in their differences in cellular and subcellular origins. Due to the paucity of the data on these damage markers in the literature, studies need to investigate the CSF levels of these markers namely MBP, GFAP, S100B and NSE in the course of acute ischemic stroke. Based on the relation between these damage markers with the baseline stroke severity, location, long-term outcome, new avenues for targeted therapy of stroke can possibly be drawn in the future [26-30].

Metabolic Imaging and Lesion Chemistry in Stroke

As discussed previously, metabolic analysis tend to be a useful approach for understanding the biochemical aspects of stroke. Magnetic resonance spectroscopy (MRS) is a chemical shift imaging method which is proven as a beneficial tool to investigate the in vivo metabolic changes in ischemic stroke. Some in vivo investigations have demonstrated altered levels of lactate, creatine, N-Acetyl Aspartate (NAA) and choline in cerebral ischemia [31, 32].Nevertheless, unfavorable sensitivity and resolution often limit the relevance of the detectable and assignable metabolites from MRS in stroke. On the other hand, high-resolution nuclear magnetic resonance (NMR) spectroscopy serves as a better alternative to capture more metabolic information owing to its capacity in simultaneously detecting a wide range of metabolites. Evaluating the correlation of such changes with physiological and genetic alterations in the brain upon stroke would shed light on the key role of chemistry in the pathophysiology of stroke [33-35].

Some approaches such as principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) are among widely used multivariate data analysis methods [17-19] which are used to explore metabolic alterations in relation to a given biological disturbance. The above approachis known to be the technical basis for so-called metabonomics. In research setup, metabonomics encompass high-resolution NMR or mass spectrometry (MS) analyses to help draw the metabolic map of samples such as blood or CSF. This approach has been used as a successful tool to diagnose the condition, understand pathophysiology, identify biomarkers and explore mechanistic aspects of toxicity [36-38].

NMR and high performance liquid chromatography (HPLC) have recently been used to detect metabolic alteration in ischemic condition. Based on some recent reports, cerebral ischemia resulted in increased level of adenosine, inosine and hypoxanthine at 4, 6 and 8 hours after the occlusion in extracellular fluid of striatum, respectively [39-41].

In addition, acetate and glutamine/aspartate increased, and decreased the ischemic tissue 6 hours after occlusion, respectively. Similarly NMR-based studies demonstrated that focal ischemia leads to a decrease in aspartate, glutamate, NAA and total creatine levels 24 hours after the occlusion. Despite the overall quality of such investigations, these works appear fragmented in terms of the metabolisms and the holistic metabolic responses of cerebral tissues to focal brain ischemia [42].

Conclusion

Future studies in this field need to define the metabolic features associated with stroke. Comprehensive insights on the metabolic analysis would provide a clearer view on the biochemical aspects of stroke; the perspective which offers future potentials for targeted therapies.

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Conflict of Interest

None declared.

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