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PATHOGENESIS AND NEURO-PROTECTIVE AGENTS OF STROKE

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ABSTRACT: Stroke remains world's second leading cause of mortality; and globally most frequent cause of long-lasting disabilities. The ischaemic pathophysiologic cascade leading to neuronal damage consists of peri-infarct depolarization, excitotoxicity, inflammation, oxidative stress, and apoptosis. Despite plethora of experimental evidences and advancement into the development of treatments, clinical treatment of acute stroke still remains challenging. Neuro-protective agents, as novel therapeutic strategy confer neuro-protection by targeting the pathophysiologic mechanism of stroke. The aim of this review is discussion of summary of the literature on stroke pathophysiology, current preclinical research findings of neuroprotective agents in stroke and possible factors that were responsible for the failure of these agents to translate in human stroke therapies.

INTRODUCTION: Stroke is a pathological phenomenon that results from a transient or permanent reduction in cerebral blood flow, which in most cases, is caused by the occlusion of a cerebral artery either by an embolus or local thrombosis; this is a rapid development of clinical signs of focal (global) disturbance of cerebral function. Symptoms of stroke last for 24 hours or longer and can lead to death, with no apparent cause other than of vascular origin [1]. The disorder is not the only world's second leading cause of mortality but also, the most frequent cause of long-lasting disabilities [2].

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Brain stroke results from either vessel occlusion (ischemic stroke) or cerebral blood-related neurotoxicity (haemorrhagic stroke). Thus, stroke is classified into Ischaemic and Haemorrhagic. While the former type accounts for 85% of all strokes, the later is almost 15% of all haemorrhagic types of strokes [3], in which intra-cerebral haemorrhage as the second most common form followed by subarachnoid haemorrhage. Ischemia is defined as a reduction in blood flow that is sufficient to alter normal cellular functions, and ischemic stroke remains at the centre stage of the disorder because of its prevalence amongst the several other types that attack the brain [4].

Brain's vulnerability towards stroke is partly due to first, its nature as a highly active metabolic and complex organ that does not store glycogen but instead, relies on glucose from the blood; secondly, due to the nature of the brain in containing high levels of the neurotoxic excitatory

neurotransmitter, glutamate; and unlike in other tissues, transient cerebral ischaemia can produce profound neuronal damage that becomes evident only after 3 days and continue progressively for months [5]. Thus, any disruption of the brain's normal function can lead to loss of homeostasis and ultimately, to neurological deficits; cognitive and visual impairments; loss of memory, balance and co-ordination; or in severe cases, death.

The staggering mortality, debilitating outcomes, burden of care-taking, and expensive cost associated with the stroke disease, have created a wide field of researches into drug development and rehabilitative efforts [6]. Intervention in the mechanistic steps in the pathophysiologic progression of stroke remains the novel therapeutic strategy for acute ischaemic stroke. Despite plethora of experimental evidence into the development of treatments that lessen the severity of the disorder, clinical treatment of acute stroke still remain challenging [7]. This is partly due to failure of therapeutic agents, that have shown potential in pre-clinical research animal stroke model, to reach clinical viability in human clinical trials, and partly due to the challenge posed by pathophysiology of stroke that is marked by incredibly complex cascade of events, each of which with distinct time frame, thereby increasing therapeutic time window of stroke intervention.

In recent years, only one drug passed all clinical trials and has been approved for thrombolytic acute ischaemic stroke [8]; yet, this recombinant tissue plasminogen activator is only available to around 5% of patients due to factors, including highly restrictive time window of administration such as necessity of administration within 4-5 hours of lesion onset and cost [9]. The others are associated risk of intra-cerebral haemorrhage post treatment and other exclusion criteria such as age and hypertension [10]. Numerous reviews of neuro-protective agents on ischaemic stroke that span both clinical and preclinical research findings have previously been published [11-18]. None of the aforementioned exclusively details a review in preclinical research findings; it is therefore expedient to review this phenomenon.

Hence, the aim of this review is to provide an overview summary of the literature on stroke pathophysiology as well as identify the recent neuro-protective agents that have shown therapeutic potential in preclinical animal stroke model with possible factors that were responsible for the failure of these agents to reach clinical viability.

Pathophysiology of Ischaemic Stroke: The pattern of pathological damage after cerebral ischaemic insult depends upon factors such as degree and duration of the impaired blood flow, the brain's inherent capability to recover and repair itself through endogenous mechanisms, state of collateral circulation in the affected area of the brain, and the health of systemic circulation.

Normal cerebral blood flow (CBF) ranges between 50-60ml/100g of brain tissue/min, depending on the different parts of the brain, and during mildest vascular ischaemia the endogenous cerebral auto-regulatory mechanism compensates for reduction in CBF by local vasodilatation, opening of collaterals, and increasing the extraction of oxygen and glucose from blood. The critical level of CBF is set at 23ml/100g/min in which electrical silence ensues and the synaptic activity is greatly diminished but rapid reperfusion up to the normal values reverses functional damage [11].

However, CBF of less than 12 ml/100g/min initiates ischaemic pathophysiologic cascade, each of which has a distinct time frame that ultimately results in irreversible neuronal injury and demise. These pathophysiologic processes leading to neuronal death includes immediate (within minutes) peri-infarct depolarization and excitotoxicity, hours later by inflammation and oxidative stress, days later by apoptosis; these are interrelated and coordinated events with each step serving as positive feedback loop to amplify insult.

Peri-infarct Depolarization: Abrupt ischaemic insult leads to loss of oxygen and glucose to the brain, thereby yielding an inefficient oxidative metabolism that curtails ATP production via oxidative phosphorelation, and subsequently loss of ATP-dependent ionic pump homeostasis in cells within the ischaemic core region termed as ischaemic prenumbra.

Failure in the functioning of sodium potassium pump in this region results in massive uncontrolled anoxic depolarization and they never repolarise. This leads to the opening of voltage-sensitive calcium channels, mitochondrial dysfunction, an abnormally extracellular buildup of excitatory amino acids, and persistently elevated intracellular calcium, thus triggering a cascade of secondary biochemical changes that will lead to neuronal demise of penumbral cells [17].

However, this necrotic core of ischaemic prenumbra is surrounded by a zone of less severely affected tissue, which is rendered functionally silent by reduced blood flow but remains metabolically active [2]; this immediate peripheral region is termed as ischaemic penumbra and the critical time period during which this volume of brain tissue is at risk is referred to as the «window of opportunity» since the neurological deficits created by ischemia can partly or completely be reversed by reperfusing the ischemic yet viable brain tissue within a critical time period of several hours.

Moreover, the core propagates spontaneous electrical waves known as peri-infarct depolarization to the ischaemic penumbra, leading to rapid depolarization of these neurons but they can repolarise at the expense of further energy consumption [16]. Evidence supported that, many neurons in the ischemic penumbra or peri-infarct zone may undergo apoptosis only after several hours or days, and, thus they are potentially recoverable for some time after the onset of stroke [2].

Excitotoxicity: Glutamate together with other related excitatory amino acids such as aspartate, are neurotoxic above homeostatic level; these amino acids are collectively called excitotoxins, and their associated neuronal damage, excitotoxicity [19]. The main excitotoxic neurotoxicity neurotransmitter glutamate is the principle neurotransmitter in central nervous system. It is the most abundant excitatory neurotransmitter in the brain whose physiological roles are enormous. Primarily includes initiation of action potentials in the postsynaptic neuron via interaction with both ionotropic and metabotropic glutamate receptors.

Under normal physiological conditions, cytosolic glutamate concentrations are approximately 10 mM, while its synaptic concentrations are in the micromolar range [16]. These appropriate physiologic levels are ensured by three distinct processes of synaptic glutamate reuptake: it can be taken up into the postsynaptic cell; it can undergo reuptake into the presynaptic cell from which it is released or; it can be taken up by a third non-neuronal cell, namely protoplasmic astrocytes.

Thus released glutamate in the synaptic cleft is cleared into the neurons and glia by sodium-dependent uptake system that keeps only micromolar levels of the glutamate in the extracellular fluid despite millimolar levels inside the neurons. Ischaemic insult resultant loss of ATP production leads to the excessive excitotoxic accumulation of glutamate in extracellular compartment via two folds; first is, the lack of ATP affects the ability of the ATP-dependent ionic pump, leading to the cytosolic rise in sodium ion and a decrease in potassium ion (anoxic depolarization); this rise in cytosolic sodium concentrations prevent the re-uptake of glutamate from extracellular fluid into the neurons and glia; secondly, the resulting anoxic depolarisation is accompanied by influx of calcium ions into the neurons via voltage-gated calcium channels, calcium ions trigger the release of glutamate from the synaptic vesicles into the synaptic cleft, thereby increasing the level in the extracellular fluid. Excessive glutamate levels can rise up to 80mM and remain at these highly neurotoxic concentrations for several hours [2].

This results in hyperexcitation of glutamate N-methyl-D-aspatate (NMDA) receptor, which is arguably the most calcium-permeable ionotropic glutamate receptor; this results in influx of calcium ion into hypoxic neuron that triggers series of cascading events that ultimately lead to neuronal demise [20, 21].

Calcium activates key number of destructive intracellular enzymes such as proteases, kinases, lipases, and endonuclease that not only allows release of cytokines and other mediators that result in the loss of cellular integrity but they also orchestrated triggering of intrinsic apoptotic pathway of neuronal death.

Specifically, calcium activation of phospholipases which hydrolyse membrane bound glycerophospholipids to free fatty acids, which facilitate free radical peroxidation of other membrane bound lipids, calcium activation of proteases that lyse structural proteins as well as nitric oxide synthase initiates free radical mechanism [13].

Inflammation: Inflammation is a classical defense response of vascularised living tissue to infection and injury, and in the CNS, the term neuroinflammtion is used to denote cellular and inflammatory responses of vascularised neuronal tissue through activation of resident cells in the brain (microglia, astrocytes and endothelial cells), the recruitment of blood-derived leukocytes including neutrophils, lymphocytes and macrophages, and a plethora of humoral factors [22]. Neuro-inflammation following focal cerebral ischaemia, supposedly has a positive effect such as increasing blood flow and removal of damaged tissue by phagocytosis but in a disease state such as stroke, the resulting inappropriate inflammation caused negative effects which by far out weight the positive effect [23].

Activation of microglia cells constitutes the first key response in acute stroke, coupled with subsequent activation of blood-borne monocytes/macropahges to yield a full blown neuroinflammatory thick rim around ischaemia infarct that becomes observable after one week in both human and animal models [24]. Microglia in the CNS constitutes 5-15% of total brain population, having share common precursor with peripheral macrophages they produced transient inflammatory changes like macrophages such as phagocytosis, inflammatory cytokine production, and antigen presentation, normally returning to their basal state when the activation stimulus is resolved.

In a disease state such as in the onset of focal cerebral ischaemia, however, the microglia response becomes inappropriately more reactive and exaggerated to produce plethora of inflammatory mediators that triggers apoptosis and exaggerate neuronal damage [25]. Microglia when transform into phagocytes can release a variety of substances many of which are cytotoxic and/or cytoprotective.

While cyto-protective substances include neuro-trophic molecules such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor I (IGF-I), several other growth factors, and anti-inflammatory factors, cytotoxic substances include pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 as well as other potential cytotoxic molecules including nitric oxide (NO), reactive oxygen species (ROS), and prostanoids. The most studied cytokines related to inflammation in acute ischemic stroke are tumour necrosis factor-α (TNF-α), the interleukins (IL), IL-1β, IL-6, IL-20, IL-10 and trans-forming growth factor (TGF)-β, While IL-1β and TNF-α are pro-inflammatory that appears to exacerbate cerebral injury, TGF-β and IL-10 are anti-inflammtory that may exerts neuro-protective effects, and IL-6 has both pro- and anti-inflammtory effects [5]. Pro-inflammatory TNF-α being one of the most key important early initiators of neuroinflammtion interacts with two receptors R1 and R2, to mediate extrinsic apoptotic death signal via Fas-associated death domain (FADD) and inflammation via nuclear factor kappa-light-chain enhancer of activated B cells (NFκB), respectively [26]. Astrocytes, like microglia, are capable of secreting inflammatory factors such as cytokines, chemotaxis cytokines (chemokines), and NO in response to cerebral ischaemia.

Following successful restoration of blood flow to an ischaemic region (reperfusion), a good outcome in salvaging neuronal tissue is anticipated; ironically, this reperfusion process adds to the degree of brain injury termed ischaemic reperfusion injury due to the activation of additional signaling pathways [16]. When leukocytes reenter a previously hypoperfused region via returning blood, they can leads mechanical occlusion of small vessels, producing additional ischemia. Leukocytes also activates vasoactive substances such as oxygen free radicals, arachidonic acid metabolites (cytokines) and nitric acid, the cumulative cellular effects of these substances are numerous but most importantly is upregulation of cell adhesion molecules on endothelial cells to increase leukocyte adherence and infiltration through the endothelial wall.

Vascular endothelium itself becomes activated in response to ischaemic hypoxia to cause activation of endothelial adhesion molecules that promotes

leucocytes adherence to the endothelial wall and consequent leukocytes exudation and infiltration in the brain parenchyma. Therefore, adhesion molecules in leukocytes and endothelial cells are membrane surface glycoprotein that are involved in leukocyte-endothelium interactions to allow for the infiltration of leukocytes through the endothelium into brain parenchyma by processes of rolling, adhesion, and trans-endothelial leukocyte migration/diapedesis.

There are three major adhesion groups that are involved in these three processes; the selectins (P-selectin, E-selectin, L-selectin), the immuglobulins super family (intracellular adhesion molecule-1 ICAM-1, intracellular adhesion molecule-2 ICAM-2, vascular cell adhesion molecule-1 VCAM-1, platelet-endothelial cell adhesion molecule PECAM, mucosal vascular addressing cell molecule-1 MAdCAM-1), and the integrins [23, 25]. Rolling involves interaction of P-selectin with P-selectin glycoprotein ligand-1 PSGL-1.

Although blood-brain barrier (BBB) confers brain with protection against systemic toxins under normal physiologic condition, during cerebral ischaemia BBB disruption results from activation of matrix metalloproteinases (MMPs) with MMP-2 (gelatinase A) and MMP-9 (gelatinase B) being implicated in cerebral ischaemia [24]. MMP-2 that is normally expressed at low levels becomes increased during cerebral ischaemia to cleaves and activates MMP-9, which degrades components of the basement membrane in the vascular wall leading to BBB disruption, thus allowing further infiltration of inflammatory mediators and other potential toxins [11]. There is also increasing evidence that initial IL-6 and TNF-α inflammatory cytokines are directly capable of expressing MMP-9 [25]. Importantly, there is also evidence of neuro-inflammation; secondly, involvement in ipisilateral hemisphere, attributable to retrograde degeneration of thalamo-cortical projection fibres [24].

Oxidative Stress: Oxidative stress is defined as the condition occurring when the physiological balance between oxidants and antioxidants is disrupted in favour of formation of oxidants and reduction in antioxidants response as seen in ischaemic stroke. Long term cerebral hypoperfusion produces abnormal levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) through multiple injury mechanisms, such as mitochondrial inhibition, calcium ions overload, reperfusion injury, and inflammation [5]. The brain derives its energy almost exclusively from oxidative metabolism in mitochondrial respiratory chain, which involves the transfer of electron and generation of by-product as free radicals/oxidants. Free radical are chemical species that has single unpaired electron around its outer orbit, and they includes ROS which are hydroxyl radicals (·OH), superoxide(O_2·−), hydrogen peroxide (H_2O_2), and RNS which are nitric oxide (NO), and peroxynitrite (OONO-).

Under normal cellular conditions, mitochondrial respiratory chain generate NADP as by-product of ATP generation by oxidative phosphorelation, this NADH through NADPH oxidase activity generate superoxide(O_2·−) which is further converted to hydrogen peroxide (H_2O_2) simultaneously or enzymatic catalysis of superoxide dismutase (SOD) by combining with hydrogen ion ($H^{+)}$. This H_2O_2 when leaves the mitochondria into the cytosol can form the hydroxyl radicals (·OH) radical either in the presence of transition metal ions (Fenton reaction) or in the presence of superoxide radical (Haber-Weiss reaction).

Under ischaemic condition, there mitochondrial inhibition of oxidative phosphorelation due to the lack of sufficient oxygen, and the oxygen depleted cell switch to glycolytic pathway of ATP production that results in lactate acid and hydrogen ion (H^+) build-up in the mitochondria and the subsequent reversal of the H^+ uniporter on the mitochondrial membrane which causes excess cytosolic H^+ accumulation and acidosis [27]. Acidosis contributes to oxidative stress by providing excessive H^+ for the successive progression in the generation of H_2O_2 and the final ·OH through aforementioned reactions, with this effect more pronounced in neurons due to inherently low anti-oxidant defense. In addition, the potent protein and lipid oxidant peroxynitrite ($OONO^–$) of RNS is favourably formed in the oxygen depleted cell by the reaction of nitric oxide (NO) and superoxide(O_2·−), thereby also contributing to oxidative stress.

Calcium overloads, as a result of glutamate mediated NMDA receptor excitotoxicity, contributes in neuronal oxidative stress at cytosolic and mitochondrial level. At cytosolic level, excessive calcium ion activation of key intracellular enzymes such as neuronal nitric oxide synthase (nNOS) via Ca^{2+} binds calmodulin, nNOS catalyses the formation nitric oxide (NO) free radical from L-arginine [5].

At the mitochondrial level, excessive calcium ion influx into mitochondrial matrix leads to the inner mitochondrial accumulation of significant amount of Ca^{2+} via mitochondrial calcium uniporter (MCU) which propagates disruption of normal bio-energetic, mitochondrial ROS, and membrane permeability [4]. Production of ROS becomes more significant during ischaemic reperfusion and post-ischaemic neuroinflammatory cellular activity of NADPH oxidase in microglia, macrophages, and neutrophils.

Apoptosis: Apoptosis is a physiological pathway of cell death which occurs under various physiological and pathological conditions initiated and triggered by either extrinsic or intrinsic pathways [28]. In the nervous system, the notion that ischemic insults cause neurons to undergo necrosis is strengthened by the implication of excitotoxicity in ischemic neuronal death; growing evidence indicates that ischemia may additionally induce programmed apoptotic neuronal death in a fashion where apoptosis becomes dysregulated [29]. While the neurons within the core infarct die by immediate necrosis due to insufficient ATP. Penumbra die by ATP requiring process of apoptosis, supporting the established evidence that cell death after cerebral ischemia occurs through the dual pathways of ischemic necrosis and apoptosis [30].

There are two distinct pathways that can initiate the caspase-dependent apoptosis: the intrinsic (or mitochondrial) pathway, and the extrinsic (or death receptor) pathway. There is an additional pathway that involves T-cell mediated cytotoxicity and perforin-granzyme-dependent killing of the cell. The perforin/granzyme pathway can induce apoptosis via either granzyme B or granzyme A. The extrinsic, intrinsic, and granzyme B or A pathways converge on the same terminal, or execution pathway [31, 32].

Intrinsic signaling pathway that initiates apoptosis involves increase in inner mitochondrial permeability due to cellular stress (such as free radicals and hypoxia) and damage, which causes release of cytochrome C from mitochondria into the cytosol leading to activation of caspase 9 by binding to the caspase-activating protein Apaf-1, and subsequent activation of caspase 3 and other effectors of apoptosis. Caspase 3 is the execution phase that initiates a caspase cascade leading to the degradation of cellular components and cell death; as such, caspase 3 activity is commonly used as an indicator of apoptosis [31].

Under normal cellular conditions, the mitochondria permeability is regulated by a balance between pro- and anti-apoptotic members of Bcl family proteins. Alternatively, mitochondria may also release apoptosis-inducing factor (AIF), which leads to apoptosis by a caspase independent mechanism [11]. The extrinsic signaling pathway initiation of apoptosis involves transmembrane receptor mediated interactions of death receptors. Activation of TNF super family cell death receptors; such as Fas and TNF receptors activates their respective ligands FasL and TNF-α to form an active complex termed FADD (Fas-associated death domain), FADD binds with inactive pro-caspase-8 to activate it through induced proximity mechanism that leads to the execution caspase [33].

Death receptors identified includes, Fas, TNFR1, DR4 and DR5, and their respective death ligands are FasL, TNFa, TRAIL, and TNFSF10 [31]. Multiple pre-existing pathophysiologic mechanisms that can induce apoptosis after cerebral ischaemia includes oxidative stress, glutamate excitotoxicity, calcium influx and pro-inflammatory cytokines [34].

Neuro-protective Agents: Various substances called ‘agents’ that recently shown to confer neuro-protective activity in cerebral ischaemia via targeting one or more pathophysiologic mechanism(s) are represented in **Table 1** below:

TABLE 1: AGENTS THAT CONFER NEURO-PROTECTION IN CEREBRAL ISCHAEMIA VIA TARGETING ONE OR MORE PATHOPHYSIOLOGIC MECHANISM (S).

Treatment	Animal Model	Findings	Proposed mechanism	References
Linagliptin	Type 2 diabetic mice bilateral common carotid artery occlusion	Significantly counteract cognitive impairment, reduction in increase of cerebral IgG extravasation and reactive microglia, suppress the increase in cerebral oxidative stress, increase in cerebral claudin-5 and decrease gp91phox	Anti-inflammatory effect by ameliorating cerebral IgG extravasation and activation of microglia. Antioxidative effect by attenuation of superoxide free radical and decrease gp91phox; a major subunit of NADPH oxidase. Prevent BBB disruption through increase in cerebral claudin-5; the main cerebral endothelial tight junction protein which plays a major role in BBB function	Ma, et al 2015 [35]
Docosahexaenoic acid (DHA)	Rat middle cerebral artery occlusion	Decrease in evans blue dye (EB) extravasation and fluorescein isothiocynate (FITC)-dextran leakage, attenuation of cortical and total infarct volume	Prevent ischemia-induced BBB disruption by reduction in EB extravasation and FITC-dextran leakage	Hong, et al 2015 [36]
2,3,5,6-tetramethylpyrazine (TMP)	Rat middle cerebral artery occlusion	Significant improvement in neurological function, increase in MAP-2 level, and enhancement in spine density of basilar dendrites	Blockade of multiple events of the injury cascade, and increase MAP-2 expression level that play a key role in neuronal dendritic plasticity	Lin, et al 2015 [37]
Ligustrazine Derivative (T-VA)	Rat middle cerebral artery occlusion	Improvement of motor functions	Antiexcitotoxic effect by stimulating mark increase in Ca^{2+}-Mg^{2+}ATP enzyme activity thereby attenuating intracellular Ca^{2+} overload. Antioxidative effect via enhancing the activities of SOD. Anti-inflammatory effect via blockade of NF-κB activation and the subsequent suppression of COX-2	Li, et al 2015 [38]
Interleukin-1 receptor antagonist (IL-1Ra)	Rat transient middle cerebral artery occlusion	Acute administration led to faster and more complete recovery than chronic administration on various motor test scores	IL-Ra promotes functional recovery through inhibiting acute proinflammatory IL-1 cytokine.	Girard et al, 2014 [39]
Panax notoginseng polysaccharides (PNPS)	Rat temporary middle cerebral artery occlusion	Significantly reduced severity of neurological deficit, infarct volume, cerebral edema and neuronal death	Supress apoptosis by increasing Bcl-2/Bax ratio and reducing the the level of cleaved caspase-3.	Jia, et al 2014 [40]
Panax notoginseng polysaccharides (PNPS)	Rat temporary middle cerebral artery occlusion	Significantly reduced severity of neurological deficit, infarct volume, cerebral edema and neuronal death	Supress apoptosis by increasing Bcl-2/Bax ratio and reducing the the level of cleaved caspase-3.	Jia, et al 2014 [41]
Lithium	Rat unilateral left common carotid artery occlusion	Significant reduction of brain injury and increment in neurogenesis. Normalisation of motor hyperactivity, anxiety-like	Anti-inflammatory effect by amelioration of delayed cytokines production and reduction in activation of resident glial cells	Xie, et al 2014 [42]

		behavior, and serum cytokine levels, including IL-1a, IL-1b, and IL-6.		
Kruppel-like zinc-finger transcriptional factor (BTEB-2/ IKLF)	Rat intracerebral haemorrhage	Significantly decrease in neuronal apoptosis	Antiapoptotic via down-regulation of neuronal apoptosis by promoting Bad phosphorylation	Liu, et al, 2015 [43]
Progesterone	Rat parmanent middle cerebral artery occlusion	Significant attenuation of infarct volume, and improvement of functional outcomes on locomotor activity, grip strength, sensory neglect, and gait impairment	Antiinflammatory effect by increasing the expression of CD-55, a cell surface protein which reduces complement factors that can trigger the debilitating inflammatory cascade	Wali, et al 2014 [44]
Withania somnifera (WS)	Rat parmanent middle cerebral artery occlusion	Significantly improve functional recovery and reduce the infarct volume	Antioxidant effect via upregulating the expression of hemeoxygenase. Antiapoptotic effect by attenuating the expression of proapoptotic proteins (PARP-1) and apoptotic inducing factors (AIF) via the PARP-1-AIF pathways.	Raghavan, and Shah 2015 [45]
Edaravone and Scutellarin	Rat intraluminal middle cerebral artery occlusion	Both drugs markedly reduce infarct cerebral tissue area, and attenuate the expression levels of TNF-α, IL-1β and NOS. They further suppress the upregulation of inflammatory cytokines, iNOS, NO and ROS in LPS-induced BV-2	Anti-inflammatory effect by inhibiting the expression levels of various inflammatory mediators in activated microglia, especially TNF-α. Antioxidant through inhibiting the inflammatory responses, ROS generation and oxidative tissue damage.	Yuan, et al 2014 [46]
Apelin-13	Rat middle cerebral artery occlusion	Significant reduction in apoptosis by decreasing positive TUNEL cells. Significant change in neurological dysfunction	Antiapoptotic via reduction positive TUNEL cells	Aboutaleb, et al 2014 [47]
Matrix Metalloproteinase-8 inhibitor (M8I)	Rat middle cerebral artery occlusion	Reduction in infarct volume, neurological score, and survival/death of neural cells. Abrogation of microglial activation and TNF-α expression on histological analysis	Anti-inflammatory effect as neutrophil collagenase matrix metalloproteinase-8 (MMP8) inhibitor that modulate neuroinflammation by abrogating microglial activation and TNF-α production Abrogation of microglial activation and TNF-α expression	Han, et al 2016 [48]
Nicotine	Rat global cerebral ischaemia	Significant increase in neuronal survival, as well as a significant reduction of enhanced expression of tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β)	Anti-inflammatory effect via α7 nicotinic acetylcholine receptor (α7 nAChR) to inhibits microglial proliferation	Guan, et al 2015 [49]
Linolenic acid	Rat photothrombotic cerebral ischaemia	Preservation in protein abundance of astrocytic glutamate transporter GLT-1, decrease in protein abundance of AQP4 and brain edema, inhibition of microglia	Multi-mechanism therapeutic target of antiexcitotoxicity via clearance of glutamate, anti-inflammatory via inhibition of microglia activation, and antiapoptotic via attenuation of cell apoptosis	Liu, et al 2014 [50]

		activation, attenuation of cell apoptosis and improvement of behavioral function recovery		
Dexamethasone (DEX)	Rat intracerebral hemorrhage	Increase in the expression of Bcl-2, level of Bax, cleaved caspase-3, and P53 proteins	Antiapoptotic via increase in Bcl-2/Bax ratio and decrease in the expression of cleaved caspase-3. Anti-inflammatory via inhibition of inflammatory response.	Lee et al 2015 [51]

CONCLUSIONS AND RECOMMENDATION: It can be seen that, numerous neuro-protective agents have shown promising efficacy in preclinical animal stroke models but conversely, fail to translate in humans, stroke therapies. Gladstone, Black and Hakim 2002 [18] identified possible reasons attributed to this drawback to include:

1. Preclinical studies have used very short time windows for drug administration, whereas clinical trials allow longer time windows.
2. Preclinical studies target the ischemic penumbra, whereas clinical trials do not.
3. Preclinical studies have demonstrated protection of gray matter, whereas clinical trials frequently enroll patients without specifying location of damage.
4. Optimal duration of neuro-protectant administration is unknown.
5. Preclinical studies have relied on infarct size to judge therapeutic efficacy, whereas clinical trials rely on behavioral outcomes.
6. Preclinical studies have relied on early outcomes, whereas clinical trials rely on late assessments.
7. Experimental stroke models are homogeneous, whereas human stroke, heterogeneous.
8. Choice of outcome measures can determine the success of a clinical trial more than actual drug efficacy.
9. Small trials are trying to answer questions that only large trials can answer.

RECOMMENDATION: The Stroke Therapy Academic Industry Roundtable (STAIR) recommendations should be strictly followed to improve the quality of stroke studies and their later translation into practice. Despite the challenges in acute stroke therapy, there is still reasonable hope of finding an effective future neuro-protective agent for human stroke.

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