Original Research Paper

Volume-8 | Issue-1 | January-2018 | PRINT ISSN - 2249-555X

Biomedical

VESICULAR DRUG TARGETING IN COMBATING CEREBRAL OXIDATIVE INJURY

Ardhendu Kumar Mandal* Infectious Diseases and Immunology CSIR-Indian Institute of Chemical Biology, India *Corresponding Author

Aparajita Ghosh Drug Development, Diagnostics and Biotechnology CSIR-Indian Institute of Chemical Biology, India

Nirmalendu Das Drug Development, Diagnostics and Biotechnology CSIR-Indian Institute of Chemical Biology, India

ABSTRACT Brain stroke is the main reason for health disability and mortality among aged pupils worldwide. Mitochondrial dysfunction, due to oxidative stress during cerebral ischemia-reperfusion, exerts inflammations and damages to the neurones of the brain. Free form of antioxidant is basically inefficient for the treatment of cerebral ischemia as blood brain barrier remains in between systemic blood and brain interstitial fluid. Furthermore, the affectivity of antioxidant compounds may be hindered by their poor permeability, low solubility and enhanced rate of efflux from the effected cells. Therefore, different active compounds encapsulated in various vesicles have been investigated in vivo to prevent brain ischemia. Our review, on the methods applied for the preparation, characterization, and efficacy of vesicular active drug compounds -delivery, has demonstrated a promising aspect, especially for nanoencapsulation in the treatment of cerebral ischemia against oxidative cell death with higher biological effectiveness and least side effects.

KEYWORDS : Cerebral ischemia; Oxidatve stress; Inflammation; Cellular damage; Vesicular drug efficacy

Introduction

Cerebral oxidative injury is the 3rd most familiar leading factor of death and senile dementia all over world. The diminished supply of oxygen and glucose to the parts of the effected brain during cerebral ischemia initiates bioenergetic failure leading to oxidative strain, inflammation and dysregulated blood-brain-barrier (BBB) that ultimately causes brain cell dying.[1] In cerebral ischemia, the production of ATP becomes hampered resulting in defective ions leakage across the cell membrane, neurotransmitters such as dopamine and glutamate - release, phospholipase activation, phospholipids hydrolysis, arachidonic acid release, and reactive nitrogen and oxygen species (RNS and ROS) elevation that altogether lead to apoptotic and necrotic cell death.[2,3] In pathogenesis of cerebral ischemia-reperfusion, loss of neuronal mitochondrial membrane integrity promotes oxidative stress through enormous productions of ROS and RNS that overpower the counter defence of the brain cells and succumb to irreversible damage.[4] Oxidative injury worsens when blood flow to brain regions i.e. cerebrum, cerebellum, hypothalamus and especially hippocampus is refurbished during reperfusion.[5,6]

Hence, it becomes needed to administer exogenous potential antioxidants as drugs to scavenge free radicals and to counter brain ischemia-reperfusion -inducted oxidative damage. But simple antioxidants therapies are not the proper approaches to attenuate cerebral ischemic damages as most of drugs when introduced into the body become diluted into the systemic circulation and also cannot traverse the BBB owing to their insolubility and poor bioavalability. Therefore, it is needed to grow a device which could impart a raised pool of antioxidant to the affected areas of the brain cells for their entire protection with reduced drug toxicity against oxidative insult.

Liposome and nanocapsule, the familiar accepted forms of drug-vehicle by virtue of their non-immunogenic, nontoxic, biodegradable and continuous drug liberating capability in the living framework, might be considered as therapeutic tools in combating cerebral oxidative damage.[7-9]

At present, there are no effective ischemia preventive agents that may be useful for treating cerebral injury. Melatonin, quercetin, CDP-choline and ascorbate have been experimented in several studies as vesicular potent antioxidants applied against cerebral ischemic injuries signifying a reliable outcome.[7-9,10]

Cerebral injury

Cerebral ischemic injury occurs as a result of an oxygen occlusion within a blood vessel for supplying blood to the brain through embolus or thrombus formation or cerebrovascular hemorrhage. The obstruction in the blood vessel occurs due to the development of gradual cholesterol deposits lining the vessel-wall that refers either to a thrombus i.e. blood clot developed at the clogged part of the vessel or to a blood clot formed at another location such as embolism in the circulatory system, whereas, cerebrovascular hemorrhage takes place due to rupturing of a weakened blood vessel for uncontrolled hypertension.

Oxidative stress

Blockage of arteries causes anoxic stage in brain cells reducing high-energy phosphate amounts.[11] The resultant membrane depolarization triggers glutamate to increase calcium-overload in the neurones which are pivotal for death.[12] Enhanced intracellular ADP, Na^+ and Ca^{2+} induce mitochondria to generate ROS, vulnerable for brain cells as containing lower levels of endogenous antioxidants.[13] During ischemia, increased activity of nitric oxide synthase (NOS), increment in the intracellular Ca^{2+} level, efflux of glutamate at synapses, and triggering of N-methyl-D-aspartate receptor (NMDAR) play a critical role for the deleterious function of NOS in making neurodamage. Moreover, ischemia conducts to the production of superoxide through the activation of nicotinamide adenine dinucleotide phosphate hydride (NADPH) oxide synthase (NOX), cyclo-oxygenase (COX), xanthine oxidase (XO), and the mitochondrial electron transport chain leakage, with also other mechanistic involvements.[14-16] The peroxynitrite, formed by the combination of superoxide and NO, and other ROS, destroy the DNA with actuating poly (ADP-ribose) polymerase-1 (PARP-1), a DNA repair enzyme, to convert β-nicotinamide adenine dinucleotide (NAD+) into long poly ADP-ribose and nicotinamide (NA). The over-activity of PARP-1, in turn, depletes cellular NAD+ levels by the impairment of glycolysis and mitochondrial respiration leading to energy failure, ATP fasting, and subsequently neuronal damage.[17] In addition, oxidative strain may also be evaluated by estimating oxidized bio molecules such as lipids, nucleic acids, proteins, nuclear factor erythroid 2-related factor 2 (Nrf2), and 8-hydroxy-2-deoxyguanosine (8-OHdG). Lipohydroperoxide (conjugated diene) derived from polyunsaturated fatty acids due to lipid peroxidation after having ischemia, alters cell membrane fluidity, enhances membrane permeability and reduces membrane ATPase activity, leading to brain cell injury.

The augmented productions of nitric oxide, peroxynitrate and superoxide occur in the surrounding of blood vessels after reperfusion takes place. Matrix metalloproteinases get activated by ROS and disrupt collagen and laminis in the basal lamina by distorting the unification of BBB and the vascular wall permeability.[18] Secondary oxidative damage appears as a consequence of brain edema directing to reduced blood perfusion and inflammation during reperfusion due to huge ROS and leukocytes influx into the injured cerebral region.[19,20]

Generation of free radicals

Oxygen (O_2) is converted to reactive O_2 species, such as O_2^-, H_2O_2 and $^{\cdot}OH$ by its univalent reduction.

$O_2 + e^- \rightarrow O_2^-$ Superoxide radical
$2O_2^- + 4H^+ \rightarrow 2H_2O_2$ Hydrogen peroxide
$H_2O_2 \rightarrow {}^{\cdot}OH + {}^{\cdot}OH$ Hydroxyl radical
In the presence of Fe^{2+}, H_2O_2 produces the very active species $^{\cdot}OH$ by the Fenton reaction mentioned below:
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + {}^{\cdot}OH$

Mechanism of lipid peroxidation

Cell possesses large amount of poly unsaturated fatty acids receptive to free radical attack as the double bonds within membrane allows removal of hydrogen atom by ROS such as $^{\cdot}OH$. The fatty acyl radicals formed in poly unsaturated fatty acids undergo molecular rearrangement to form more stable conjugated diene which can crosslink fatty acids within cellular membrane. This process of auto oxidation of fatty acid occur involving three sequences – 1. Initiation, 2. Propagation, and 3. Termination.

In a lipoperoxide free lipid system, the initiation of a peroxidative sequence points out to the attack of a $^{\cdot}OH$ having abstracting capability of a hydrogen atom from a methylene group ($-CH_2-$) with very high mobility. The attack generates ROS from polyunsaturated fatty acid while carbon radical formed inclines to stabilize by molecular arrangement for forming a conjugated diene.

In propagation, conjugated dienes, under aerobic condition, are capable of binding with O_2 for forming a peroxidal radical, $LOO^{\cdot}$, having also the ability to abstract H from another adjacent lipid molecule causing an autocatalytic chain reaction.

Formation of H_2O_2 in termination stage is generally gained by the reaction of a peroxyl radical with α-tocopherol, the principal lipophilic chain breaking molecule in the cell membrane. In addition, any kind of lipid free alkyl radicals may react to a lipoperoxide for forming non-initiating and non-propagating species (Figure 1).

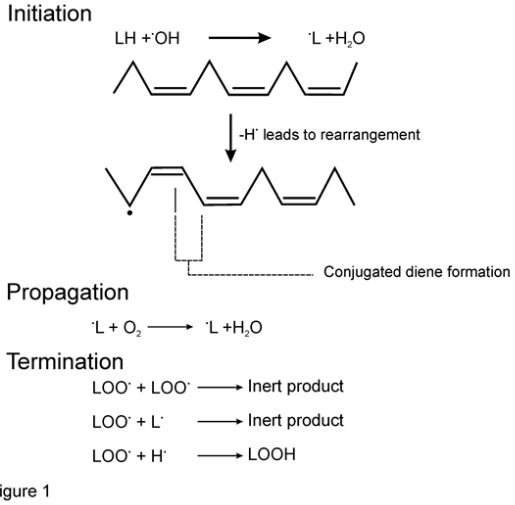

Figure 1

Fig 1. Mechanism of lipid peroxidation.

Defence mechanisms

To counter ROS, the body has evolved several methods to deactivate them, before they can cause tissue damage by two ways:

1. Primary defence against ROS, 2. Secondary defence against ROS

For primary defence against ROS, superoxide dismutase (SOD), catalase and peroxidase, the antioxidant enzymes lead to catalytic removal of ROS. Cu and Zn containing SOD participate in scavenging superoxide in the cytosol whereas Mn carrying SOD in the mitochondrial matrix. Glutathione peroxidase catalyses reduced glutathione (GSH) and hydroperoxide to oxidized- glutathione disulphide (GSSG) and the reduced hydroperoxide, 2/3rd of which is cytosolic and 1/3rd mitochondrial. Catalase located in microperoxisomes or peroxisomes, catalyses the breaking up of H_2O_2 to H_2O and O_2, preventing cells from oxidative injury by $^{\cdot}OH$ and H_2O_2.[21]

For secondary defence against ROS (Figure 2), micro molecules react with free radicals to make another scavenger radical compound for producing lesser harmful antioxidant radical species. Generally, reduced glutathione, ascorbate and α-tocopherol function as cellular antioxidants while α-tocopherol, existed in plasma lipoproteins and cell membrane, acts as a chain breaker antioxidant. At first, when α-tocopherol radical is generated, it migrates to the membrane surface and becomes regenerated to α-tocopherol by the reaction with ascorbate. The eventual ascorbate radical can be reconverted to ascorbate by the reduction with GSH, involved in direct ROS scavenging and the productive GSSG can be reconverted to GSH via NADPH-glutathione reductase activity.

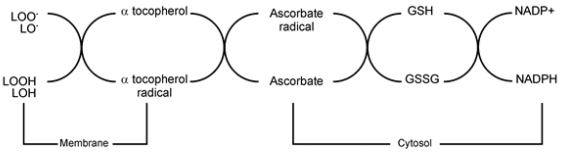

Figure 2

Fig 2. Secondary defence mechanism.

In this aspect, in the presence of catalytic iron and copper ions, ascorbate, despite of its antioxidant capability, may produce $^{\cdot}OH$ owing to its metal reducing capability which is similar to O_2^- generation together with H_2O_2[22] while ascorbate (0.2-4 mM) may take part as a cellular reducer to exchange O_2^- in Heber-Weiss reaction. Thus, these antioxidants can participate in combined form to function as cellular antioxidant.

Furthermore, lipid peroxidation can be regulated at the initiative stag through quenching singlet oxygen or scavenging free radicals while the propagatory chain reaction may be interrupted through scavenging of peroxyl radicals by the phenolic components (AOH).[23]

Sources of free radicals in cerebral ischemia

ROS generation from mitochondria

The role of mitochondria as apoptotic signal transducer and central integrator is significant during the ischemic onset due to diminished glucose level and oxygen supply in the neuronal cells causing electron leakage and reducing intermediates accumulation to produce ROS.[24] Free radical generations become higher during the state IV metabolic stage of mitochondria manifested by low ADP, ATP synthesis and electron flow, low oxygen consumption and high NADH / NAD^+ ratio in contrast to stage III.[25] Due to lack of ADP, succinate-derived electrons can reversibly be transported to complex I to produce increased oxygen levels correlated as the major pathophysiologically relevant mitochondrial ROS-generating site.[26]

Mitochondrial nitric oxide ($NO^{\cdot}$) acts significantly as modulator of ROS generation because of existing few reactive $NO^{\cdot}$ redox metal centres in the electron transport chain for mitochondrial oxygen consumption due to inhibition of cytochrome c (cyt c) oxidase activity.[27,28]

An update has demonstrated the participation of p66Shc protein for mitochondrial ROS generation to form cyt c-molecular complex by abstracting electrons to reduce oxygen for forming O_2. The inter-mitochondrial membranous P66Shc, becomes responsible for downstream p53 target as well as indispensable for the enhanced ROS generation, cyt c release, mitochondrial trans-membrane potential dissipation and apoptosis.[29] In mitochondria-dependent apoptosis, molecular signalling activates the apoptotic activators and effectors - liberations i.e. apoptosis-inducing factors or cyt c to cause cell death.[30]

Xanthine oxidase, the source of ROS

Ischemic damages, occurred due to oxidative stress during cerebral reperfusion, emerge because of mitochondrial disbalance causing seize in the oxidative phosphorylation and inducting energetic equilibrium-breakdown i.e. increase in the levels of hypoxanthine, inosine and adenosine.[31,32] Basically, xanthine dehydrogenase

converts hypoxanthine to uric acid and xanthine, and thus itself can be transmuted to xanthine oxidase (XO) by utilizing oxygen as electron receiver to generate anions.[33] In hypoxia, XO metabolizes xanthine and hypoxanthine to produce O_2^- and H_2O_2 related to oxidative stress to cause cerebral injury during ischemia-reperfusion.[34]

NADPH oxidase

The membrane bound NADPH oxidase (NOX) enzyme, found in leukocytes and vascular tissues, is judged to be a pivotal factor for anomalous ROS burst during ischemic insult. Triggered NADPH oxidase reacts with enhanced O_2^- production to produce hydroxyl radical, and peroxynitrite with the nitric oxide interaction.[35] NOX2 and NOX4, the NOX isoforms, located hugely in the hippocampus CA1 and cerebral cortex, take part chiefly in oxidative stress-persuaded brain cell death during ischemia-reperfusion.[36-38] Ca^{2+} influx via NMDAR, activates PKCζ and phosphorylates p47phox and triggers its translocation inducing the activation of NOX enzyme, the primary source of O_2^- generation after the activation of neuronal glutamate receptor for excitotoxic damage.[39]

NO synthases

Nitric oxide (NO) takes part in ROS mediated inflammation which is depended upon cellular source of NO and various isoforms of the nitric oxide synthase (NOS) after having ischemic cerebral injury.[40,41] Neuronal system contains three types of NOS while endothelial NOS (eNOS) keeps up the cerebral blood flow, inducible NOS (iNOS / NOS2) and neuronal NOS (nNOS / NOS1) may produce more NO by well defined stimuli in glial cells.[42] After cerebral ischemic insult, NO, liberated immediately from eNOS, promotes calcium dependent vasodilation and inhibits microvascular aggregation and adhesion as a protective mechanism [43] while NO production by over-activated nNOS and eventual release by de novo iNOS expression contribute maximal to the cerebral injury. NO also produces highly reactive peroxynitrite, involved in late neurotoxicity. The initial increase in NO after the onset of cerebral ischemia-reperfusion occurs by the activation of nNOS, governed by the intracellular calcium signalling.[44] Furthermore, iNOS when activated by cytokine upregulation after cerebral ischemic impose, contributes its greater activity to brain cell injury in the NF-kB-dependent way than that of nNOS suggesting as the prime target on endothelial damage.[45]

Oxidative stress-induced inflammation

Inflammation takes place in the recovery after ischemic brain injury which may worsen the ischemic state.[46] It is intermediated by cytokines, leukocytes and microglia, having pro-inflammatory characteristics with destructive or promoting consequences as ROS generation.[47-49] The inflammatory mediators, induced by ROS, utilize leukocytes to attach the vascular endothelial cells augmenting their assembly. Therefore, leukocytes, having superoxide-generating system and NADPH oxidase, are infiltrated into the ischemic regions from the systemic circulation within hours after the onset of brain ischemia.[50,51] Likewise, myeloperoxidase carrying neutrophils, generate highly reactive hypochloric acid with the participation of Cl^- and H_2O_2 implicated in the cerebral ischemic pathogenesis.[52] Moreover, dendritic glutamatergic neurons derived COX-2 becomes highly-activated and accumulated in microglia, astrocytes, neuron, vascular and inflammatory cells invading the brain to cause cellular damage during cerebral ischemia probably due to a mechanism of COX-2-dependent glutamate excitotoxicity mediated Ca^{2+} dysregulation.[53]

Pathways for neuronal cell death

Initially, ROS can attack directly on proteins, nucleic acids, lipids and other cellular macromolecules and subsequently, in the signalling leading to apoptotic cell death after induction of brain ischemia (Figure 3).[54,18] Mitochondria maintain the key contribution during apoptosis by liberating cyt c from the inter membranous space to the cytoplasm. Cyt c combines apoptotic protease activity factor 1 in the cytosol and makes a multimeric complex with the participation of dATP. This complex recruits procaspase-9 which becomes triggered by autocleavage. The upstream triggered caspase-9 initiates and activates caspases- 3,2,6,8 and 10 while the enforcer caspase-3 strikes the cell framework, resulting apoptosis by cleaving substrate proteins e.g. PARP and causing remarkable damage to DNA.[55-57] Furthermore, mitochondria-derived activators of caspases like Bid, Bax and Bim which are released from mitochondria, neutralize the activity of inhibitor proteins and promote the caspase activation by disrupting mitochondrial membrane potential and actuating the cyt c release through the permeability transition pore.[58,59]

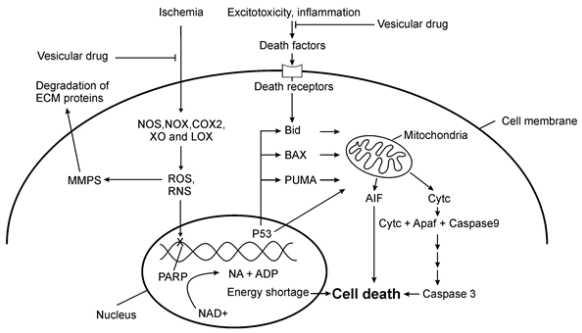

Figure 3

Fig 3. Pathways for neuronal cell death and its inhibition.

p53, the tumor suppressor gene, mediates apoptosis while it makes bond to specified transcripts and DNA sequences such as BH3-interacting domain death agonist (Bid), Bcl-2-associated X protein, pro-apoptotic protein genes, and p53-upregulated modulator of apoptosis (PUMA).[60] In this concern, apoptotic stimuli may translocate p53 to the mitochondrial membrane by forming a repressive complex with preventive Bcl-X_L after cerebral ischemia.[61] PUMA, thus, makes bond directly to antagonize anti apoptotic Bcl-2-associated X protein and Bak activity, and induces caspase activation resulting mitochondrial dysfunction with the maintenance of a critical role as mediator of apoptosis induced by various stimuli.[62]

The p53 signalling is also correlated to neuronal apoptosis via a downstream PIDDosome protein, i.e. p53-induced death domain (PIDD) and pro caspase-2. The p53-induced caspase-2 triggering via PIDDosome -caspase-2, promotes cellular apoptosis by the activation of Bid monitored during cerebral ischemia.[63]

Recent studies have revealed that adiponectin, the hormone, has a role in the pro-apoptotic signalling connected to p38 mitogen-activated protein kinase (p38MAPK) and AMP-activated protein kinase (AMPK) after having cerebral ischemia.[64]

Excitotoxic damage after ischemia

Oxidative stress, glutamate excitotoxicity and intracellular Ca^{2+} imbalance are interlinked to a series of biochemical cascades occurred after cerebral ischemia.[65] The reduced ATP production following ischemia contributes to bio-energetic stress resulting decreased Na^+ / K^+ ATPase activity and imbalanced neuronal Na^+ and K^+ levels i.e. an increase in intracellular Na^+ instead of K^+ causing membrane depolarization.[66] Mitochondrial Ca^{2+} homeostasis is maintained by the Ca^{2+} ATPase membrane activity through calcium uniporter and Na^+ / Ca^{2+} exchanger.[67] The increased amount of cytosolic Ca^{2+} create cellular stress by over-accumulating in the mitochondrial matrix, ensuing mitochondrial disorder, swelling, permeability transition, and the cell death factors release as well as producing more ROS involved in cell death signalling.[67,68] The spontaneous neurotransmitter glutamate release from synaptic vesicles, and its assembly in the extracellular spaces after the onset of cerebral ischemia, is mediated by the elevated cytosolic Ca^{2+} level through Na^+ / glutamate transporters promoting neurotoxic injury where glutamate makes the disarrangement of Na^+ levels with the membrane depolarization, and the subsequent influx of H_2O and Cl^-.[69,66,70] The osmotic gradients are developed owing to the influx of H_2O into the neuronal cells while their swellings form an edema that enhances intracranial pressure after the onset of cerebral ischemia-reperfusion. Thus, a coordinated interlink exists among free radical generation, rise in Ca^{2+} levels, and glutamate accumulations during hypoxia / re-oxygenation due to the fall in ATP levels having inability to translocate accumulated Ca^{2+} into the cytosol through membrane ATPases.[71] The enhanced level of extracellular glutamate also interferes with the cystine uptake via the cystine / glutamate antiporter. The reduced cystine level in the neuronal cells conducts to the intracellular glutathione (GSH) decrement causing severe cellular oxidative damage by generating ROS after ischemic insult.[72]

Role of drugs as antioxidant for ischemic therapy

The endogenous antioxidant status in brain tissue after having ischemia reperfusion, especially in ageing, become low showing poor

antioxidant defence mechanism against free radicals-induced injury. Therefore, it is essential to introduce biological antioxidant to protect injuries from free radical attack in ischemia.[73-75] However, endogenous antioxidant therapy is not a fruitful perspective to encounter the cerebral ischemic injury as free radicals generated by ischemia-reperfusion lead to their rapid consumption and depletion.[76] In this concern, it is needed to administer potent exogenous antioxidants as drug for free radical scavenging to check cerebral ischemia-reperfusion inducted oxidative stress specially for aged individuals. Quercetin, CDP choline, ginkgo biloba and melatonin, studied earlier, are known as strong free radical scavengers which are useful for combating ischemic injuries.[8,9,77,78]

Vesicles as drug carrier

Most of the drugs cannot traverse BBB owing to their low oral stability and insolubility, and also for their dilution in the systemic circulation. Therefore, it is needed to develop a strategy for delivering raised pool of drug in the brain area for entire neuronal cells protection in combating oxidative damage. Liposome- or poly lactide nanocapsule-mediated drug targeting to brain cells is important not only for their non toxic nature, biodegradability, smaller sizes, non immunogenicity, sustained drug-releasing ability and both hydrophobic and hydrophilic drug carrier capability but also for assisting the encapsulated drug to reach the effected tissue significantly.[9,8] Furthermore, because of the existence of mannosyl/fucosyl receptors on the neuronal cells -surface, sugar-grafted vesicles become efficient in the neuronal site specific drug targeting for protecting cerebral ischemic damage.[9]

Targeting of vesicles

Vesicles may be targeted in the brain cells by passive and active targeting. In passive targeting, natural anatomical structures and physiological process of the host are taken into consideration, directing in vivo distribution and disposition, while passive targeting of vesicles may be facilitated by their alteration of composition, surface charge, and size.[7] In active targeting, there is chemical or physical modifications of vesicular surface which help the vesicles to be targeted towards a particular cell basically through a receptor mediated endocytosis for glycoside or glycolipid bearing vesicles and antibody or peptide grafted vesicles.[9,79]

Efficiency of vesicular drugs

Drug delivery to the neuronal cells, is challenging as it has to cross BBB located at the brain capillaries with an accumulation of various cell types such as microglias, endothelial cells, astrocytes and pericytes. After ischemia, vigorous decrements in blood flow in the core cerebral lesions cause cells death within minutes. The up-regulation of cell adhesion molecules and the activation of cytokines, sucs as TNF-α and IL-1 have been monitored in cerebral ischemia.[80] Lymphocytes usually penetrate the BBB and releases mainly metallo-proteinases which in turn contribute to open BBB.[81,82] Owing to this opening, adhesion and migration of monocytes, macrophages and neutrophils, take place in the injury-site for altering the BBB- function and structure.[83,84]

As BBB imposes a lot of confinements in the process of drug targeting towards brain cells, most of the drugs, e.g. antibiotics and anti carcinogenic agents, designed for the treatment of brain diseases, cannot pass the BBB.[85]

At present, central nervous system disorders depict the most frightful circumstances in the biomedical science owing to the BBB-hurdle for the transport of a drug. Different carriers, such as, niosomes, nanoparticles, microspheres and liposomes, are used to keep up their concentrations at a therapeutic desired range to enhance half-lives, solubility, permeability and stability for delivery of drug to brain through BBB. Accordingly, vesicles have been configurationally acclimatized to transport various drugs, enhance delivery effectiveness and decline side effects through proper targeting. Liposome- or poly lactide- nanocapsule- mediated drug delivery to neuronal cells has a significant importance as efficient among other delivery devices, especially during cerebral ischemia-reperfusion.[8,9]

Importance of characterization for vesicles

Liposomes composed of phosphatidyl ethanolamine (PE), cholesterol, dicetyl phoaphate (DCP)/stearyl amine and drug, and nanocapsules made up of poly-lactide-co-glycolide, didodecyl dimethyl ammonium bromide (DDAB), drug and / or poly ethylene glycol (PEG) were characterized by scanning electron microscopy (SEM) / transmission electron microscopy (TEM) and atomic force microscopy (AFM). Based on rigidity and size of vesicular composition in the biological system, $t_{1/2}$ of liposome was found comparatively less than nanocapsule.[7] Our earlier studies observed by using SEM/TEM and AFM demonstrate that the size of nanocapsules (6-100 nm) was smaller than liposomes (60-200 nm) while both possessed a spherical shape.[7] Topological AFM analysis illustrated lesser heights of nanocapsules (~2 nm) compared to liposomes (~4 nm).[7] Although both liposomes and nanocapsules were spherical, nanocasules were narrower in size compared to liposomes indicating more $t_{1/2}$.[7]

It has also been observed that the encapsulation efficiency as well as loading of a drug was lesser in nanocapsules in comparison to liposomes.[7] In our previous study, the concentration of drug release from colloidal nanocapsule in PBS and rat plasma was estimated where a sharp release of drug component was observed at 24 h followed by sustained release, but for liposomal formulation, a pattern of steady release was monitored [7] because of more rigidity of poly-lactide than lipid molecules in biological system.

Effect of vesicular drugs against ischemia-reperfusion induced ROS generation in different rat brain zones

Intracellular ROS level was estimated in different regions of brain mitochondria.[9] The over-production of ROS causes damage in macromolecules and activates different pathways. The ROS, produced, are determined by using the membrane-permeable fluorescent probe (5-(and -6)-chloromethyl-2′, 7′-dichloro-dihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA). The uncharged H_2DCFDA readily diffuses through the membrane bi-layer and becomes hydrolyzed by intracellular esterase to yield trapped H_2DCF inside the cell. Then H_2DCF becomes oxidized to highly fluorescent dichloro fluorescein (DCF) by H_2O_2 or other peroxides generated in the cells, which correlates the fluorescence intensity to be proportional to the amount of H_2O_2 produced by the mitochondria of different brain zones of both aged and young rats. The increased ROS production in mitochondria by the cerebral ischemia-reperfusion –induction was reduced significantly by liposomal drug treatment and maximally by mannose grafted liposomal / or nanocapsulated drug treatments.[8,9]

Effect of vesicular drug on ischemia-reperfusion induced lipid peroxidation

Conjugated diene in the brain- mitochondria and homogenate lipid-membrane of young and aged rats was estimated by extracting in chloroform:methanol mixture (2:1, v/v). The extracted lipid was evaporated to dryness and re-dissolved in n-cyclohexane for assaying at 234 nm.[8,9] The increment of peroxidized lipid in the brain cell and mitochondrial membrane occurred in cerebral ischemia-reperfusion was reduced by the treatments of liposomal and nanocapsulated / or mannosylated liposomal drugs.[8,9]

Effect of vesicular drugs on ischemia-reperfusion induced endogenous antioxidant level

The cytosolic fraction [8,9] of rat brain homogenate was used to determine the level and activities of GSH, SOD, catalase, GST, GPx and GR. The alteration of the antioxidant defence levels, occurred by the initiation of cerebral ischemia-reperfusion, was reduced by the treatment of liposomal drug whereas maximum reduction was monitored by the treatment of nanocapsulated /or mannosylated liposomal drug.[8,9]

Effect of vesicular drug on ischemia-reperfusion induced cerebral edema

The cerebral edema, occurred by the ingress of plasma water into aged and young rats brain in ischemic condition, was quantified and demonstrated as water content percentage in the whole brain.[9] Thirty minute cerebral ischemia performed by occlusion of the bilateral common carotid artery following 30 minute reperfusion resulted a marked enhancement in the brain water-content of aged and young rats which was protected significantly by the pre-treatment with mannosylated liposomal drug suggesting improvement of edema-formation induced by cerebral ischemia.[9]

Effect of vesicular drug on ischemia-reperfusion induced proteins and / or genes associated in signal transduction

Cyt c, associated weakly with the brain cell mitochondria-inner membrane, is released to cytosol playing a crucial role in inducing cell death signalling after having brain ischemia.[9] The cyt c -release was

restricted by pre-treating the young and aged rats with mannosylated liposomal drug subjected to ischemia-reperfusion.[9]

The inducible nitric oxide synthase (iNOS) that produces toxic nitric oxide (NO), is over-expressed in cerebral ischemia and develops brain pathologies through production of transcriptional genes.[8] In our earlier study, ischemic insult with reperfusion for 30 min, 24 h and 72 h demonstrated the up-regulation of iNOS protein expression and its significant down-regulation by nanocapsulated drug especially in the hippocampus region of both aged and young rats.[8]

Caspase-3, an interleukin-1β-converting enzyme-like protease, is known to play a key role in initiating apoptosis while its activated form cleaves and inactivates proteins such as PARP, histone H1 in maintaining processes for neuronal integrity.[86-88] Ischemic induction following reperfusion of 30 min, 24 h and 72 h showed an increment of caspase-3 activation and its down-regulation by prior nanocapsulated drug treatment, minimizing the activation of caspase-3 signaling especially in hippocampal area of both young and aged rats (Figure 3).[89-91,8]

Future aspects for ischemic therapy

The physiopathology of cerebral ischemia focuses a series of complex and interconnected mechanisms providing potential molecular targets as a therapy. The pathway of nuclear factor erythroid 2-related factor 2 with antioxidant–response element (Nrf2-ARE) has been recently reported as the main cellular defence system under oxidative strain while activated Nrf2 targets antioxidant –response element genes for maintaining redox-homeostasis and influencing the inflammatory responses such as glutathione peroxidase, heme oxygenase and 1-ferritin activities. It has been demonstrated that a lot of natural and synthetic potent molecules have been used to induce the activity of Nrf2 for protecting the brain cells against ischemic insult.[92]

A lot of antioxidants like NXY-059, Vit E and C as free radical scavenger to target cellular mitochondria, have been investigated to improve the antioxidant defences against cerebral ischemia, but failed to demonstrate suitable outcomes.[93-96] It may be for the disparities between experimental and clinical investigations i.e. the discriminations in subjects i.e. rodents vs. humans, therapeutic window i.e. within 6 h post ischemia vs. pre or post treatments, age i.e. 69 years vs. juvenile animals and comorbidity i.e. 77% hypertension, 46% cardio-embolic ischemia, 33% ischemic heart disease and 25% diabetes vs. healthy animals. Therefore, further investigations of the therapeutic windows and usage of aged animals with comorbidities may direct the fruitful translation to clinical usage.

In this context, simple treatment of drug as antioxidant may not be also effective approach to combat ROS-generated oxidative stress because of its dilution in the circulatory system. Moreover, most of the drugs are toxic, lipophilic, poor bio-stable and cannot cross BBB barrier. Therefore, liposomes and nanocapsules encapsulated with drug, recognized as drug carriers, have been investigated in earlier studies[7] while colloidal poly (lactide-co-glycolide) nanoparticles having smaller sizes, longer shelf life, higher cellular uptake and longer drug releasing capability have demonstrated as better biological efficient vehicle compared to liposomes for encapsulating small quantity of drug and their maximal target to specific site of interest to overcome the barriers in animal study [7] which needs future clinical investigations.

Conclusions

Oxidant-overproduction has provided evidences as the chief contributing factor in the pathophysiological process after occurring brain ischemia and reperfusion. The biochemical cascades as well as signal transductions, activated by oxidative strain, have been focussed for the future target in ischemic therapy by aiming the attenuation of the oxidative stress effects. Both of ROS and RNS, and NOX induce cerebral damage following different biochemical signals which offer various targets for ischemic therapy. The complex mechanisms interconnected in the pathogenesis of cerebral ischemia and the development of unique antioxidant therapies to combat the disease has been implicated on the basis of the involvement of oxidative strain in ischemic insult. In this context, vesicular drugs have been evaluated by their more / maximum ameliorating biological efficacies compared to free drugs evolved in the destructive neurochemical signalling that are controlled with oxidative stress mechanisms in cerebral ischemia.

References

1. Brouns R, De Deyn PP. The complexity of neurological processes in acute ischemic stroke. Clin Neurol Neurosurg 2009; 111:483-495.
2. Horst GJ, Postigio A. Stroke: prevalence and mechanisms of cell death. In: Horst GJ and Korf J, eds. Clinical Pharmacology of Cerebral Ischemia. Towata, NJ: Humana Press, 1997;1-30.
3. Ryter SW, Kim HP, Hoetzel A, Park JW, Nakahira K, Wang X, et al. Mechanisms of cell death in oxidative stress. Antioxid Redox signal 2007; 9: 49-89.
4. Linnea RF, Jeffrey NK. Oxidative and cerebral endothelial cells: Regulation of the blood-brain-barrier and antioxidant based interventions. Biochim Biophys Acta 2012; 1822: 822-829.
5. Chrissobolis S, Faraci FM. The role of oxidative stress and NADPH oxidase in cerebrovascular disease. Trends Mol Med 2008; 14: 495-502.
6. Squire LR, Schacter DL. Neuropsychology of memory 3rd edition. New York: Guilford Press, 2002.
7. Sana S, Ghosh S, Das N, Sarkar S, Mandal AK. Vesicular melatonin efficiently downregulates sodium fluoride-induced rat hepato- and broncho- TNF-α, TGF-β expressions, and associated oxidative injury: a comparative study of liposomal and nanoencapsulated forms. Int J Nanomed 2017; 12: 4059-4071.
8. Ghosh A, Sarkar S, Mandal AK, Das N. Neuroprotective role of nanoencapsulated quercetin in combating ischemia-reperfusion induced neuronal damage in young and aged rats. PLoS One 2013; 8: e57735.
9. Ghosh S, Das N, Mandal AK, Dungdung SR, Sarkar S. Mannosylated liposomal cytidine 5′ diphosphocholine prevent age related global moderate cerebral ischemia reperfusion induced mitochondrial cytochrome c release in aged rat brain. Neurosci 2010; 171: 1287-1299.
10. Sarkar S, Mukherjee A, Swarnakar S, Das N. Nanocapsulated ascorbic acid in combating cerebral ischemia reperfusion-induced oxidative injury in rat brain. Curr Alzheimer Res 2016; 13:1363-1373.
11. Yager JY, Kala G, Hertz L, Juurlink BH. Correlation between content of high-energy phosphates and hypoxic-ischemic damage in immature and nature astrocytes. Brain Res Dev Brain Res 1994; 82: 62-68.
12. Kahlert S, Zundorf G, Reiser G. Glutamate-mediated influx of extra-cellular Ca2+ is coupled with reactive oxygen species generation in cultured hippocampal neurons but not in astrocytes. J Neurosci Res 2005; 79: 262-271.
13. Coyle J, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science 1993; 262: 689-695.
14. Choi DW. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci 1988; 11: 465-469.
15. Iadecola C, Yang G, Ebner TJ, Chen G. Local and propagated vascular responses evoked by focal synaptic activity in cerebellar cortex. J Neurophysiol 1997; 78: 651-659.
16. Siesjo BK. Historical overview. Calcium, ischemia, and death of brain cells. Ann NY Acad Sci 1988; 522: 638-661.
17. Altmeyer M, Hottiger MO. Poly (ADP-ribose) polymerase 1 at the crossroad of metabolic stress and inflammation in aging. Aging 2009; 1: 458-469.
18. Taylor JM, Crack PJ. Impact of oxidative stress on neuronal survival. Clin Exp Pharmacol Physiol 2004; 31: 397-406.
19. Fritz HG, Bauer R. Secondary injuries in brain trauma: effects of hypothermia. J Neurosurg Anesthesiol 2004; 16: 43-52.
20. Baskaya MK, Dogan A, Rao AM, Dempsey RJ. Neuroprotective effects of citicoline on brain edema and blood-brain barrier breakdown after traumatic brain injury. J Neurosurg 2000; 92: 448-452.
21. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44-84.
22. Urbanski NK, Beresewicz A. Generation of .OH initiated by interaction of Fe2+ and Cu+ with dioxygen; comparison with the Fenton chemistry. Acta Biochimica Polonica 2000; 47: 951-962.
23. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv 2015; 5: 27986-28006.
24. Abramov AY, Scorziallo A, Duchen MR. Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. J Neurosci 2007; 27: 1129-1138.
25. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol 2005; 25: 29-38.
26. Kushnareva Y, Murphy AN, Andreyev A. Complex 1-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P) + oxidation-reduction state. Biochem J 2002; 368: 545-553.
27. Brown GC, Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett 1994; 356: 295-298.
28. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Lett 1994; 345: 50-54.
29. Trinei M, Giorgio M, Cicalese A, Barozzi S, Ventura A, Migliaccio E, et al. A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. Oncogene 2002; 21: 3872-3878.
30. Brandes RP. Triggering mitochondrial radical release: a new function for NADPH oxidases. Hypertension 2005; 45: 847-848.
31. Ishii T, Asai T, Urakami T, Oku N. Accumulation of macromolecules in brain parenchyma in acute phase of cerebral infarction / reperfusion. Brain Res 2010; 1321: 164-168.
32. Vanlangenakker N, vanden Berghe T, Krysko DV, Festjens N, Vandenabecle P. Molecular mechanisms and pathophysiology of necrotic cell death. Curr Mol Med 2008; 8: 207-220.
33. Papi A, Controli M, Gasparini P, Bristot L, Edwards MR, Chicca M, et al. Role of xanthine oxidase activation and reduced glutathione depletion in rhinovirus induction of inflammation in respiratory epithelial cells. J Biol Chem 2008; 283: 28595-28606.
34. Terzi C, Kuzu A, Alar AK, Kale IT, Tanik A, Koksoy C. Prevention of deleterious effects of reperfusion injury using one-week high dose allopurinol. Dig Dis Sci 2001; 46: 430-437.
35. Loukogeorgakis SP, vanden Berg MJ, Sofat R, Nitsch D, Charakida M, Haiyee B, et al. Role of NADPH oxidase in endothelial ischemia / reperfusion injury in humans. Circulation 2010; 121: 2310-2316.
36. Chen H, Song YS, Chan PH. Inhibition of NADPH oxidase is neuroprotective after ischemia-reperfusion. J Cereb Blood Flow Metab 2009; 29: 1262-1272.
37. Raz L, Zhang QG, Zhou CF, Han D, Gulati P, Yang LC, et al. Role of Rac1 GTPase in NADPH oxidase activation and cognitive impairment following cerebral ischemia in the rat. PLoS One 2010; 5: e12606.
38. Zhang QG, Raz L, Wang R, Han D, De Sevilla L. Estrogen attenuates ischemic oxidative damage via an estrogen receptors alpha mediated inhibition of NADPH oxidase activation. J Neurosci 2009; 29: 13823-13836.
39. Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, et al. NADPH

oxidase is the primary source of superoxide induced by NMDA receptor activation. Nat Neurosci 2009; 12: 857-863.
40. Gambaryan S, Kobsar A, Hartmann S, Birschmann I, Kuklencordt PJ, Muller-Esterl W, et al. NO-synthase- / NO-independent regulation of human and murine platelet soluble guanylyl cyclise activity. J Thromb Halmost 2008; 6: 1376-1384.
41. Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric oxide production and signalling in inflammation. Curr Drug Targets Inflamm Allergy 2005; 4: 471-479.
42. Rosa SC, Judas F, Lopes MC, Mendes AF. Nitric oxide synthase isoforms and NF-kappa B activity in normal and osteoarthritic human chondrocytes:regulation by inducible nitric oxide. Nitric Oxide 2008; 19: 276-283.
43. Bulhak AA, Jung C, Ostenson CG, Lundberg JO, Sjoquist PO, Pernow J. PPAR-alpha activation protects the type 2 diabetic myocardium against ischemia-reperfusion injury: involvement of the PI3-kinase / Akt and NO pathway. Am J Physiol Heart Circ Physiol 2009; 296: 719-727.
44. Izci Y. Splenectomy may be a prophylactic treatment for cerebral ischemia? Med Hypotheses 2010; 75: 347-349.
45. Keynes RG, Garthwaite J. Nitric oxide and its role in ischemic brain injury. Curr Mol Med 2004; 4: 179-191.
46. Guo MF, Yu JZ, Ma CG. Mechanisms related to neuron injury and death in cerebral hypoxic ischemia. Folia Neuropathol 2011; 49: 78-87.
47. Ahmed M, Graham SH. Inflammation after stroke: Mechanism and therapeutic approaches. Transl Stroke Res 2010; 1: 74-84.
48. Madinier A, Bertrand N, Mossiat C, , Prigent-Tessier A, Beley A, Marie C, et al. Microglial involvement in neuroplastic changes following focal brain ischemia in rats. PLoS One 2009; 4: e8101.
49. Wang H, Hitron IM, Iadecda C, Pickel VM. Synaptic and vascular associations of neurons containing cyclooxygenase-2 and nitric oxide synthase in rat somatosensory cortex. Cereb Cortex 2005; 15: 1250-1260.
50. Amantea D, Nappi G, Bernardi G, Bagetta G, Corasaniti MT. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. FEBS J 2009; 276: 13-26.
51. Zheng Z, Yenari MA. Post-ischemic inflammation: molecular mechanisms and therapeutic implications. Neurol Res 2004; 26: 884-892.
52. Stocker R, Keaney JF. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004; 84: 1381-1478.
53. Iadecola C, Gorelick PB. The Janus face of cyclooxygenase-2 in ischemic stroke: Shifting toward downstream targets. Stroke 2005; 36: 182-185.
54. Ozkul A, Akyol A, Yenisey C, Arpaci E, Kiylioglu N, Tataroglu C. Oxidative stress in acute ischemic stroke. J Clin Neurosci 2007; 14: 1062-1066.
55. Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, et al. Differential requirement for caspase 9 in apoptotic pathways in vivo. Cell 1998; 94: 339-352.
56. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev 2001; 15: 2922-2933.
57. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Penninger JM, et al. Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 1998; 94: 739-750.
58. Gross A, Jockel J, Wei MC, Korsmeyer SJ. Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis. EMBO J 1998; 17: 3878-3885.
59. Ottvai ZN, Milliman CL, Korsmeyer SJ. Bnl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993; 74: 609-619.
60. Green DR, Kroemer G. Cytoplasmic functions of the tumor suppressor p53. Nature 2009; 458: 1127-1130.
61. Endo H, Kamada H, Nito C, Nishi T, Chan PH. Mitochondrial translocation of p53 mediates release of cytochrome c and hippocampal CA1 neuronal death after transient global cerebral ischemia in rats. J Neurosci 2006; 26: 7974-7983.
62. Yu J, Zhang L. PUMA, a potent killer with or without p53. Oncogene 2009; 27: S71-S83.
63. Niizuma K, Endo H, Nito C, Myer DJ, Kim GS, Chan PH. The PIDDosome mediates delayed death of hippocampal CA1 neurons after transient global cerebral ischemia in rats. Proc Natl Acad Sci USA 2008; 105: 16369-16374.
64. Thundyil J, Tang SC, Okun E, Shah K, Karamyan VT, Li YI, et al. Evidence that adiponectin receptor 1 activation exacerbates ischemic neuronal death. Exp Transl Stroke Med 2010; 2: 15.
65. Choi DW. Glutamate neurotoxicity in cortical cell culture is calcium dependent. Neurosci Lett 1985; 58: 293-297.
66. Lipton P. Ischemic cell death in brain neurons. Physiol Rev 1999; 79: 1431-1568.
67. Nicholls DG. Mitochondrial calcium function and dysfunction in the central nervous system. Biochim Biophys Acta 2009; 1787: 1416-1424.
68. Thayer SA, Miller RJ. Regulation of the intracellular free calcium concentration in single rat dorsal root ganglion neurones in vitro. J Physiol 1990; 425: 85-115.
69. Lee J, Auyeung WW, Mattson MP. Interactive effects of excitotoxic injury and dietary restriction on microgliosis and neurogenesis in the hippocampus of adult mice. Neuromol Med 2003; 4: 179-196.
70. Won SJ, Kim DY, Gwag BJ. Cellular and molecular pathways of ischemic neuronal death. J Biochem Mol Biol 2002; 35: 67-86.
71. Perez Velazquez JL, Frantseva MV, Carlen PL. In vitro ischemia promotes glutamate-mediated free radical generation and intracellular calcium accumulation in hippocampal pyramidal neurons. J Neurosci 1997; 17: 9085-9094.
72. Schubert D, Piasecki D. Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. J Neurosci 2001; 21: 7455-7462.
73. Tasdemeroglu E, Chistenbery PD, Ardell JL, Chronister RB, Taylor AE. Effects of superoxide dismutase on acute reperfusion injury of the rabbit brain. Acta Neurochir Wien 1993; 120: 180-186.
74. Truelove D, Shuaib A, Ijaz S, Richardson S, Kalra J. Superoxide dismutase, catalase and U78517F attenuate neuronal cell damage in gerbils with repeated brief ischemic insult. Neurochem Res 1994; 19: 665-671.
75. Yamaguchi K, Vemaster D, Itoh Y, Wtanabe S, Fukuruchi Y. In Vivo measurement of superoxide in the cerebral cortex anoxia reoxygenation and ischemia-reperfusion. Keio J Med 2002; 51: 201-207.
76. Geetha T, Malhotra V, Chopra K, Kaur IP. Antimutagenic and antioxidant / prooxidant activity of quercetin. Ind J Exp Biol 2005; 43: 61-67.
77. Qin XF, Lu XJ, Ge JB, Xu HZ, Qin HD, Xu F. Ginkgolide B prevents cathepsin-mediated cell death following cerebral ischemia / reperfusion injury. Neuroreport 2014; 25: 267-273.
78. Sarkar S, Mukherjee A, Das N, Swarnakar S. Protective roles of nanomelatonin in cerebral ischemia-reperfusion of aged brain: Matrix metalloproteinases as regulators. Exp Gerontol 2017; 92: 13-22.
79. Mandal AK, Das N. Sugar coated liposomal flavonoid: a unique formulation in combating carbontetrachloride induced hepatic oxidative damage. J Drug Target 2005; 13: 305-315.
80. Zhang DD, Zou MJ, Zhang YT, Fu WL, Xu T, Wang JX, et al. A novel IL-1RA-PEP fusion protein with enhanced brain penetration ameliorates cerebral ischemia-reperfusion injury by inhibition of oxidative stress and neuro-inflammation. Exp Neurol 2017; S0014-4886: 30151-30156.
81. MA, Lo EH. Junctional complexes of the blood-brain barrier:permeability changes in neuroinflammation. Prog Neurobiol 2002; 68: 311-323.
82. Bolton SJ, Anthony DC, Perry VH. Loss of the tight junction proteins occluding and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo. Neurosci 1998; 86: 1245-1257.
83. Huang SH, Jong AY. Cellular mechanisms of microbial proteins contributing to invasion of the blood-brain barrier. Cell Microbiol 2001; 3: 277-287.
84. Wolka AM, Huber JD, Davis TP. Pain and the blood-brain barrier: obstacles to drug delivery. Adv Drug Deliv Rev 2003; 55: 987-1006.
85. Hawkins BT, Davis TP. The blood-brain barrier / neurovascular unit in health and disease. Pharmacol Rev 2005; 57: 173-185.
86. Fernandes-Alnemri T, Litwack G, Alnemri ES. CPP32, a novel human apoptotic protein with homology to Caenorhabditis elegans cell death protein Ced-3 and mammalian interleukin-1 beta-converting enzyme. J Biol Chem 1994; 269: 30761-30764.
87. Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, et al. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 1995; 376: 37-43.
88. Rosenthal DS, Ding R, Simbulan-Rosenthal CM, Vaillancourt JP, Nicholson DW, Smulson M. Intact cell evidence for the early synthesis, and subsequent late apopain-mediated suppression, of poly (ADP-ribose) during apoptosis. Exp Cell Res 1997; 232: 313-321.
89. Chen J, Nagayama T, Jin K, Stetler RA, Zhu RL, Graham SH, et al. Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. J Neurosci 1998; 18: 4914-4928.
90. Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, et al. Inhibition of interleukin 1β converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci USA 1997; 94: 2007-2012.
91. Endres M, Namura S, Shimizu-Sasamata M, Waeber C, Zhang L, Gonez-Isla T, et al. Attenuation of delayed neuronal death after mild focal ischemia in mice by inhibition of the caspase family. J Cereb Blood Flow Metab 1998; 18: 238-247.
92. Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. J Trans Med 2009; 7: 97-108.
93. Behl C, Moosmann B. Oxidative nerve cell death in Alzheimer's disease and stroke: antioxidants as neuroprotective compounds. Biol Chem 2002; 383: 521-536.
94. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P. Intake of flavonoids, carotenoids, vitamin C and E, and risk of stroke in male smokers. Stroke 2000; 31: 2301-2306.
95. Subramanian S, Kalyanaraman B, Migrino RQ. Mitochondrially targeted antioxidants for the treatment of cardiovascular diseases. Recent Pat. Cardiovasc Drug Discov 2010; 5: 54-65.
96. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. N Engl J Med 2007; 357: 562-571.