A molecular approach to epilepsy management; from current therapeutic methods to preconditioning efforts

Abstract

Epilepsy is a most common and chronic neurological disorder characterized by recurrent unprovoked seizures. The key aim in treating patients with epilepsy is the suppression of seizures. An understanding of focal changes that are involved in epileptogenesis may therefore provide novel approaches for optimal treatment of the seizure. Although the actual pathogenesis of epilepsy is still uncertain, recently growing evidence declare that microglia and astrocytes activations, oxidative stress and reactive oxygen species (ROS) production, mitochondria dysfunction, and damage of blood–brain barrier (BBB) are involved in its pathogenesis. Impaired GABA-ergic function in the brain is probably the most accepted hypothesis regarding the pathogenesis of epilepsy. Clinical neuroimaging of patients and experimental modeling have demonstrated that seizures may induce neuronal apoptosis. Apoptosis signaling pathways are involved in the pathogenesis of several types of epilepsy such as temporal-lobe epilepsy (TLE). The quality of life of patients is seriously affected by treatment-related problems and also by unpredictability of epileptic seizures. Moreover, the available antiepileptic drugs (AED) are not significantly effective to prevent epileptogenesis. Thus, novel therapies that are proficient to control seizure in people who are suffering from epilepsy are needed.

The preconditioning method promises to serve as an alternative therapeutic approach, because this strategy has demonstrated the capability to curtail epileptogenesis. For this reason, understanding of molecular mechanisms underlying brain tolerance induced by preconditioning is crucial to delineate new neuroprotective ways against seizure-damage and epileptogenesis.

In this review, we summarize the work to date on pathogenesis of epilepsy, and discuss recent therapeutic strategies in treatments of epilepsy. We will highlight that novel therapy targeting such as preconditioning process hold great promise. In addition, we will also highlight the role of gene reprogramming and mitochondrial biogenesis in the preconditioning-mediated neuroprotective events.

Keywords: Epilepsy; seizure; inflammation; neuroprotection; tolerance; oxidative stress; mitochondria dysfunction; apoptosis; preconditioning
Abbreviations:

AAV Adeno-associated virus
A1R Adenosine A1 receptors
ADK Adenosine kinase
ATP Adenosine triphosphate
AMPK AMP-activated protein kinase
AED Antiepileptic drugs
AIF Apoptosis-inducing factor
BBB Blood–brain barrier
BMC Bone marrow mononuclear cells
BMMCs Bone marrow mononuclear cells
BDNF Brain-derived neurotrophic factor
CNS Central nervous system
DG Dentate gyrus
DZ Diazoxide
ESNPs Embryonic stem cell-derived neural progenitors
FGF Fibroblast growth factor-2
GalR1 Galanin receptors type 1
GalR2 Galanin receptors type 2
GABA Gamma-aminobutyric acid
GFAP Glial fibrillary acidic protein
GDNF Glial-derived neurotrophic factor
GIRK G-protein-mediated inward rectifier potassium channels
HSV Herpes simplex virus
IRF3 Interferon regulatory factor 3
IFN-β Interferon-beta
IL-11 Interleukin-1
IL-1β Interleukin-1 beta
IL-1R | Interleukin-1 receptor
---|---
IL-10 | Interleukin-10
IL-6 | Interleukin-6
KA | Kainic acid
LPS | Lipopolysaccharide
MSC | Mesenchymal stromal cells
mtDNA | Mitochondrial DNA
MPTP | Mitochondrial permeability transition pore
TFAM | Mitochondrial transcription factor A
MyD88 | Myeloid differentiation primary response protein-88
NSC | Neural progenitors
NPs | Neural stem cells
NPY | Neuropeptide Y
NMDAR | N-methyl-D-aspartate receptor
NF-κB | Nuclear factor kappa-light-chain-enhancer of activated B cells
NRF-1 | Nuclear respiratory factor-1
PT Z | Pentlenetetrazol
PI3K | Phosphoinositide 3-kinase
PG C- 1α | Proliferator-activated receptor gamma co-152 activator 1-alpha
QA | Quinolinic acid
ROS | Reactive oxygen species
SV | Simian virus
Ship1 | Srho homology2-containing inositol phosphatase-1
SE | Status epilepticus
SOD | Superoxide dismutases
TRIF | TIR-domain-containing adaptor protein
Tollip | Toll interacting protein
TLR | Toll-like receptor
TNF-R | Tumor necrosis factor receptors
TNF-α | Tumor necrosis factor- alpha
1. Introduction

Epilepsy is considered as one of the oldest neurological conditions caused by repeated sudden abnormal neuronal discharges in the central nervous system (CNS) and affects around 50 million people worldwide [1]. Epilepsy mostly develops because of brain insults, but the molecular mechanisms underlying the process of epileptogenesis are poorly understood [2]. Numerous precipitating events, such as head trauma, stroke, initial/acute prolonged seizure (status epilepticus, SE), and cerebral infections can cause epilepsy and trigger seizures [3]. Prolonged seizures are believed to contribute to neuronal death in the hippocampus, that is considered to initiate epileptogenesis [4]. A variety of studies have provided strong support to the hypothesis that chronic brain inflammation play a key role in the pathophysiology of several CNS diseases, including seizures and epilepsy [5,6]. Inflammatory processes consists of the production a cascade of pro-inflammatory mediators [7], such as cytokines and related molecules [8,9]. However anti-inflammatory molecules are also induced to combat inflammation process [10,11]. An increasing body of evidence suggests that microglia and astrocytes activations with oxidative stress and reactive oxygen species (ROS) production [12,13], mitochondria dysfunction [14,15], and blood–brain barrier (BBB) breakdown [16,17] may constitute crucial mechanisms in the pathogenesis of seizures and epilepsy. Furthermore, a significant increment in morbidity and mortality with epilepsy has also been reported, but the exact mechanisms by which seizures induce death remain largely elusive [18].

During the last decade, development of new treatment approaches like; antiepileptic drugs (AED) therapy, [19-21], surgery, [22-24], cell therapy [25-27], gene therapy [28-30], and brain stimulation [31,32] have made great advances in the control of seizures. However, approximately one-third of all patients with epilepsy continue to have seizures that are intractable and experience intolerable side effects to currently available treatments [33]. Therefore, despite optimal treatment with recent therapeutic approaches, epilepsy still continues to be a major health problem [34]. Therefore, it is necessary to investigate novel treatments with improved efficacy for both prevention of and diminution of seizures in epileptic patients.
Recently, some surveys support that preconditioning provides an alternative treatment to control seizure and epileptic disorders [35,36]. Preconditioning is an endogenous protective mechanism that increases cellular tolerance to subsequent serious injury induced by either the same insult or other kinds of stimulations that their severity is near to sub-threshold levels of irreversible damage [37].

In current work, we are going to review recent findings on pathogenesis of epilepsy, then we discuss the prospects of current therapeutic strategies in treatments of epilepsy, and finally, we discuss preconditioning as a new method to trigger neuroprotection that would result in seizure reduction.

2. Clinical and pathological features

According to the etiology, epilepsies can be divided into three major categories: idiopathic, symptomatic (acquired), and presumed symptomatic (cryptogenic) [38]. Idiopathic epilepsy is a genetic model that manifests spontaneous epilepsy from gene mutations and to alteration of basic neuronal regulation [39]. Symptomatic (or acquired) epilepsy is epileptic seizures arise from more identifiable structural lesions of the brain [38]. Cryptogenic epilepsy is believed to be symptomatic that cause is not determined [38,40].

However there is a vast number of diseases as well as genetic and enviromental factors that can predispose to epilepsy; but the etiology of epilepsy is unclear for over 50% of the patients, this condition is known as idiopathic epilepsy. Pathology of idiopathic epilepsy is yet to be elucidated. Inherited vulnerability to prolonged seizures (SE) is etiologically involved in some other forms of epilepsy that are a consequence of imbalance in a number of factors including acute metabolic abnormalities (hypo- or hyper-glycemia, or electrolyte imbalances), acute neurological insults such as infections (e.g. meningitis, encephalitis), head trauma, tumors, stroke, fever, and drug use [41,42,6,43,44,3,45].

Epilepsy is characterized by periodic abnormal electrical activity that leads to cell loss or neurodegeneration and consequently causes inflammation in the brain [3]. It is believed that
prolonged and frequent occurrence of seizures cause changes in the stoichiometry of the N-methyl D-aspartate receptor (NMDAR) in the hippocampus, leading to loss of hippocampal neurons, thereby causing disruption of hippocampally mediated behaviors and irregular nerve fiber sprouting [46,4,2].

Consistently, in most animal models of epilepsy, the first insult triggers a cascade of robust cellular events; such as alterations in neurotransmitter receptors, necrotic and apoptotic neuronal death in which, after a delayed period of epileptogenesis, eventually may lead to chronic abnormal discharges, hyperexcitability and seizures [33].

The influences of seizures on brain structure and function are not yet fully understood. In fact, it cannot be said if repeated seizures with short duration are able to affect hippocampal neural circuitry to cause epileptogenesis or lead to significant cognitive deficiencies [4]. Nevertheless as the seizure threshold is low for some brain structures such as hippocampus, massive cell loss of pyramidal cells in the CA1, CA3 and CA4 subfields consequently develop as a result of seizures [47,48,12]. It is evident that seizures originate from different areas of the brain. It is typical for newly generated neurons to migrate into ectopic areas such as hilus and the molecular layer of the dentate gyrus (DG) as a consequence of an acute seizure. It has been suggested by some studies that abnormal migration of nascent neurons will lead to creation of a neural circuitry in hippocampus after acute seizures, SE or head injury [47].

Consistently it has been suggested that temporal-lobe epilepsy (TLE) in humans and in animal models could be associated with a loss of neurons in CA1-CA3 areas of the hippocampus and astrogliosis, also it has been indicated that improper connections between neurons may happen afterwards, as a consequence of continuous sprouting-pruning [24]. In addition, structural changes in the dentate gyrus have been the subject of studies on alterations in synaptic circuitry that are induced in experimental animal models and are also seen in human TLE [49].

As far we know, various factors contribute to the pathogenesis of epilepsy, however, the actual pathogenesis of epilepsy that results in cell death remains unclear [50,1,51]. One of the most accepted hypothesis for pathogenesis of epilepsy is dysregulation of excitatory (glutamatergic) and inhibitory (gamma-aminobutyric acid, GABAergic) systems which disturb the tightly regulated balance between neuronal excitation and inhibition in the brain and eventually lead to
hyperexcitability in epilepsy [52-54]. Cellular changes induced by epilepsy that are generally distinguished in hippocampus especially in SE models, include gliosis, neuroinflammation, neurogenesis, neurodegeneration, neurogenesis, axonal damage or sprouting, BBB damage, changes in extracellular matrix, functional dendritic plasticity, alteration in voltage and ligand-gated ion channels in individual neurons, and invasion of inflammatory cells into brain tissue [55-57]. Briefly it can be said that epilepsy is characterized by some well known cellular and molecular properties such as dysregulation in mitochondrial and ion channels function as well as neurotransmitter imbalance, in coming sections we briefly summarize involvement of these abnormalities in the pathogenesis of epilepsy.

2.1. Dysfunction of ion channels and epilepsy

It is well accepted that ion channels play essential roles in cellular functions like cellular signal transduction and neuronal signaling; therefore it is conceivable to conclude that dysfunction of ion channels might participate in neurological disorders. Generally, dysfunction of ion channels is called channelopathies, such as sodium channelopathies, potassium channelopathies, and calcium channelopathies [58]. These are inherited disorders that are caused by mutations in genes encoding different ligand-gated and voltage-gated ion channels [59].

It has been demonstrated that CNS channelopathies is involved in neurological disorders such as forms of ataxia, migraine, and epilepsy. Consistently molecular studies have demonstrated that mutations in genes encoding ion channel proteins in brain that lead to hyperexcitability of neurons contribute in the pathophysiology of various epilepsy syndromes. For instance mutations in the genes encoding subunits of GABA\(_A\) receptors that impair intracellular trafficking, therefore lead to the retention of channel subunits in the endoplasmic reticulum (ER) is one of these channelopathies that is identified in epilepsy [60]. In addition it is believed that mutations in sodium (Na\(^+\)) channels would cause impairment in inactivation of these channels, and this
could be involved in the pathology of several forms of epilepsy [61]. Mutations in the neuronal nicotinic acetylcholine receptor (nAChR) (a4, CHRNA4, and b2, CHRNB2, subunits), and the voltage-gated potassium (KCNQ2, KCNQ3) are other examples of channelopathies that are linked to different forms of epilepsies [62]. These examples imply that imbalance of ion charges because of channelopathies, are key factor of pathogenesis in human epilepsy [63].

2.2.Oxidative stress

Oxidative stress is a result of imbalance of oxidants and activity of defense systems against it. Lipopolysaccharide (LPS) and cytokines as well as several other factors are believed to induce oxidative stress through promoting production of ROS and this causes oxidative-mediated cellular injury [64]. Since brain tissue consumes high amounts of oxygen and therefore produces high amounts of free radicals compared to other tissues, therefore it is particularly more vulnerable to oxidative stress. In this regard different studies suggest that the oxidative stress induced by acute neurological insults such as stroke and seizures play key roles in brain injuries seen in these pathologies [13]. In order for cells to maintain their normal function it is crucial to keep ROS in low levels, therefore increasing ROS for longer periods of time carries an inherent risk of developing neurodegeneration such as those cases observed in epilepsy [12]. Consistently studies performed on neurochemical and enzymatic activities suggest that excitotoxic stimulation in SE induces production of higher amount of ROS that will eventually lead to overwhelming of the whole antioxidant capacity [65].

When molecular oxygen reacts with unpaired electrons that have escaped the electron transport chain, superoxide is produced which leads to ROS generation. The generated superoxide is able to react and damage macromolecules such as nucleic acids, proteins and lipids [12]. it is also capable of opening the mitochondrial permeability transition pore, which will cause energy
failure and release of pro-apoptotic factors (e.g. cytochrome c) into the cytoplasm [66]. This would eventually result in induction of neuronal apoptosis [67].

2.3. Mitochondrial dysfunction

Mitochondrion is a highly dynamic organelle that is able to sense and react to changes in cellular environments rapidly and with outstanding characteristics, and therefore its dysfunction leads to a wide range of neurological issues [14].

It has been indicated that most of cerebral adenosine triphosphate (ATP) consumption is used for electrogenic activity of neurons, therefore excitability of neurons and survival depends mostly on sufficient energy supply by the mitochondria [68]. Impaired ATP production is not only the consequence of mitochondrial dysfunction, but some other detrimental events such as increased generation of free radicals, oxidative stress, induction of permeability transition and disrupted intracellular calcium homeostasis will also happen that cause neurons to go through apoptosis or necrosis [15,69,70].

The largest complex of mitochondrial respiratory chain, complex I (NADH-ubiquinone oxidoreductase) play a significant role player in oxidative phosphorylation [15]. Inhibiting of this complex leads to a disturbance of mitochondrial energy metabolism [68], and excessive generation of free radical, which are direct inhibitors of the mitochondrial respiratory chain, so a vicious circle is formed which result in oxidative cellular damage [68]. In comparison to other complexes, complex I is significantly more susceptible to both oxidative and nitrosative stress and is therefore considered an immediate target for reactive nitrogen species and ROS. Contrarily, this complex is itself the major source of intracellular ROS, especially in brain mitochondria [71,72]. Therefore mitochondrial dysfunction and its resulting oxidative stress, is considered to play an essential role in chronic epilepsy and the subsequent epileptogenesis [73]. Accordingly, surveys on animals also recommend that impaired mitochondrial calcium buffering, extra production of free radicals along with increased production of peroxynitrite and
nitric oxide (NO) after prolonged seizures can lead to neuronal death in vulnerable brain areas [68]. Seizures mediated increment in ROS production can induce oxidative damage to protein, mitochondrial DNA (mtDNA), and lipid, and result in mitochondrial dysfunction and then leads to epileptic encephalopathy and epileptogenesis [74].

The role of mitochondrial impairment in susceptibility to seizure has been investigated in humans and in several models of experimentally induced seizures [15]. For instance, it has been shown those patients who suffer from inherited mitochondrial diseases are known to be more susceptible to epileptic seizures. In addition patients with TLE are sometimes found to have deficiencies in mitochondrial Complex I in the seizure foci [75,70]. Consistently it has been shown that complex I activity in the hippocampus is reduced after experimental induction of status epilepticus [75,68]. Moreover many factors that increase risks of epileptogenesis such as trauma, neonatal or adult hypoxia, and infections are also believed to play a role in mitochondrial dysfunction. This further confirm that mitochondrial impairment potentially contributes in seizure-induced brain damage [71,70]. Prolonged seizures (SE) and glutamate excitotoxicity are also believed to cause oxidative stress and mitochondrial dysfunction in some cases and this in turn is suggested to play a pivotal role in epileptic brain damage [69,70,76]. On the other hand mitochondria is shown as an important source of reactive species produced during excitotoxicity [69,70].

2.4. Failure in the regulation of Gamma-aminobutyric acid (GABA)

Impaired GABA-ergic function has been postulated to contribute to multiple neurodegenerative disorders, including autism, schizophrenia, and seizure susceptibility in some forms of epilepsy [77,78]. GABAergic system has a counter-balancing effect on the neuronal excitation and
therefore it can suppress the epileptiform discharges [79]. GABA_A and GABA_B receptors are known as two major receptor types for GABA-ergic system [80]. Recent observations suggest that alteration in GABA_A receptor subtypes have often been linked to epileptic seizures [81-83].

GABA_A receptors that are ubiquitously expressed throughout the CNS are members of a ligand-gated ion channel superfamily assembled from subunits of seven different classes: α(1–6), β(1–3), γ(1–3), δ, ε, θ, and π and it has been shown that changes in their expression and function are implicated in virtually all aspects of brain function [84,85].

Studies both on human and experimental animals have revealed that dysregulation of GABA_A receptors play an important role in the pathogenesis of epilepsy, for instance linkage studies has shown that genes encoding GABA_A receptor subunits of the α, β, γ, and δ families are involved in absence epilepsy (Lü et al., 2004; Marini et al., 2003; Wallace et al., 2001). Consistently postmortem studies also have shown that GABA_A receptor subunits are altered in epileptic patients [84,86]. Binding of GABA to the GABA_A receptor leads to the opening of channels thus allowing an influx of negatively charged chloride ions (Cl\(^{-}\)) leading to hyperpolarisation of postsynaptic membrane [81]. Pathological changes in chloride homeostasis can affect GABA_A receptor-mediated transmission thus switching GABA-ergic signaling from hyperpolarizing to depolarizing in epileptic tissue [87]. The basis of this change remains unclear [78].

2.5. N-methyl-D-aspartate (NMDA) receptor contributes to epileptogenesis

NMDAR are important subtype glutamate receptors that play a crucial role in both pathological and physiological processes [82]. NMDAR can result in excitotoxic damage through consequent excessive calcium (Ca\(^{2+}\)) influx occurring via the receptor-associated ion channel and subsequent necrosis or apoptosis [88,89]. NMDARs are which are involved in various intracellular cascades and participate in several functions associated with synaptic plasticity and pathological conditions are composed of at least seven subunits, these include a ubiquitously expressed NR1
subunit, four distinct NR2 subunits (named as NR2A, NR2B, NR2C and NR2D) and two NR3 subunits (NR3A and NR3B) [90,91].

NMDAR activation may be an important mechanism in the pathogenesis of a wide range of neuropathological disorders including schizophrenia, stroke, depression, multiple sclerosis, Huntington’s disease [92], Parkinson’s disease [93], Alzheimer’s disease [94,95], and epilepsy [96,92]. Several surveys have displayed a differentially increased expression of NR2B in the hippocampus of patients with epilepsy [97,82].

Some studies demonstrated that NMDA receptor antagonists have elicited protective effect in neuronal damage induced by seizure and in the development of epilepsy [98,99]. In addition, NMDA antagonists may provide anticonvulsant activity to suppress seizure in several epilepsy models. For instance, the competitive NMDA antagonists 3-[2-carboxypiperazin-4-yl]-propyl-1-phosphonate (CPP) and 2-amino-7-phosphonoheptanoic acid (AP7), and the noncompetitive antagonists phencyclidine (PCP) and (+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801) are shown to be effective in preventing seizure [100].

2.6. Cell death

As mentioned seizures are acute forms of brain injuries that trigger a large number of changes and initiate cascades of cellular events, including receptor and synaptic alteration, gene expression, and induction of cell death pathways [101,68]. In contrast to studies suggesting a predominance of necrosis after seizure, some studies show that apoptotic cell death plays a main role in seizure to cause brain damage. Hence, it is important to perform more research on cell fate especially after a SE episode [101]. Intracellular level of ATP is a key factor that is responsible to direct cells toward alternate death pathways. Since ATP is associated with development of apoptosis, depletion of ATP to below significant critical levels may prevent the apoptosis process [101]. From a morphological point of view, apoptosis is a distinct process and is a genetically controlled cell death that leads to loss of nearly half of neural cells during early development of brain. On the other hand, it also is believed to be a normal feature in the
development of the nervous system, and also seem to play a major role in neurodegenerative diseases [102].

Apoptotic pathways are shown to be involved in neuronal death seen in both human temporal-lobe epilepsy as well as experimental models of SE [101,103]. The mechanism that leads to seizure-induced neuronal cell death is controversial. The sequential SE in mice and rats markers that may represent morphologic and biochemical features are noticeable in apoptotic cell death [104,105,101].

Two comprehensively characterized pathways including the intrinsic pathway and the extrinsic pathway can induce programmed cell death (apoptosis) [106]. Intrinsic (Mitochondrial) pathway of apoptosis is activated by signals originating from inside the cell, and can be related to transient opening of the non-specific mitochondrial permeability transition pore (MPTP). Induction of MPTP under cellular stress conditions causes collapsing of mitochondrial transmembrane potential and initiates release of cytochrome c and other pro-apoptotic factors into the cytosol [107,106,101].

Complex I of the respiratory chain is believed to be part of MPTP [75,108]. There is a growing body of evidence that suggests after release of cytochrome c induced by occurrence of seizures it binds to pro-caspase 9 and apaf-1 and forms a complex known as apoptosome which in turn leads to activation of caspase-3 [109,110].

Mitochondrial involvement in apoptosis occurs at two levels, the maintenance of ATP production and mitochondrial membrane permeability which is enforced by controlling the release of specific pro-apoptotic molecules such as cytochrome c, and apoptosis inducing factor (AIF) into the cytosol [76,110]. Another signaling molecule that participates in mitochondrial release of cytochrome c is a multi domain pro-apoptotic BAX, consistently it has been reported BAX-deficient neurons are resistant to excitotoxicity [76].

In the extrinsic (receptor mediated) pathway of apoptosis, caspase-3 activation is believed to be involved. In this pathway, certain pro-apoptotic ligands bind to cell surface receptors called “death receptors” including tumor necrosis factor receptors (TNF-R) and Fas which then results in activation of caspase-8 by the death adaptor Fas-associated protein with death domain (FADD) [106]. BAX that is considered as a pro-apoptotic protein is an important mediator of
apoptotic cell death through extrinsic pathway. In a number of studies, increased expression and translocation of BAX as well as activation of caspase-3 are reported after SE by using immunohistological techniques [68,111].

2.7. Neuroinflammation

Experimental evidence have shown that neuroinflammation plays a crucial role in a number of acute and chronic brain disorders, such as neuropathologies and neuronal death in seizures, for instance it has been reported that systemic inflammation is capable of imposing significant effects on normal brain function and may particularly cause epileptogenesis [112,44,42]. However, the molecular mechanisms leading to such events are mostly undetermined. Although it is common to study epileptic seizure and neuroinflammation separately, they are not mutually exclusive and all those factors that increase the risk of occurrence of epilepsy (traumas, and infections, etc), can happen in different levels of CNS inflammation. Animal studies conducted in this regard indicated that administering chemoconvulsants, electrical stimulation or kindling rapidly triggers onset of inflammatory responses which are followed by acute seizures in glial cells in those brain areas that are affected by epileptic activity [41].

Epileptic seizures in turn, can be associated with neuroinflammatory responses through activation of brain immune cells including microglial cells and release of pro-inflammatory cytokines and related molecules [6,5]. Different surveys reported the seizures induced modifications in cytokines, cytokine-mRNA and leukocyte subsets in brain areas of experimental models. For instance, it has been reported that after onset of SE or seizures a significant enhancement in expression of inflammatory cytokines and/or their mRNA could be detected in the hippocampal structures [9]. Consistently, leukocyte–endothelium interactions which eventually leads to leukocyte recruitment in the brain parenchyma has also been known to play a key role in the cascade of epileptogenesis [1]. When microglia are in resting phase they help maintain homeostasis in the brain, however their activation have been shown to be associated
with neurodegeneration and hippocampal cell death, especially during seizures in the CA1, CA3 and the DG hippocampal sub regions [113]. It has been shown that induction of epilepsy through kindling by pentylenetetrazol (PTZ) in rats causes moderate neuronal cell loss in CA1 and CA3 regions of hippocampus [18]. Similarly, neuronal loss and microgliosis were also detected in regions such as the hippocampus and amygdale after pilocarpine treatment [2], pilocarpine induced neuronal damage is also observed in the cortex [114]. In addition to mentioned studies, preexisting inflammation in the brain also can predispose brain to epileptic seizures, accordingly it has been shown that the threshold to pentylenetetrazole induced seizures can be decreased by systemic adminstration of LPS in a dose dependent manner, this seizures promoting effect of LPS was prevented by anti-inflammatory drugs [115]. Administration of bacterial endotoxin LPS has been used to induce chronic neuroinflammation in animals, LPS stimulates the production of several glial cytokines, although a direct effect through the Toll-like receptor (TLR) might be also possible. [116]. Traditionally, TLRs are considered as innate immune receptors and their actions are mediated through two main pathways: Myeloid differentiation primary-response protein-88 (MyD88) and TIR-domain-containing adaptor protein (TRIF). the first pathway, results in activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [35,117,118], and induction of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), Interleukin-1 beta (IL- 1β), and Interleukin-6 (IL-6) that associated with inflammation and then contributed to brain damage [7,119,120,117]. Second pathway leads to Interferon regulatory factors 3 (IRF3) activation, and thereby induces Interferon-beta (IFN-β) (Fig.1) [35,119,120,117,118].
Fig. 1: Schematic diagram of TLR4 signaling cascades through proinflammatory and/or preconditioning stimuli. Activation of TLR4 is associated with the MyD88-dependent and/or TRIF (MyD88-independent) pathways, which finally results in a proinflammatory or anti-inflammatory response.
2.7.1. Blood - Brain Barier disruption

The BBB is high-resistance vascular bed of the CNS that provide a physical and metabolic barrier to regulate and protect CNS microenvironment [121]. Several lines of evidences are available showing that inflammation could alter BBB integrity and this altered BBB integrity particularly disorganized interaction of leukocyte-endothelium can cause extravasation of leukocytes and permeabilization of BBB which will promote spontaneous seizures development [122,57]. Hippocampus is known to be the source of the electrical discharges during spontaneous epileptic seizures and when such events happen, these epileptic attacks in rat hippocampus is shown to be associated to enhanced BBB permeability to serum albumin [41,1]. The importance of BBB disruption in the pathology was further confirmed in animal model of meningitis; in which leukocyte migration through the brain endothelium disrupt BBB and this trigger neuroinflammatory responses that eventually induce severe seizures [50]. These reports suggest that disruption of BBB has a significant role in the pathogenesis of epilepsy [16,17].

Different insults that occur during seizures such as increased local blood pressure, inflammatory responses and free radical formation as well as leukocyte recruitment, cytokine and interleukin production and/or loss of tight junction molecules may participate in seizure induced disruption of BBB integrity. As a result of BBB leakage, some serum proteins may accumulate in the brain and hyper excitability can emerge [16].

2.7.2. Astrocytes and microglia activation

Local macrophages (microglia and astrocytes) are recognized to be the major sources of proinflammatory molecules in the brain. Prolonged activation of glial cells which occurs in some cases as a pathological or protective response to CNS insults in both human and experimental animal models is believed to be a potential contributor to epileptogenesis [123,124,5]. Any disruption in neuro-supportive role of glial cells and overproduction of pro-inflammatory cytokines that leads to morphological changes and expression of specific markers could be...
potentially harmful for CNS [5]. Nevertheless, microglial activation is a complicated process that involves changes in electrophysiological and pharmacological properties, migration, proliferation, and release of a variety of mediators [42]. These inflammatory reactions are associated to different brain disorders, such as Parkinson’s and Alzheimer’s diseases, ischemia, and epilepsy [42]. Secretion of pro-inflammatory mediators from activated microglial cells can directly trigger neuronal apoptosis pathways, augment oxidative stress and cause inflammatory response to be potentiated [125]. Accordingly, activation of astrocytes and microglial cells in SE models induced by administration of pilocarpine is known to be a possible mechanism of epileptogenesis and BBB alterations [57]. Furthermore, CNS inflammation is in turn associated with increased BBB permeability, a process which is believed to contribute in both induction of seizures and development of epilepsy with chronic seizure generation. Moreover, leakage in BBB results in neuronal hyper-synchronization and mediates epileptiform activity moderately through exposing astrocytes and neuronal cells to blood albumin or potassium ions, respectively [116,1]. During BBB permeabilization, serum-derived albumin diffuses into the brain’s extracellular space and is rapidly transported into astrocytes (via a specific receptor-mediated mechanism) where it induces a rapid (within hours) up-regulation of the astrocytic marker glial fibrillary acidic protein (GFAP) [17]. Astrocytes expressed primarily GFAP which is a major intermediate filament protein and is also one of the markers of astrogliosis activation. Its over-production is highly related to the generation of inflammation and enhance of neurotoxic molecules including TNF-α, IL-10, and IL-1β, this suggest that astrocytic dysfunction is one of the key factor in injury induced epileptogenesis [113,125,126].

2.7.3. Cytokines

In both developing and mature brain, pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 are released within the brain in specific circumstances [127,50]. The actions of these molecules, is to modify neuronal excitability both in acute and long-term period [50].
Consistently in rodents models with perturbation of the cytokine systems, activating transcriptional and posttranslational intracellular pathways are shown to affect neuronal excitability and cell survival [41]. Findings in experimental models of epilepsy report a rapid onset rise in levels of cytokines in glia and neurons after acute seizures [128,124]. Among the pro-inflammatory cytokines, IL-1β seems to play an important role in the progression of epileptic process [116]. In recent years, it has been demonstrated that IL-1β pathway is involved in the enhancement of epileptogenesis induced by inflammation. Accordingly in the brain, Interleukin-1 (IL-1) and Interleukin-1 receptor (IL-1R) are rapidly upregulated after seizures [124]. This increment of the pro-inflammatory cytokines in microglia and astrocytes may also result in recruitment of adaptive immune system cells in epileptic tissue [129].

Experimental and clinical evidences also indicated that inflammatory cytokines, particularly IL-1β, been raised up in the CNS and plasma during epilepsy. It has been shown that IL-1β could enhance the extracellular concentrations of glutamate by inhibition of glial reuptake. Moreover, IL-1β could also increase the function of NMDA receptor by NR2B subunit phosphorylation, resulting in an increased calcium influx. On the other hand it is well documented that glutamatergic neurotransmission play an essential role in kindling phenomenon. These imply that involvement of IL-1β in the epileptogenesis may be due to its effects on glutamatergic pathway [116] (Fig. 2).

In addition, the activation of IL-1β may disturb the tight-junction organization or activation of nitric oxide synthase and matrix metalloproteinases in endothelial cells of BBB, and by this means it may contribute to elevated permeability of the BBB that occurs after SE [128,16,124]. Over-expression of other cytokines such as TNF-α, and IL-6 is also associated with seizure activity [44]. In astrocytes, it was reported that production of IL-6 increases the sensitivity to induce seizures by glutamatergic agonists and a constitutive loss of GABA in the hippocampus, that might be lead to develop seizures [130]. Activation of TNF-α also is shown to increase extracellular glutamate levels, in such way that it can affect neuronal excitability and contribute to seizure susceptibility [41].
Fig. 2: Schematic representation of how the activated inflammatory pathway contributes to pathogenesis of epilepsy.
3. Treatment

Brain insults such as trauma, stroke, hypoxic conditions and epileptic seizures are among the most usual causes for mortality and long-term disability worldwide. Patients affected by these conditions are common to have persistent, mild cognitive deficits even after recovery. Usually two major remediation approaches against brain insults are considered:

(a) Administration of neuroprotective drugs to suppress biochemical pathways that lead to death (e.g. calcium channel blockers, NMDA receptor antagonists, and anti-inflammatory drugs).

(b) Administration of neurotrophic factors that is able to induce proliferation of dendritic spines, synaptogenesis, and regeneration of neuronal cells [131].

Nevertheless treating an epileptic individual entails dealing with some uncontrollable factors that cannot be modified such as etiology of the seizures, age of onset, and location of the epileptogenic area [132].

In this coming section we have reviewed the advantages (Table 1) and disadvantages (Table 2) of currently available treatments and the latest investigations for management of epilepsy, including antiepileptic drugs (AEDs), surgery, brain stimulation, cell therapy, and gene therapy.
Table 1: Advantages of therapeutic approaches in epilepsy

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Advantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiepileptic drugs (e.g., felbamate,</td>
<td>Absence of hypersensitivity reactions in modern AED</td>
<td>[133]</td>
</tr>
<tr>
<td>gabapentin, lamotrigine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>– Safe</td>
<td>[26, 145, 146]</td>
</tr>
<tr>
<td></td>
<td>– Effective</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>– Successful</td>
<td></td>
</tr>
<tr>
<td>Cell therapy (e.g., stem cell transplantation)</td>
<td>– An effective delivery system for neurotransmitters (e.g., GABA and growth factors)</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>– Unique ability to differentiate into various somatic cell types</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Unique potential to repair neural circuits</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– The enormous donor source (donor cell types, such as the hippocampal precursor cells, and neural stem cells derived from diverse human sources)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Responsiveness for a wide spectrum of genetic manipulations for transplantation</td>
<td></td>
</tr>
<tr>
<td>Gene therapy (e.g., adenosine kinase,</td>
<td>– Limited immune response induced after intraparenchymal delivery</td>
<td>[159]</td>
</tr>
<tr>
<td>galanin, and neuropeptide Y)</td>
<td>– Various techniques have been utilized for gene expression in a specific region of the brain</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Disadvantages of therapeutic approaches in epilepsy

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiepileptic drugs (e.g., felbamate, gabapentin, lamotrigine)</td>
<td>– Suppress seizures without influencing the underlying biologic process of [epileptogenesis&lt;br&gt;– Side effects such as: Sleepiness&lt;br&gt;Dizziness&lt;br&gt;Poor memory concentration&lt;br&gt;Weight gain/loss&lt;br&gt;Long-term bone health issues</td>
<td>[29]</td>
</tr>
<tr>
<td>Surgery</td>
<td>Failure of epilepsy surgery due to: [Wrong initial hypothesis&lt;br&gt;– Incorrect localization of the seizure focus]</td>
<td>[166]</td>
</tr>
<tr>
<td>Cell therapy (e.g., stem cell transplantation)</td>
<td>– Various and long methods for preparing the cells prior for transplantation&lt;br&gt;– The graft location in the host brain; the age and type of donor cells and the host; the type of brain injury; and the interval between acute injury and transplantation</td>
<td>[30]</td>
</tr>
<tr>
<td>Gene therapy (e.g., adenosine kinase, galanin, and neuropeptide Y)</td>
<td>– Technical challenge for genetic material delivery to the brain because of BBB, which limits access to the CNS&lt;br&gt;– Not appropriate for use due to only a limited region of the brain is required</td>
<td>[159]</td>
</tr>
</tbody>
</table>
3.1. Antiepileptic drugs (AEDs)

The AED selection for pediatric epilepsy is not a simple task [20]. Numerous variables need to be taken into consideration for choosing an AED. The choice is usually made based on evidence of efficacy and effectiveness for the seizure type or the epilepsy syndrome; however other individual-specific variables also need to be considered, including age, sex (with special reference to childbearing potential), safety, tolerability, ease of use [133], comorbidities, concomitant medications [134,20], pharmacokinetics (in consideration of the current or likely future need for concomitant medication for comorbidity), and finally cost [133]. Phenytoin, Phenobarbital, Primidone, Carbamazepine, Valproic acid and Benzodiazepines along with new AEDs included Neurontin (gabapentin), Topamax (topiramate), Felbatol (felbamate), Gabitril (tiagabine), Keppra (levetiracetam), Lamictal (lamotrigine), Trileptal (oxcarbazepine), Lyrica (pregabalin), Zonegran (zonisamide), and g-vinyl GABA are the most frequent AEDs which are extensively administered [20] [19]. Current AEDs might be able to suppress seizures, but they are not capable of preventing transformation of a normal brain to an epileptic one [21]. Thus new AEDs need to be developed, particularly those AEDs that are used to treat pediatric epilepsies and developing brains [135,136].

Most of current AEDs target ion channels and transmitter receptors, and are only effective in only 60–70% of epileptic patients, this means that almost one-third of patients with epileptic seizures do not successfully respond to current treatments. In addition, even these ADEs which suppress seizures, do not influence the underlying biologic process of epileptogenesis [29]. It has been mentioned that transport of AEDs by drug efflux transporters such as P-glycoprotein at the BBB and its subsequent extruding of AEDs from their intended action sites might be the reason why epileptical pharmaco-resistance take place [137].
3.2. Surgery

The procedure of surgery is the final option for treating those patients with symptomatic epilepsy that are resistant to AEDs and uncontrolled by other medication [29,138]. In general, the aim of surgery is to remove the presumed epileptogenic region with minimal possible postsurgical neuropsychological deficit, while preserving eloquent cortex in patients with pharmacologically-uncontrolled focal epilepsy [139,22].

The choice of surgical procedure usually depends on type of seizures, the location and extension regarding underlying pathology, and also its relation to functionally relevant cortex [138]. The expected result is a long-term seizure-free period and health improvement. Conducting surgery on extra temporal epilepsy become more effective in recent years, to the extent that a seizure free state can be achieved in 47% to nearly 70% of the cases [23,138].

The timing to perform the surgery has been rather controversial however it is usually arranged based on the drug resistance and duration of occurrence of epileptic seizures [140]. Those patients who suffer from epilepsy for longer periods are more probable to have developed side effects after the surgery such as cognitive side effects which are mostly observed in older patients [141].

Patients with medically refractory TLE are often advised to be treated through surgery [139,24]; however it is often limited to those with unilateral hippocampal sclerosis and sufficient functional reserves contralateral to the proposed surgical site [24]. For treatment and support of people with temporal lobe epilepsy, selective amygdalo-hippocampectomy through the transsylvian route was established as a current surgery in various epilepsy centers. Additionally, a treatment option for patients experience persistent postoperative seizures, a reoperation utilizing an extended resection type, such as anteromedial temporal lobectomy can be suggested [139].
3.3. Brain stimulation

Stimulation of deep brain structures (Deep brain stimulation, DBS) is a treatment option for resistant epilepsy, that its use dates back to the 1950 and since then numerous reports have verified its antisiezure effects. This method provide an adjustable and reversible way to modulate epilepsy symptoms, properties that are not available in surgery method. To date, different neural targets have been studied for DBS both in animal models and clinical studies and promising results are reported. These targets include cerebellum, centromedian nucleus of the thalamus (CMT), hippocampus, anterior nucleus of the thalamus (ANT), subthalamic nucleus (STN), the caudate nucleus (CN) (reviwied in [142,143]).

3.4. Cell therapy

One the most useful alternative treatments of epilepsy is to introduce new cells or to modulate neurogenesis endogenously, for the damage to the epileptic circuits to be reconstructed and also to bring function of the neurotransmitters such as GABA and growth factors back into balance [144]. Increased excitatory neurotransmission which is found in the epileptic hippocampus is mentioned to be due to decreased number of GABAergic interneurons or lack of inhibition function and reduced numbers of GABAergic terminals. As an example, seizures can be suppressed by transplanting those cell types that can continuously release inhibitory transmitters such as GABA in proper brain areas [145,146,26]. Some evidence have shown that there is therapeutic benefits in transplantation of embryonic stem cell-derived neural progenitors (ESNPs) differentiated into GABAergic interneurons into the hilus of the dentate gyrus in rodents with TLE [147]. While transplanting cell in to the intranigral area of fetal GABAergic cells has the potential to immortalizing GABAergic cells; transgenic cell lines have been shown to decrease the intensity of established epileptic seizures induced by kindling, pilocarpine, or kainic acid [148]. Administering an agonist of GABA to this nucleus bilaterally halts
propagation of convulsive seizures that are induced by administration of bicuculline and of non-
convulsive generalized seizures and as a result protects the brain against the development of
convulsive and non-convulsive seizures [146]. The most proper form of cell therapy is believed
to be local delivery of therapeutic compounds by bioengineered cells at desired brain areas.
Intrinsic factors that regulates cell survival, migration and differentiation are not clearly
understood, however with cell transplantation, a stable and potentially more physiologic
concentration of the transmitter could be achieved without risk of any complication or systemic
side effects [27], despite these benefits, potential tumorigenesis effects of cell therapy is a great
concern which could limit the advantages/disadvantage of this method [149].

3.4.1. Stem cell transplantation

Transplantation of stem cells as a potential and innovative strategy is being continuously
improved within the last few years as a mean to treat a wide spectrum of severe neurological
conditions such as epilepsy that were once thought to be incurable [145,146,26]. Accordingly,
stem cells therapies can be used to repress epileptogenesis by compensating the missing
inhibitive interneurons and also promoting their intrinsic neurogenesis or secreting some
neurotrophic factors in neuroprotection [150]. A number of studies also have displayed the
therapeutic potential of mesenchymal stromal cells (MSC) [151] and bone marrow mononuclear
cells (BMC) [152,153] in CNS disorders like ischemia by using different experimental models
[24].

Different strategies for cell transplantation are used in animal models of epilepsy; one of these is
transplantation of bone marrow mononuclear cells (BMMCs). Consistently it has been revealed
that BMMCs are capable to reduce seizure frequency as well as learning and spatial memory
impairments induced by chronic epilepsy [154]. In agreement with this report, it is also showed
that BMMCs are able to reduce neuronal loss, affect the reorganization of the hippocampal
network in the acute phase of experimentally induced epileptic seizures and finally prevent the
development of chronic seizures [24]. Moreover, it has been shown that convulsion in acute
epilepsy is effectively inhibited by transplantation of BMMCs. Interestingly, lowering levels of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 while increasing levels of anti-inflammatory cytokine IL-10 have also been reported in the brain and serum of BMMC-treated rats [11]. Other sources of stem cells such as neural stem cells and neural progenitors (NSC/NPs) can also be targeted at focal areas of epileptogenesis. NSC can be expanded in culture from potential and diverse sources including the embryonic stem cells [147], adult stem cell sources, non-neural tissues [155], and the fetal stem cells [156]. Because of NSC/NPs ability to integrate into host brain circuitry, and their considerable capacity in self-renewal, and plasticity, these cells are considered as a promising candidate in neurorestorative therapy [157].

3.5. Gene therapy

Clinical administration of drugs for epilepsy, are only able to alleviate symptoms while causing adverse side effects. In addition, as previously mentioned, AEDs are only effective in 60-70 percent of cases. Therefore there is a need for more localized and more effective treatments. Inducing expression of endogenous antiepileptic or antiepileptogenesis agents by means of gene therapy is a relatively novel approach to control the disease development and achieve stable and long-term suppression of seizures in epileptic patients [28]. For treatment of intractable focal epilepsies, gene therapy can be used as a realistic and novel strategy alternative to surgery [29]. In the nervous system, gene therapy involves the transfer of genes and their expression into brain tissue, generally neurons [158].

In order for a gene to be expressed in a specific brain region, numerous techniques can be used such as non-viral vector delivery, transplantation of cells in an ex vivo method (immortalized cells, fetal cells, and fibroblasts), liposomes and viral vector delivery including retrovirus, Simian virus (SV), lentivirus, herpes simplex virus (HSV), adenovirus, and adeno-associated virus (AAV) vector delivery. These types of vectors enable long-term gene expression without significant toxicity, and can transduce non-dividing cells [158]. Furthermore some essential
factors such as efficiency of gene delivery, ability to regulate transgenic expression, and the level and stability also need to be considered while conducting gene therapy [159]. Recently, most studies on gene therapy that are associate with epilepsy involve limbic brain structures including entorhinal cortex or piriform cortex, and the hippocampus, where activity of seizure is induced by electrical kindling stimulation, pilocarpine, or kainic acid (KA) (as a reflective of human TLE) [160].

Different surveys have demonstrated the efficacy of viral vector gene therapy for suppression of seizure [160]. As an example, neurotrophic factors like glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF-2) and their receptors have been considered as antiepileptic gene transfer targets to repair changes that occur after seizures and neuronal cell death [161]. Over expressed GDNF in the rat hippocampus using adenovirus (Ad) in KA model [160] or over expression of BDNF and GDNF using herpes-based vectors in pilocarpine model lead to reduced neuronal cell death related to seizure [162], prevent mossy fibers sprouting to the rat dorsal hippocampus [21], and lessen hippocampal neuroinflammation [163]. But the most well-studied gene therapy technique for treating epilepsy is to target adenosine kinase (ADK), galanin and neuropeptide Y (NPY) [30].

### 3.5.1. Adenosine kinase (ADK)

Adenosine is a neuromodulator that is believed to have anticonvulsant and neuroprotective properties. It is released endogenously during seizures, ischemia, and hypoxia and its receptors mediate its antiepileptic and neuro-protective properties, among these receptors, adenosine A1 receptors (A1R) is the most abundant in critical regions for epileptogenesis, such as hippocampus [164]. Adenosine kinase (ADK) is the primary enzyme that participates in adenosine metabolism and by this way ADK play a key role in the regulation of adenosine concentration [165]. Additionally, recent studies have indicated that in experimental model and human TLE, ADK made in astrocytes is the main regulator of adenosine level in adult brain. Increased ADK
activity leads to astrogliosis and astrocyte dysfunction and it may be linked directly to the development of epileptogenesis, higher seizure activity and increase the susceptibility to brain damage [166-169]. On the other way, reduced levels of ADK augment endogenous adenosine and results in lower seizure activity [166]. As a powerful anticonvulsant, activation of A1R often mediates the inhibitory effect of adenosine that profoundly inhibits excitatory glutamatergic transmission [158]. Thus, ADK is a logical target to suppress epileptic seizures [168,170].

For better understanding of the link between epilepsy in clinic part and ADK expression, these findings should generalize into different animal models and validate in appropriate patient populations [170]. For example, in treatment of epilepsy based on gene therapy that specifically targets ADK, AAV8 vectors are designed to express Adk- cDNA in either sense or antisense orientation under the control of a an astrocyte-specific gfaABC1D promoter to over-express or knock down ADK in astrocytes, respectively. It has shown that generation of seizure was using the ADK–over-expressing sense vector and suppression of seizure was using the ADK-knockdown antisense vector. This finding demonstrated that targeted knock down of ADK makes a rational and effective approach for therapy of epilepsy [168].

3.5.2. Galanin

Galanin is an endogenous neuropeptide with a wide distribution in the CNS and is involved in the modulation of a number of physiological processes such as seizure sensitivity [171]. Generally, galanin has inhibitory effect in the CNS by increasing potassium or reducing calcium conductance, the magnitude of this inhibition is in fact sufficient to suppress hippocampal seizure activity via local infusion [172]. Consistently, injection of galanin into the dentate gyrus on the side of perforant-path stimulation has been shown to be protective against self-sustaining SE, even at those stages that SE seems to be resistant to GABAergic drugs such as diazepam [173].

Two types of galanin receptors; type 1 (GalR1) and type 2 (GalR2) are expressed in hippocampus [174], GalR1 is found in the ventral cornu ammonis (CA) areas while GalR2 is in
the ventral and dorsal DG [175]. However, these receptors are linked to different signaling cascades; The stimulation of both hippocampal GalR1 and GalR2 produces anticonvulsant activity. Like other members of the G-protein-coupled receptor family, the signaling pathway mediated by galanin receptors is complex, with apparently only definite cascades relevant to their anticonvulsant effects. Most probably, attachment of GalR1 to G protein opens G-protein-mediated inward rectifier potassium channels (GIRK) or ATP-sensitive K channels, which in turn leads to presynaptic inhibition of glutamatergic transmission [176]. Accordingly, Over-expression of the galanin through viral vectors has been demonstrated to suppress generalized seizures effectively in many animal models of epilepsy [175]. For instance, AAV–mediated expression and constitutive secretion of galanin attenuates sensitivity of focal seizure, prevents neuronal cell death induced by seizure, and blocks limbic seizure activity induced by kainic acid (KA) [172,177].

3.5.3. Neuropeptide Y (NPY)

Neuropeptide Y (NPY) is a 36-amino acid peptide with a wide distribution in various forebrain areas. Functional studies on epileptic tissues indicated that NPY and some of its analogs are capable of strongly inhibiting seizure activity [178-180].

NPY participates in a wide range of physiological activities such as regulation of feeding behavior, blood pressure, circadian rhythms, anxiety, memory processing and cognition, which raise the possibility of side-effects of peptide overexpression. Nevertheless most of these functions are conducted in areas other than the hippocampus. The mechanism or mechanisms through which NPY decreases spontaneous seizures and halts their progression involves the inhibition of presynaptic glutamate release by activating of NPY-Y2 receptors at glutamatergic neural terminals. In chronic forms of epilepsy, this inhibitory action can be increased in hippocampal areas of human and rodent where the density of NPY-Y2 receptors is elevated on mossy fiber terminals [29]. The initial experimental attempts to use neuropeptide gene therapy to
suppress seizure activity established that adeno-associated virus (AAV)-mediated neurotrophic expression shows anti-ictal effects on acutely induced seizures. Injection of the neurotropic recombinant adeno-associated viral (rAAV) vector which contains the recombinant NPY gene into the rat hippocampus provokes a long-range expression of NPY in specific areas and in turn it results in reduction of acutely induced seizures and SE and delays kindling acquisition. It seems these effects are highly dependent on the extent of NPY enhancement in fibers of hippocampus and dentate gyrus [178-180].

4. Preconditioning: as a novel neuroprotective strategy in epilepsy

Preconditioning is an adaptive cellular response in which an insignificant or sub-lethal noxious stimulus protects the brain from a subsequent more serious insults [181,131,182,35]. Essentially any injury paradigm, such as progressive depression, hypothermia and hyperthermia, metabolic inhibition, inflammation, and epilepsy will induce preconditioning if applied below the threshold of cell damage [131]. Rapid tolerance happens in a short period of time and is independent of new gene transcription from rapid biochemical and structural changes in brain including synapse remodeling, ion channel activation, ubiquitin-mediated down-regulation of pro-apoptotic proteins, resulting in temporary and potent neuroprotection [183-185]. Delayed (classical) tolerance which evolves over 1–3 days, depends on transcription of new gene and de novo protein synthesis. It then induces a neuroprotected state that can persists for days [184-186]. A variety of treatments and stimuli such as LPS are capable of inducing a neuronal tolerance state, cross-tolerance (tolerance against other factors) and preconditioning brain tissue against harmful insults [35] [186].

Although the underlying mechanism for preconditioning is not completely understood, however it is believed that preconditioning can induce signaling cascades which in turn activate effector mechanisms leading to neuroprotection. In this way attenuation of cell damage pathways including ionic unbalance, oxidative and nitrosative stress, metabolic dysfunction, excitotoxicity, inflammation as well as processes related to necrosis and apoptosis which lead to cell
death have been suggested. Although details of signaling cascades in up-regulation of genes involved in survival pathways or down-regulation of those involved in cell death pathways are not completely known, some studies have shed new light towards this direction [187].

It has been shown that in order for the brain to be in a tolerant state, 24 h is required for establishment and the synthesis of new protective proteins that might be involved in the process. However the details of the role that these proteins play in this process are still unclear, it has been suggested that up-regulation of antioxidant enzymes activity is implicated to play an essential role [182]. It has also been indicated that in a preconditioned brain the level of neuroinflammatory mediators that are usually present in response to injury will drop significantly [35].

In animal models, epileptic tolerance has been shown to happen in multiple epileptic preconditioning models such as bicuculline, electroshock, kindling, and KA induced epileptic preconditionings. In these models preconditioning has been shown to develop when the brain is already exposed to one or more brief non-harmful seizure episode(s) which strongly reduce hippocampal damage upon SE [188]. Gene profiling by microarray has shown that down-regulation of genes that code for proteins is a preserved mechanism underlying tolerance process [189,185,188]. On the other hand up-regulation of miRNAs is shown to be a probable mechanism of action although changes made to epigenetic and transcriptional processes are also important [188]. Neuroprotection by inducing epileptic tolerance is strongly associated with suppression of transcription of genes involved in ion channels, calcium signaling, and excitability [184].

4.1. Gene reprogramming:

It is has been established that reprogramming of genes involved in cellular response to excitotoxic insults, plays a important role in tolerance and preconditioning of tissues [113]. Accordingly evidence from genomic studies have demonstrated that diverse stimuli which trigger preconditioning mediated neuroprotection may share common process which depends on
a fundamental reprogramming of the response to injury. This reprogramming process can induce novel neuroprotective pathways which leads to the synthesis of new proteins that fundamentally change the molecular and genetic response to subsequent injury [190,191].

For example in ischemic tolerance, preconditioning leads to a fundamental reprogramming event and triggers endogenous responses that protect the brain against a subsequent severe ischemic insult. It has been proposed that ischemic preconditioning could regulate miRNA expression and then serve as novel effectors of altered protein expression that leads to ischemic tolerance [192,193]. In line with these reports, low dose LPS preconditioning also leads to protection against subsequent high dose LPS induced cell death via a process referred to as “reprogramming”. It is believed that such reprogramming leads to sustained or enhanced production of anti-inflammatory mediators such as TNFR1 and IL-10. Pretreatment with low dose LPS in animals confirm such reprogramming and indicate that protection may relatively be due to suppression of extra production and release of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 during subsequent exposure to large dose of endotoxin [194,35,10]. In addition induction of beneficial neuroprotective and anti-inflammatory pathways that increase cell survival could be another way that participates in this process. Such mechanisms can be amenable for further development in drug discovery [10]. One of the TLRs that detect foreign pathogens is TLR4, which is believed to participate preconditioning by low dose administration of LPS as well as in the preconditioning-induced tolerance [194,186,35].

Recently, studies suggest that neuroprotection which induced by TLRs appear to be activated through reprogramming the genomic response to the damage-associated molecular pattern molecules (DAMPs), which are released in reaction to injury [117,118]. Consistently, it has been demonstrated that preconditioning with LPS protects hippocampus structures against seizure-induced neurodegeneration [35]. It is believed that this reprogrammed state, might be brought about by up-regulation of the TLR mediated IRF3 pathway [195,117].

IRF3 is key modulator of type I interferon gene regulation [117] and it may have neuroprotective effects [194]. IRF3 can induce anti-inflammatory mediators and may affect the regulation of IFN-β that have been associated with neuroprotection [195,117,35]. Thus, it would be expected that the enhanced TLR4 signaling to TRIF–IRF3–IFN contribute to neuroprotection [194].
epileptic tolerance, neuronal death caused by status epilepticus can be reduced by prior delivery of mild, brief non-harmful seizures using a variety of preconditioning paradigms [189,196,184]. It may evoke protective adoptions and also can function to precondition brain against damage induced by subsequent prolonged seizures [184,197]. Consistent with mentioned results a microarray analysis of hippocampal genes after preconditioning has also revealed changes in expression of those genes that are thought to be related to protein turnover and tissue structure, signaling, and neuronal excitability. It has been reported that more than 70% of differentially regulated genes were down-regulated in epileptic tolerance and it is not surprising that many subunits of ionotropic glutamate receptor, calcium channels, and calcium-regulated enzymes were among those genes [184]. However, these results clearly show that gene suppression also play an important role in preconditioning mediated tolerance against epilepsy, but the exact mechanism gene reprogramming toward gene suppression still remained to be elucidated. It is suggested that it may be a function of the preconditioning stimuli that increase the transcriptional silencing or other post-transcriptional mechanisms [190,184].

4.2. Mitochondrial biogenesis

Mitochondrial biogenesis is an essential role player in maintaining mitochondrial homeostasis necessary for accomplishment of physiological needs of a eukaryotic cell [198]. As mentioned previously in section 2.2, dysregulation in mitochondrial biogenesis play role in epilepsy pathogenesis, evidences are also available showing that preconditioning paradigms could improve mitochondrial biogenesis and by this way protect CNS, in present section we review current evidences indicating that preconditioning could protect CNS via affecting mitochondrial biogenesis.

Induction of ischemic-tolerant state by various preconditioning stimuli can induce an ischemic-tolerant state that may also induce mitochondrial biogenesis. For instance LPS preconditioning temporarily increases expression of some components which are essential components of mitochondrial biogenesis, such as nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (TFAM) [74] as well as copy numbers of mtDNA. Moreover, additional
protein contents of mitochondria and its functionality including citrate synthase activity, increased cellular ATP content, and maximal respiration capacity are also enhanced. Interestingly, TFAM knockdown interestingly could stop induction of mitochondrial biogenesis and also neuroprotective preconditioning effects of LPS [14]. These cellular events are coordinated by multiple signaling pathways. LPS suppresses NRF-1 and TFAM expression by AMP-activated protein kinase (AMPK) inhibition, while nuclear translocation of NRF-1 in turn induces expression of TFAM and is dependent on phosphoinositide 3-kinase (PI3K)/Protein Kinase B (Akt) signaling. In neuronenriched cortical cultures, LPS affects TLR4 and induces extra production of ROS thereby resulting in sub lethal oxidative stress which contributes to AMPK activation. Indirectly, activated AMPK in turn leads to the increased transcription of NRF-1 which is then translocated into the nucleus through activation of Akt where transcription of the TFAM gene is increased. Finally TFAM protein is synthesized in the cytoplasm and then enters mitochondria to induce mitochondrial biogenesis [14]. There is growing body of evidence that has indicated the existence of a fundamental role in mitochondrial biogenesis for peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) [74, 199, 200]. Induction of PGC-1α which is a transcriptional co-activator is essential for biogenesis of mitochondria via activation of both nuclear and mitochondrial transcription factors [199, 200]. The translocation and increased expression of PGC-1α can promote genes transcription that are included in substrate transport, oxidative phosphorylation and oxidation of lipids typically contribute to mitochondrial biogenesis [199, 74]. Up-regulation of PGC-1α and also its transcriptional cascade and subsequent mitochondrial biogenesis have been reported in several neurological diseases such as transient brain hypoxia, sustained brain hypoxia, and hypoxic-ischemic brain injury. The underlying mechanism is suggested to be for compensation of mitochondrial oxidative damage and dysfunction. Regardless of strong evidence in support of a critical role for PGC-1α-mediated mitochondrial biogenesis in neurons, alterations in mitochondrial biogenesis and related factors during chronic recurrent seizures could not be established [199, 74]. Content of mtDNA and mitochondrial-encoded proteins, such as for oxidative phosphorylation are fundamental for normal functioning of mitochondria and their deficiency leads to
mitochondrial diseases. Function of mitochondria is under control by mtDNA, and some other factors including those that regulate mtDNA transcription and replication. TFAM regulates the maintenance of mtDNA in many cells during mitochondrial biogenesis. TFAM which is translocates into mitochondria is an important nuclear-encoded transcription factor to activate mtDNA transcription and replication through binding to the mtDNA promoter region. Therefore, TFAM may be a good target for therapeutics in some diseases [74,198]. So mitochondrial biogenesis is a novel mechanism contributing to the neuroprotection afforded by sublethal LPS pre-conditioning [14].

4.3. LPS preconditioning

LPS as a potent stimulus for production of pro-inflammatory cytokines in the circulation and the brain then mediates most of its effects by IL-1, TNF-α, and IL-6. LPS-induced neuroprotection is a time and dose-dependent phenomenon which begins 24 h after administration of LPS and lasts for almost 7 days and disappears by 14 days. The LPS dose required to achieve preconditioning depends on the route of administration and also on the animal models and might range from 0.02 to 1 mg/kg [35]. Evidences are available showing that LPS mediated preconditioning of microglia can be protective in rodent seizure models [201,202,35]. Consistently it has been demonstrated that microglial activation could affect the threshold and/or sensitivity to pilocarpine-mediated seizures and downstream pathology [113]. However, the mechanisms by which microglia preconditioning act through to induce protection is demonstrated against other pathological states like ischemia [113,203], but its potential for protection in epilepsy is controversial and still remain to be elucidated. In one study, it has been demonstrated that LPS administration prior to the pentylenetetrazole (PTZ)-induced seizures could be beneficial by elevating the plasma levels of NO and IL-6 and reducing BBB permeability [202].
Consistent with this result, it has been reported that in epileptic rats, LPS-preconditioning procedure affects animal behaviour and induces effective neuroprotection in the hippocampal CA1, CA3 and DG sections [35].

It has been argued that activation of TLR4 by low doses of LPS can lead to mild inflammatory response. This response includes production of TLR4 inhibitors such as PI3K. The level of these endogenous inflammatory inhibitors remains at up-regulated levels until occurrence of the subsequent ischemic insult. Existence of such inflammatory inhibitors will suppress the innate immune system and consequently reduce inflammatory response and then results in attenuation of damage in the brain [186]. Administration of LPS triggers the activation of TLR4 associated pathways including MyD88 dependent and TRIF (MyD88 independent) pathways which induce NF-κB and IRF3, and thereby activates IFN-β [120,35,118,117]. Protection by preconditioning using low dose LPS [36,35,118] causes activation of TRIF signaling pathway to enhance the activity of IRF3, thereby increasing anti-inflammatory/type I IFN gene expression [194,120] which then leads to increased presence of NF-kB inhibitors, including Toll interacting protein (Tollip), Src homology 2-containing inositol phosphatase-1 (Ship1), and p105 to suppress NF-κB activity [118]. Modulations of TLR4 and activation of TRIF inhibit the activation of NF-kB, decrease inflammatory responses, and play a key role in attenuating brain damage thereby reducing neuronal death [194,120].

Several studies have shown that preconditioning in parallel with activation of microglia, as occurs when rat brain is stimulated by administration of LPS, leads to release of nerve growth factor (NGF) and BDNF from microglia/macrophages, these molecules being capable of promoting neuronal recovery from injury. Preconditioning induced by LPS has the potential to accumulate protective factors and initiate a compensatory or adaptive response to excitotoxic insult [186]. However, while the potential role of protection by preconditioning against seizure is controversial and not well understood [113], some recent approaches have recommended that preconditioning with LPS can be protective in rodent seizure models [36,35,113]. Although LPS has been shown to be protective in kindling-induced models of seizure, its effects are only evident when LPS is administered daily for 16 days. Neuronal protection against cell death was observed after LPS preconditioning 72 h prior to induction of seizure [113].
4.4. Other preconditioning stimuli

Diazoxide (DZ) which is a highly selective opener of the mitochondrial ATP-sensitive potassium (mitoKATP) channel protects neurons against SE-induced neuronal cell death through reduction of oxidative stress. DZ inhibits excessive production of ROS and subsequent loss of mitochondrial membrane potential which is followed by release of mitochondrial cytochrome c in primary mesencephalic neurons. The mechanism behind the protection of hippocampal neurons against oxidative injury induced by seizure is still unknown. It is believed that DZ acts by suppressing the action of NMDA and relatively rising superoxide dismutases (SOD) level through PI3K/Akt pathway. Consistently it has been shown that in hypoxia-reoxygenation injury, DZ as MitoKATP channel openers might also activate the PI3K/Akt pathway. The pathway related to PI3K is described to play a significant role in cell survival by both enhancing the anti-apoptotic proteins expression and inhibiting the activity of pro-apoptotic ones [13].

It is reported that induction of preconditioning through administration of a sub-convulsive dose of NMDA results in neuroprotection against seizures and against neuronal death in the hippocampus after an intra-cerebroventricular infusion of quinolinic acid (QA) in mice, however the underlying mechanism is still unclear [187].

Administering brief seizures (i.e. epileptic/seizure preconditioning) initiate some endogenous protective pathways in the brain. These pathways can temporarily generate a damage-refractory or tolerance state against subsequent SE episodes which could otherwise be harmful. Changes in expression of miRNAs, which are a class of non-coding RNAs that act post-transcriptionally to regulate mRNA translation has recently been reported to be involved in epileptic tolerance[188].

Ischemic preconditioning has been practiced in many different organs including the brain. Brief periods of ischemia-hypoxia that might act through chemical activation or enhancement of endogenous protection processes are able of protecting brain areas such as hippocampus, against damages caused by subsequent seizures or prolonged episodes of ischemia [189]. It has optimistically been reported in some studies that ischemic-hypoxic preconditioning
accomplished by kindling or brief seizures is able to protect brain from damages induced by seizures [189].

5. Conclusion

Over the past years investigations have revealed several mechanisms participating epilepsy and seizure-induced brain damages. These findings have expanded our knowledge about the role of microglia and astrocytes activations, oxidative stress, mitochondria dysfunction, and BBB disruption in the pathogenesis of epilepsy. The growing research and extension efforts into the actual mechanisms of pathogenesis related to epilepsy should be greatly encouraged, since the understanding of such mechanisms should ultimately pave the way for future development of effective treatments to stop the seizures. During the last decade, numerous treatment options such as new AEDs therapy, surgery, cell therapy, and gene therapy are tested for patients with epilepsy. Although many of those therapeutic methods to prevent epilepsy have been marketed during the last decade, many epileptic patients have not had an adequate response to these medications or have adverse effects. Therefore, novel treatments should be designed to be more efficacious and safe. As noted above, precondition therapy is a new approach that is expected to enhance our ability to effectively treat people with epilepsy. Since significant risk factors are associated with uncontrolled seizures, the development of new treatment tools should be continued which are more effective than before.
This is a Post-print of published article: Amini, E., Rezaei, M., Ibrahim, N. M., Golpich, M., Ghasemi, R., Mohamed, Z., Raymond, A. A., Dargahi, L., Ahmadiani, A. (2014). A Molecular Approach to Epilepsy Management: from Current Therapeutic Methods to Preconditioning Efforts, Molecular neurobiology, 1-22. DOI: 10.1007/s12035-014-8876-5

References:


43


116. Auvin S, Shin D, Mazarati A, Sankar R (2010) Inflammation induced by LPS enhances epileptogenesis in immature rat and may be partially reversed by IL1RA. Epilepsia 51 (s3):34-38
intraventricular erythropoietin-infusion ameliorates spontaneous recurrent seizures by suppression of abnormal mossy fiber sprouting. Brain Research 1295:203-217


10.1186/1742-2094-7-81


