



## Review article

# Repurposing metformin to treat age-related neurodegenerative disorders and ischemic stroke

Sejal Sharma<sup>1</sup>, Saeideh Nozohouri<sup>1</sup>, Bhuvaneshwar Vaidya, Thomas Abbruscato<sup>\*</sup>

Department of Pharmaceutical Sciences, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center (TTUHSC), Amarillo, TX, USA

## ARTICLE INFO

## Keywords:

Metformin  
Anti-aging  
Neurodegenerative diseases  
Ischemic stroke  
Tobacco smoking  
Electronic cigarette vaping  
Diabetes

## ABSTRACT

Aging is a risk factor for major central nervous system (CNS) disorders. More specifically, aging can be linked to neurodegenerative diseases (NDs) because of its deteriorating impact on neurovascular unit (NVU). Metformin, a first line FDA-approved anti-diabetic drug, has gained increasing interest among researchers for its role in improving aging-related neurodegenerative disorders. Additionally, numerous studies have illustrated metformin's role in ischemic stroke, a cerebrovascular disorder in which the NVU becomes dysfunctional which can lead to permanent life-threatening disabilities. Considering metformin's beneficial preclinical actions on various disorders, and the drug's role in alleviating severity of these conditions through involvement in commonly characterized cellular pathways, we discuss the potential of metformin as a suitable drug candidate for repurposing in CNS disorders.

## 1. Introduction

Aging, a major risk factor for neurodegenerative diseases (NDs), is time dependent anatomical and physical change that reduces the functional and physiological capacity of a living organism [1]. NDs represent a wide number of diseases among which Alzheimer's disease, Parkinson's disease and Multiple Sclerosis are the commonly encountered ones that affect more than 6 million people in the United States [2–6]. They are generally associated with microvascular degeneration, neurovascular disintegration and blood brain barrier (BBB) dysfunction [7–9], and represent a substantial danger to human health because of their complex pathophysiology involving loss in memory and cognition which can affect a person's ability to speak, walk and even breathe [10,11]. Another central nervous system (CNS) disorder, ischemic stroke is one of the leading causes for mortality and long-term disability in the US with limited therapeutic approaches [12]. The most common cause for ischemic stroke is the partial or complete blockade of blood flow to the brain from a clot occlusion resulting in loss of neurological function [13,14]. Unfortunately, there are few or no efficient treatments available for slowing down the progress of these diseases, which can be attributable to age as one of their major risk factors.

Over the last two decades, there have been limited drugs approved as

a first line treatment by the Food and Drug Administration (FDA) for NDs and ischemic stroke with probability of just 3% for CNS drugs getting launched after entry into the phase-I clinical trial [15–18]. Therefore, development of effective therapies is pivotal but only will arise from extensive understanding of mechanism of each disease. Additionally, repurposing of drugs that have shown significant results in pre-clinical studies could accelerate developing effective therapies [19]. Drug repurposing is a strategy that involves searching for new applications of prevailing therapeutics that will allow drugs to reach a greater number of patients for a wider indication. This procedure can bypass several steps of drug development including determination of mechanism of action, formulation and pharmacokinetics which would take on average 10–17 years in contrast to development time of 3–12 years for repurposed drugs [20,21]. Interestingly, there are 103 compounds listed by [geroprotectors.org](http://geroprotectors.org) to facilitate drug repurposing approaches in targeting aging related CNS diseases that have already been approved for use in humans [22]. This database compiles data of existing substances through pharmacological modeling and biostatistical analysis. One of them is the most promising anti-diabetic drug, metformin, because of substantial experimental studies supporting its beneficial effects in CNS diseases.

<sup>\*</sup> Corresponding author at: Chair and University Distinguished Professor of Pharmaceutical Sciences at TTUHSC, Jerry H. Hodge School of Pharmacy, Office 314, 1300 Coulter Drive, Amarillo, TX 79106, USA.

E-mail address: [thomas.abbruscato@ttuhsc.edu](mailto:thomas.abbruscato@ttuhsc.edu) (T. Abbruscato).

<sup>1</sup> These authors contributed equally to this work.

## 2. Metformin

Metformin, class biguanide, is a synthetic derivative of French Lilac (*Galega officinalis*), a herbal plant traditionally employed in Europe for diabetes treatment [23]. In 1957 French diabetologist Jean Sterne first published the drug's properties and result of administration in humans for diabetes [24]. The drug was first approved for use in UK in 1958 and became available in the British National Formulary in the same year [25]. Metformin was first approved by the FDA in the year 1994 for treatment of type 2 diabetes mellitus (T2D). In 2009, metformin got approval by American Diabetic Association (ADA) and the European Association for the Study of Diabetes (EASD) as a first line oral anti-diabetic drug because studies showed drastic improvement in morbidity and mortality [26]. Metformin lowers the glucose level in the blood not by sensitizing insulin secretion from beta cells of the pancreas but inhibition of peripheral and hepatic glucose production. Thus, the drug does not cause hypoglycemia [27]. Moreover, there have not been any major safety concerns on the usage of the drug for over sixty years [28]. The only exception is that the drug has been reported to cause lactic acidosis in high doses. As a result, the drug is not recommended in severe liver impairment and chronic kidney disease patients [29]. Outside of its application in the treatment of diabetes, metformin has anti-inflammatory, anti-cancer, cardioprotective, hepatoprotective and antioxidant properties and currently it is being investigated as a drug directly acting on the CNS [30–32]. With its mild side effects and a multi-action drug profile, metformin is a favorable candidate for repurposing.

Considering metformin's pharmacokinetic profile, there have been numerous studies that are able to demonstrate a clear picture of how the human body pharmacokinetically processes this drug [33–36]. Metformin is slowly and incompletely absorbed from the gastrointestinal tract at doses of 0.5–2 g per day and the bioavailability after oral administration is 50–60%. Additionally, the drug is not bound to plasma proteins and has an apparent volume of distribution ( $V_d$ ) of approximately 600 L after an oral administration of 2 g. Moreover, this high value of  $V_d$  of metformin suggests that the drug can be absorbed by various tissues across the body. The drug is not affected by any significant biotransformation by liver or biliary secretion and finally it is eliminated through active renal tubular secretion with an elimination half-life of 4–5 h.

Metformin has capacities to balance survival and death signaling in the primary neurons which improves energy metabolism, oxidative stress and proteostasis [37]. Pre-clinical studies have suggested that the AMP-activated protein kinase (AMPK) dependent mechanisms of the drug are responsible for the antioxidative effects in aging related CNS disorders [31,38,39]. Basically, the drug exerts its activities with two proposed mechanisms; AMP-activated protein kinase (AMPK) dependent and AMPK independent manner [40]. Firstly, Metformin inhibits the complex I in mitochondrial respiratory chain and decreases cellular respiration. This increases ADP:ATP and AMP:ATP ratios which activates the AMPK and leads to increased production of ATP and decreased consumption [41]. Also, AMPK activation by metformin inhibits phosphorylation of acetyl-CoA carboxylase (ACC) that increases  $\beta$  oxidation and fatty acid uptake which ultimately results in improved lipid metabolism and insulin sensitivity [42]. Furthermore, AMPK activation promotes glucose consumption and inhibits glucose output [43]. Secondly, an AMPK-independent mechanism of metformin inhibits mitochondrial glycerophosphate dehydrogenase which decreases gluconeogenesis [44]. Moreover, metformin's role in reducing hyperglycemia as well as hyperinsulinemia, known accelerators of aging, makes it an attractive anti-aging drug [31].

The NF- $\kappa$ B pathway is one of the most prominent inflammatory mediators of aging. A bioinformatic analytical study from various aged tissues showed that NF- $\kappa$ B is the most associated transcription factor altered in gene expression during aging [45]. Similarly, reducing NF- $\kappa$ B activity has been reported to decrease accelerated aging in a mouse

model of progeria [46]. In fact, its activation leads to transcription of gene coding for survival signals, inflammatory cytokines, cell cycle modulators and finally angiogenic and growth factors, which eventually creates a replenishing tumor growth environment [47]. Metformin inhibits the phosphorylation of I $\kappa$ B and IKK $\alpha$ / $\beta$  by preventing the translocation of NF- $\kappa$ B to the nucleus [48]. These effects support AMPK independent activation and further provide evidence for metformin's potential as an anti-aging drug and possibly as an anti-neoplastic agent.

The mechanistic target of rapamycin (mTOR) is a nutrient response pathway whose inhibition extends lifespan in animal models and ensures protection against age related conditions. Thus, drugs that target the mTOR pathway can be possible therapeutic options in treatment of aging related diseases [49]. Metformin has been demonstrated to depress the mTOR pathway in both an AMPK dependent, where it inhibits protein synthesis via mTOR and suppresses ATP consumption, and an AMPK independent manner through downregulation of insulin like growth factor-1 (IGF-1). This leads to cell growth, proliferation and autophagy [50].

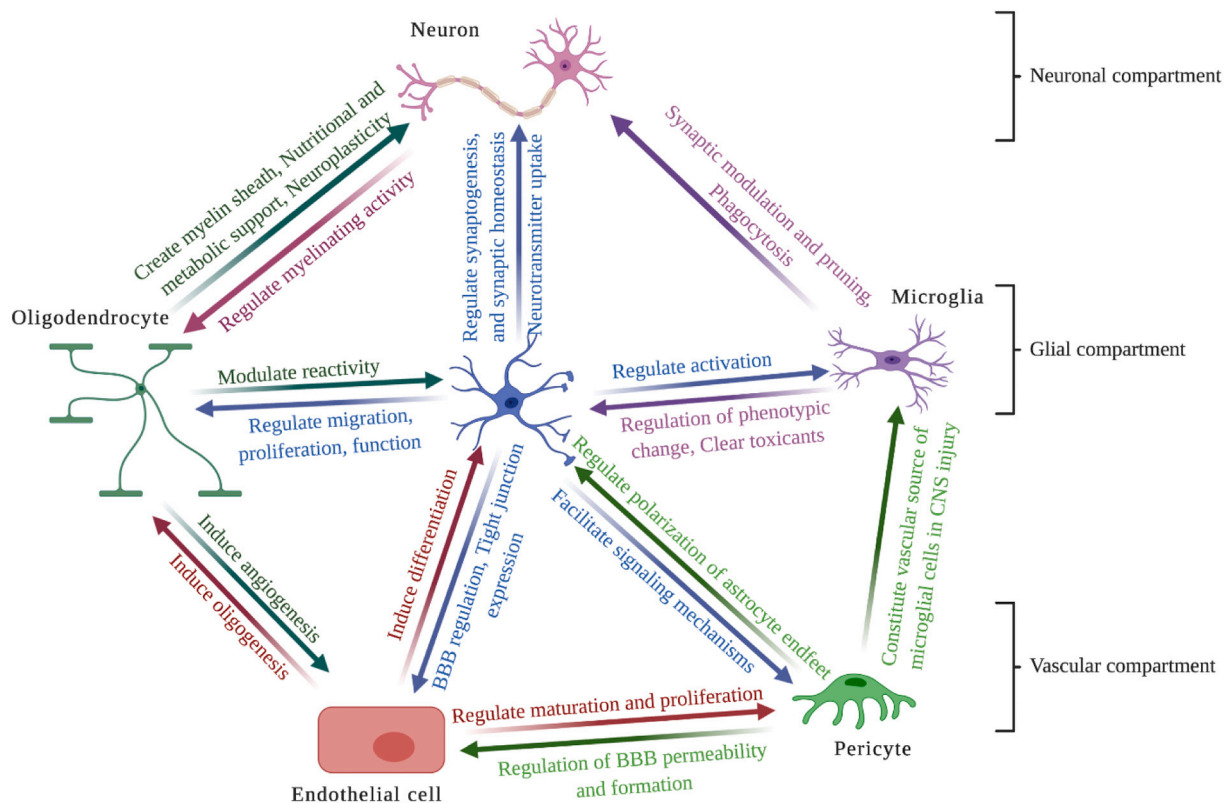
## 3. The neurovascular unit (NVU) and CNS disorders

The BBB is the major site of blood and CNS exchange at the brain microvessel endothelium level [51]. It is a dynamic structure regulates several mechanisms within the brain that involve changes in tight junctional function and activity of enzymes and transporters present at the brain microvascular endothelium. This dynamic barrier accounts for synaptic signaling, neurotransmission, restricted entry of macromolecules into the brain, protection from neurotoxins and selectively provide the necessary supply of solutes and nutrients to the brain [52]. Brain endothelial cells (BECs) are the primary anatomical unit of the BBB, providing a physical separation between the blood and CNS. The “barrier” function of the NVU is conferred by four distinct phenotypes of the endothelial cells that include expression of efflux transporters, metabolic enzymes, tight junctional proteins and reduced levels of pinocytosis [53,54]. This BBB phenotype of BECs, is greatly influenced by the surrounding cells that function collectively as the NVU. The other important cell types for the BBB induction and maintenance are pericytes, which share a basement membrane with the brain endothelium and astrocytes, and reside in close proximity to the BBB [55]. Neurons, the extracellular matrix, microglia and oligodendrocytes are the other components of the NVU that are responsible for maintenance of normal BBB function under both physiological and inflammatory conditions [56]. Fig. 1 illustrates the paracrine interactions between NVU cells and their effects on each other.

### 3.1. Pathophysiological changes in the components of NVU with aging in CNS diseases

The global decrease in cerebral blood flow as well as neurovascular uncoupling are considered as hallmarks of normal aging and neurodegenerative diseases [57]. There is reduced expression of specialized tight junction proteins such as occludin, claudin-5 and occludens-1 in the NVU that causes BBB dysfunction [58–60]. Additionally, pericyte deficiency results from their detachment from endothelial cells [9]. This causes neurotoxic macromolecules to leak or accumulate into the brain. Moreover, there is accumulation of iron in astrocytes which reduces expression of ceruloplasmin in the CNS [61]. Excessive iron can generate free radicals and cause BBB alteration. Furthermore, detachment of astrocytic end foot from the BECs results in weak communication between neurons and the endothelium, eventually causing neurovascular uncoupling and hence neurodegeneration [62].

Data from a human study showed significant increase in albumin leakage through the BBB in aged healthy individuals compared to young healthy population [63]. Similarly, morphological changes in the BBB and leakage of albumin and IgG to the brain parenchyma were confirmed for brain regions that encompass cognitive functions, such as



**Fig. 1.** Functional interactions among neurovascular unit components.

Interactions between the neuronal, glial and vascular compartments within the NVU are crucial for maintaining normal function of the brain. Also, different cells within each compartment are functioning and interacting cooperatively to regulate brain homeostasis.

the hippocampus [64]. Furthermore, some brain regions of old senescence-accelerated prone mouse strain 8 (SAMP8), showed increased transport of TNF- $\alpha$  [65]. Also, in both physiologically aged and SAMP8 mice, the BBB has decreased expression of GLUT-1 transporter, which results in decreased transport of necessary glucose to the brain [66]. The activity of efflux transporter, P-glycoprotein, is also decreased in experimental models of aging which results in reduced elimination of toxins from brain to the blood capillaries [67].

Hypoxia increases the permeability of various macromolecules such as albumin and dextran across the BBB [68,69]. This occurs as a result of increased exposure to free radicals and/or inflammatory cytokines at the levels of tight junction proteins. Angiogenic factors like vascular endothelial growth factor (VEGF) and hypoxia-induced factor 1- $\alpha$  decrease with age. Brain-derived neurotrophic factor, a growth factor secreted by both neurons and BECs, which contributes to synaptic plasticity, decreases with age. Furthermore, age-related decreases in nitric oxide bioavailability alters BECs sensitivity to VEGF. Fig. 2 illustrates the effects of aging on the NVU components and the subsequent outcomes.

### 3.2. Regional brain distribution of metformin

Pre-clinical studies have shown that with a single oral dose of 50–150 mg/kg, metformin can cross the BBB [70,71] and it was able to restore brain AMPK activity [72,73]. However, because of disturbances of AMPK activity in inflammatory CNS conditions such as NDs and ischemic stroke, it is important to determine the drug's specific pharmacological target within the brain. The analysis of brain specific distribution of metformin under normal and inflammatory conditions in one study showed a higher level of drug accumulation in pituitary gland, olfactory bulb, striatum and hypothalamus [74]. In contrast, the hippocampus, cerebellum and frontal cortex showed low accumulation of the drug in inflamed brain samples. Furthermore, it was suggested that

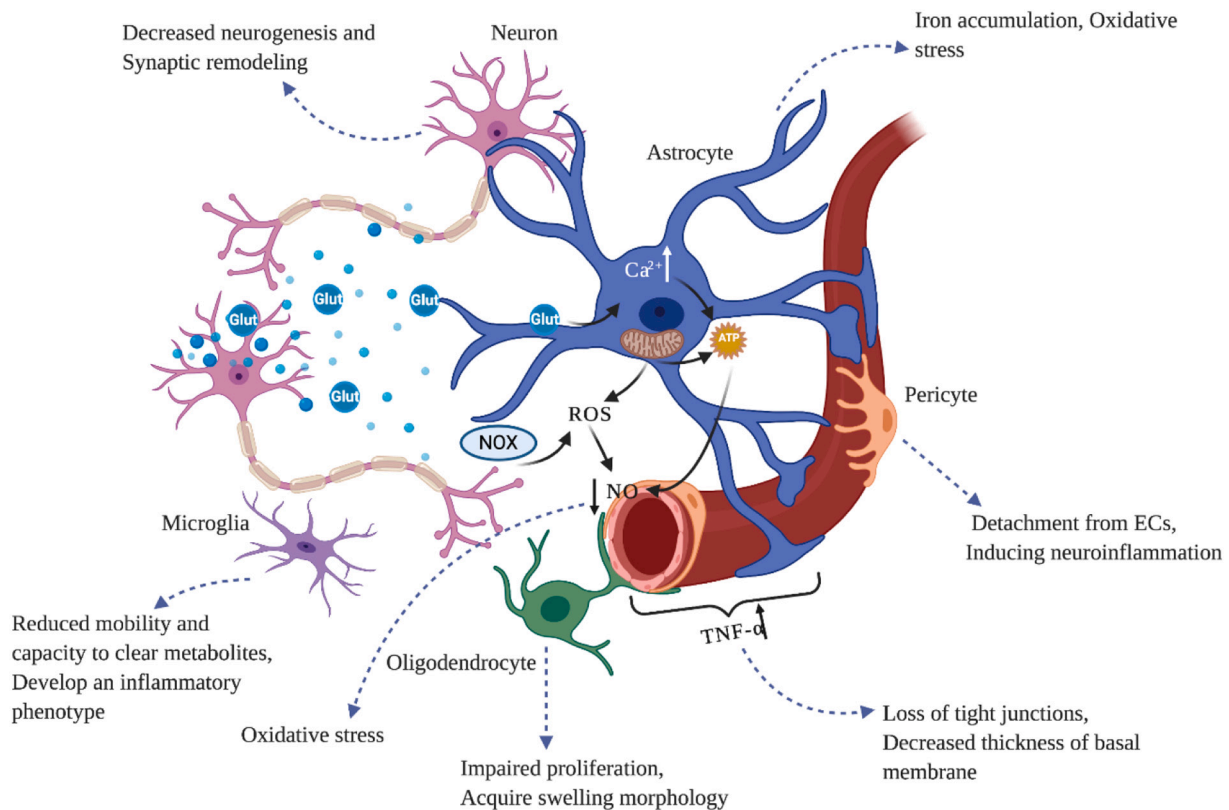
various concentrations of the drug in different parts of brain may result from either up or down regulation of determinants of intracellular accumulation of the drug, such as membrane-bound organic cationic transporters or mitochondria. It is apparent that future studies are required to determine the regional brain distribution of metformin and decipher the drug's action in molecular level.

## 4. CNS disorders and role of metformin

### 4.1. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia and progressive neurodegenerative disorder that causes memory loss and cognitive dysfunction. In fact, with growing evidences of AD's pathogenesis it could be suggested that impaired insulin signaling is responsible for neurodegeneration [75]. Interestingly, studies have shown that metformin has potent anti-inflammatory actions and it ameliorates AD-associated neuropathological changes and decreases cognitive impairment in diabetic mice model and also in patients with T2D [76,77]. A study showed that oral and intranasal metformin administration can improve memory and cognition in AD patients [78]. Furthermore, another study compared data of the pro-neurogenic potential of metformin to that of donepezil, a first-line acetylcholinesterase inhibitor, in an aluminum chloride induced mouse model of neurodegeneration [79]. The study concluded that the mice treated with metformin exhibited an enhanced number of post-mitotic NeuN positive cells compared to that treated with donepezil. Therefore, metformin mediated neurogenesis could eventually be a potential treatment option in neurodegenerative diseases that cause cognitive impairment.

Pathologically, AD is characterized by deposition of intracellular neurofibrillary tangles and extracellular amyloid- $\beta$ (A $\beta$ ) plaques. The extracellular deposition of A $\beta$  plaque surrounded by microglia and



**Fig. 2.** Effects of aging on the NVU components and their interactions. Aging can influence all the cells within the NVU and results in neurovascular uncoupling and neurodegeneration. In endothelial cells, aging induces the secretion of pro-inflammatory factors such as TNF- $\alpha$ , IL-1B and VCAM-1, tight junctional protein disruption as a result of chronic neuroinflammation, and BBB impairment. It also increases astrocytic reactivity and impairs the role of astrocytic endfoot in connecting neurons and endothelial cells. BBB disruption and loss of communication with other cells result in neuronal dysfunction and neurodegeneration. Therefore, the regenerative capacities of neurons including synaptic plasticity, neurogenesis and synaptogenesis decrease significantly.

astrocytes is reported to be due to overactivation of glia which causes excessive release of pro-inflammatory factors such as IL-1 $\beta$  and TNF- $\alpha$  [80,81]. However, activation of glia is important for the clearance of A $\beta$  deposition through phagocytosis, linking dual role for neuro-inflammation on A $\beta$  pathology [82,83]. Epidemiological studies have shown that long term use of anti-inflammatory agents has helped in reducing risk of developing AD [84]. However, with these agents, contradictory results in AD treatment have been observed, highlighting the need for more specific anti-inflammatory therapies. Interestingly, metformin administration was shown to attenuate spatial memory deficits, decrease A $\beta$  plaque load and chronic inflammation via activation of microglia and astrocytes as well as pro-inflammatory mediators in the hippocampus and cortex of APP/PS1 mice [82,83,85,86]. These studies suggested that metformin can enhance functional recovery via regulating AMPK, mTOR and NF- $\kappa$ B signaling pathways in hippocampus, thus improving neurologic defects.

#### 4.2. Parkinson's disease

Parkinson's disease (PD) is one of the most common NDs that is accompanied by progressive loss of dopaminergic neurons in the substantia nigra compacta and aggregation of Lewy bodies and Lewy neurites in various parts of the affected brains [87,88]. Mitochondrial dysfunction, oxidative stress, neuroinflammation, ubiquitin proteasome system and disturbed proteostasis due to impaired autophagy-lysosomal pathway are the hallmarks of PD [89,90]. A previous study demonstrated that metformin reduces the number of nonfunctional mitochondria and ROS generation by promoting mitophagy in an AMPK-dependent manner [91]. Additionally, metformin was shown to activate the ATF2/CREB pathway in an AMPK-independent manner and

stimulate peroxisome proliferator-activated receptor gamma coactivator-1a (PGC-1a) and its target genes [92]. PGC-1a is a transcriptional cofactor that is actively involved in regulation of mitochondrial anti-oxidative defense mechanism [92]. Moreover, in the mid brain of MPTP mouse model of PD, metformin restored anti-oxidative mediators such as superoxide dismutase, catalase and glutathione [93]. PD is characterized by various motor and non-motor symptoms such as rigidity, bradykinesia, freezing of gait, cognitive abnormalities and depression [94].

The effect of metformin on the substantia nigra, a critical brain structure affected in PD, has not been studied in detail. Nevertheless, data from a study suggests effect of metformin on microglial activation in the substantia nigra in a pro-inflammagen lipopolysaccharide (LPS) at both the cellular and molecular levels [95]. This study supports that metformin inhibits microglia activation as measured by immunoreactivity markers and it minimizes expression of pro and anti-inflammatory cytokines by phosphorylation of mitogen activated protein kinase (MAPK) as well as by ROS generation through inhibition of NADPH enzyme. However, metformin failed to protect the dopaminergic neurons of the substantia nigra in response to intranigral LPS.

### 4.3. Multiple sclerosis

Multiple sclerosis (MS) is a chronic demyelinating disease characterized by delayed remyelination of axons making it susceptible to irreversible degeneration. This substantiates progressive neurologic decline as associated with later stages of MS and can extend over decades [96]. The delayed remyelination occurs with aging due to delayed differentiation of oligodendrocytes progenitor cells (OPCs) to oligodendrocytes, the myelin forming cells of the CNS [97,98]. In fact, studies



demonstrate that regulatory mechanism that control OPC differentiation are nonfunctional in aging brain [99,100]. Additional studies found that chronically demyelinated MS lesions contain OPCs that were undifferentiated and increase in number of OPCs in white matter lesions of aged animals [101,102]. These OPCs did not contribute in remyelination, indicating that differentiation of OPCs into oligodendrocytes is crucial for remyelination. It has also been shown that, remyelination could be enhanced by adding pro differentiating factors lacking in aged brain [103,104]. However, aged OPCs differentiate very slowly and become unresponsive to pro-differentiation signals. The diminished functional capacity is associated with hallmarks of cellular aging such as mitochondrial dysfunction, unfolded protein response, autophagy, NF- $\kappa$ B and p-38 MAPK signaling. When metformin was used as a pharmacological approach targeting endogenous OPCs, it stimulated remyelination via AMPK-dependent mechanism and led to increased mitochondrial function as required for differentiation of OPCs. Also, metformin reduced oxidative stress with activation of antioxidative defense in oligodendrocytes exposed to cytokines via AMPK activation. Hence, metformin has potential to limit neurologic deficits in MS and related neurodegenerative diseases. In Table 1 we have summarized some of the effects of metformin treatment in NDs from experimental studies.

#### 4.4. Ischemic stroke

Ischemic stroke can result in cerebral ischemia/reperfusion (I/R) injury, which is characterized by transient loss followed by rapid return of the blood flow to the brain and can lead to a higher amount of neuronal death, enhanced brain infarction and substantial cognitive dysfunction compared to ischemia alone [113,114]. The ischemic cascade follows in seconds to minutes after the blockage and is comprised of multiple, continuous biochemical events leading to severe focal hypoperfusion, excitotoxicity and oxidative damage eventually leading to BBB dysfunction and inflammation [115]. White matter injury, as well as damage and death of astrocytes, also contribute to cerebral impairment [116,117]. Inflammation initially causes release of harmful radicals and cytokines; however, it also enables synaptic remodeling that helps to remove damaged tissues [118]. Similarly, glial cells promote angiogenesis and neurogenesis to regulate the BBB, but conversely forms glial scar that may further prevent neuronal plasticity [119]. Below we have discussed the pathways through which metformin exerts its protective effect in ischemic stroke.

##### 4.4.1. AMPK activation

As with NDs, AMPK activation is believed to be metformin's key pathway involved in ischemic stroke. There are several studies suggesting that metformin acts through the AMPK signaling pathway in reduction of the cerebral infarct area and neuronal apoptosis in ischemic rat models [39,120,121]. Metformin's AMPK activation in suppression of post ischemic neuroinflammation has been reported to be either because of NrF2 anti-oxidant pathway activation or inhibition of NF- $\kappa$ B cascade [38]. An in-vitro study showed that metformin treatment following oxygen-glucose deprivation and reperfusion (OGD/R) reduces NF- $\kappa$ B and intercellular adhesion molecule-1 (ICAM-1) in BECs [38]. Similarly, metformin reduced TNF- $\alpha$  induced inflammation in BECs by activation of AMPK [122]. Furthermore, metformin treatment improved apoptosis of primary fetal rat derived hippocampal neurons that were subjected to OGD [123]. An in-vitro study showed metformin's effect in primary rat cortical astrocytes subjected to OGD and found out that AMPK is critical for the regulation of apoptosis in a caspase independent manner [124].

AMPK consists of an  $\alpha$  catalytic subunit and a  $\beta$  and  $\gamma$  regular subunits and all of them play important roles in regulation of energy metabolism [125]. All these subunits are highly expressed in neurons, glia and astrocytes of the NVU. Studies have shown that diverse cellular combination of AMPK subunits may account for various effects of AMPK

**Table 1**

Effect of Metformin treatment in Neurodegenerative diseases in experimental animal models and cultured cells.

Neurodegenerative disease	Description of study	Outcome(s)	References
Alzheimer's disease	- Male <i>db/db</i> mice	- Metformin mitigated the increase of total and phosphorylated tau and decreased the JNK activation.	[77]
	- 200 mg/kg/day intraperitoneal metformin for 18 weeks	- Attenuated the reduction of synaptophysin	
	- Primary cortical neurons and Neuro2a neuroblastoma cells treated with metformin	- Metformin significantly increased the generation of intracellular and extracellular AB species	[71]
		- Upregulated transcription of B-secretase (BACE1)	
		- In combination with insulin, metformin enhances insulin's effect in reducing AB level	
	- In vitro model of "type 3 diabetes"	- Metformin ameliorated neuronal insulin resistance	[105]
	- Differentiation of neuronal cell line Neuro-2a under prolonged presence of insulin and treatment with metformin	- Insulin sensitization by metformin prevented AD-associated neuro-pathological changes	
	- <i>db/db</i> mice treated with 200 mg/kg metformin by oral gavage	- Metformin decreased AB influx across the BBB and decreased level of AB in hippocampus	[106]
		- Significant reduction of nuclear NF- $\kappa$ B p65 of brain micro-vessel endothelial cells	
		- Suppression of caspase-3 activity and inhibited neuronal apoptosis	
Parkinson disease	- Primary cortical neurons treated with up to 2.5 mM metformin for 1–24 h	- Metformin induces PP2A activity by inhibiting mTOR activity and reduces tau phosphorylation	[107]
	- Human neural stem cells exposed to AB and treated with metformin	- Decreased A $\beta$ -mediated mitochondrial deficiency	[108]
		- Significant restoration of mitochondrial morphology	
		- Rescued cell viability through AMPK pathway activation	

(continued on next page)

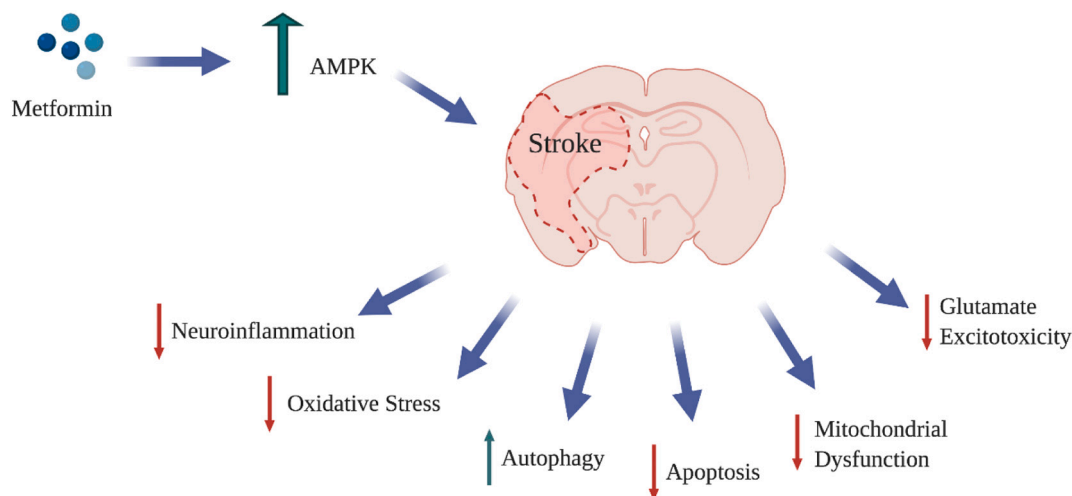
Table 1 (continued)

Neurodegenerative disease	Description of study	Outcome(s)	References
	- Adult male swiss albino mice	- Long-term metformin treatment resulted in significant improvement of the locomotor and muscular activities	[91]
	- Induction of parkinsonism in mice	- Brain-derived neurotrophic factor significantly increased in metformin treatment group	
	- Post metformin 500 mg/kg treatment for 21 days	- Attenuated degeneration of substantia nigra compacta dopaminergic neurons by inhibiting microglial overactivation induced neuroinflammation	
	- Ten-week-old male C57BL/6 mice	- Elevated striatal dopaminergic levels and improved motor impairment	
	- Induction of parkinsonism in mice	- Metformin rescued tyrosine hydroxylase-positive neurons and attenuated astroglial activation in the nigrostriatal pathway.	[109]
	- Treatment with metformin 5 mg/mL in drinking water for 5 weeks	- Metformin restored dopamine depletion and behavioral impairments exerted by MPTP	
	- 10-week-old adult male C57BL/6 mice	- Metformin ameliorated MPTP-induced synuclein phosphorylation which was accompanied by increased methylation of protein phosphatase 2A (PP2A), a phosphatase related to $\alpha$ -synuclein dephosphorylation	
	- MPTP injection (30 mg/kg/day) for the first 7 days to induce PD	- Metformin's AMP activation induced microtubule mediated autophagy and eliminated mitochondrial reactive oxygen species	
	- Post metformin (200 mg/kg/day) for the next 7 days	- Reduced MPP+ induced cytotoxicity and neuronal apoptosis	[91]
	- In-vitro pre-metformin treatment in SH-SY5Y cells	- Increased localization of precursor oligodendrocytes and their renewal in the corpus	
Multiple Sclerosis	- C57BL/6 J mice were administered with 0.2% cuprizone for 5 weeks for		[110]

Table 1 (continued)

Neurodegenerative disease	Description of study	Outcome(s)	References
	demyelination induction.	callosus via AMPK/mTOR pathway	[111]
	- Post metformin treatment of 50 mg/kg/day for 1 weeks	- Reduced brain apoptosis markers and attenuated motor dysfunction	
	-Male C57BL/6 mice were fed with 0.2% cuprizone plus metformin by oral gavage of 100 mg/kg body weight in saline every day from day 0 until to the end of 6 weeks one time in a day	- The myelinated axons within corpus callosum of cuprizone-induced demyelination animals increased after administration of metformin	
		- Metformin ameliorated the oxidative stress induced by cuprizone	
		- Metformin upregulated expression of mitochondrial biogenesis genes	[112]
		- Metformin ameliorated the oxidative stress induced by cuprizone	
		- Astrogliosis and microgliosis were decreased after metformin administration while it enhanced the number of oligodendrocytes	
		- Metformin reduced Th17 and increased Treg cell percentages along with the levels of associated cytokines	
	- Female C57BL/6 wild-type mice, 8–10 weeks of age	- Metformin inhibited activation of mTOR and its downstream target, HIF-1 $\alpha$	
	- Induction of experimental autoimmune encephalomyelitis induced by subcutaneous injection of 250 $\mu$ g of MOG35–55 peptide		
	- 100 mg/kg Metformin dissolved in saline solution was intraperitoneally administered for 20 days		

activation following stroke. For instance, in an experimental stroke model, systemic gene depletion of  $\alpha$ -2 subunit confers neuroprotection while cell-specific deletion of  $\alpha$ -2 subunit in astrocytes after cerebral ischemia has been found to be detrimental [126]. Additionally, administration of metformin caused increased neuronal damage in post-ischemic conditions [127]. The differential effects of metformin are contributable to several causes that include type and length of ischemia, dosage, duration and route and timing of administration of metformin and co-existing conditions like diabetes [128], chronic kidney diseases [129] and tobacco smoking [130]. Fig. 3 illustrates the neuroprotective effects of metformin in ischemic stroke through AMPK signaling pathway.



**Fig. 3.** Neuroprotective effects of metformin in ischemic stroke through AMPK signaling pathway. During an ischemic event, a series of neuroprotective mechanisms are stimulated by activated AMPK in order to maintain the energy homeostasis of the brain. Activated AMPK promotes autophagy, reduces neuroinflammation and oxidative stress by decreasing the levels of inflammatory factors (NF- $\kappa$ B, TNF- $\beta$ , IL-1 $\beta$ , IL-6) and ROS production respectively. Also, it restrains apoptosis, glutamate excitotoxicity through inhibiting glutamate release and mitochondrial dysfunction therefore, promoting energy metabolism and glucose uptake.

#### 4.4.2. Alleviation of oxidative stress mediated inflammation

ROS induced tissue damage is one of the most important components of cerebral I/R injury [131]. An I/R event leads to detrimental effects to the BBB, causing increased leucocyte infiltration into the brain tissue due to the upregulation of ICAM-1 and activation of microglia that leads to increased production of ROS and RNS [132]. Therefore, alleviating the damage caused by I/R injury induced by oxidative stress can be promising in the treatment of ischemic stroke. A study showed that metformin treatment significantly increased endogenous antioxidants enzymes such as superoxide dismutase (SOD) and glutathione (GSH) levels in the brain tissue [133]. This reversed the oxidative injury caused by increased levels of ROS. Moreover, metformin treatment following I/R injury leads to reductions in both the expression of ICAM-1 and subsequent leucocyte infiltration. This was confirmed with decreased myeloperoxidase+ and GrL+ cells after I/R event in brain tissue [38].

An I/R event leads to upregulation of microglia M1 signature genes including IL-1 $\beta$  and CD32, leading to inflammation. It was shown that post metformin treatment reversed the polarization of microglia M1 to M2 signature genes, including CD206 and arginase-1, as well as anti-inflammatory cytokines IL-4 and IL-10, resulting in reduced brain inflammation [120,134]. Moreover, a study conducted in LPS stimulated microglial cells, used to mimic an I/R event and treated with metformin, showed that the cells produced higher levels of anti-inflammatory cytokine, interleukin-10, compared to control group [120]. This suggests that metformin could have anti-inflammatory activity following I/R injury in microglial cells. Also, elevated numbers of angiogenic structures were seen with bEnd.3 cells when they were exposed to medium that contained metformin-treated microglia.

#### 4.4.3. Neurogenesis

It has been shown that, following an ischemic injury, there is an increased production of neurons and subsequent neuroblast migration to damaged area of the brain as a part of endogenous repair mechanism [135]. Hence, therapies that promote post-ischemic neurogenesis can be a potential target for ischemic stroke. A study tested long-term neuroprotection effects of metformin following hypoxic ischemia injury and found reduced neuronal degeneration in the CA1 region of hippocampal area [136]. The CA1 region of hippocampus is highly sensitive to HI injury. Similarly, metformin treatment reversed increasing levels of cleaved caspase 3, promoted anti-apoptosis protein BCL-2 expression, inhibited pro-apoptosis protein BAX in the cortex and hippocampus.

Another study confirmed that metformin promoted neuroblasts

proliferation and differentiation in the hippocampal area [137]. It was supported that metformin enhances regulation of neurogenesis and has a likely contribution in treatment of cerebral ischemic injury. An I/R event can also induce activation of astrocytes leading to increase in production of glial fibrillary acidic protein (GFAP). This causes formation of glial scar in the brain that eventually reduces regeneration of neurons, impeding recovery after an ischemic event [138,139]. Also, metformin treatment post I/R injury reduces GFAP+ cells, suggesting the regeneration of neurons and thereby functional recovery [140].

#### 4.4.4. Other important pathways

PI3K/Akt1/JNK3/C-Jun pathway is one of the crucial signaling pathways in cell survival. Cellular growth factors such as platelet derived growth factor can stimulate release of phosphatidylinositol 3 Kinase (PI3K) after activation of Akt1, which simultaneously inactivates c-Jun N-terminal kinase-3 (JNK-3) molecule that is found in heart and brain. JNK-3 is associated with expression of apoptotic proteins and is negatively correlated with neuronal survival in stroke model [141]. Interestingly, a study has demonstrated the involvement of this pathway when metformin improved impairment of hippocampal controlled behaviors and reduced cell apoptosis in an I/R rat model. Thus, PI3K/Akt1/JNK3/C-Jun pathway incorporates metformin's neuroprotective role in hippocampal deficits caused by I/R injury [142]. Additionally, pre-treatment of BECs isolated from a diabetic rat model with metformin and exposure to OGD/R, resulted in reduced formation of nitrotyrosine and p85 (PI3K regulatory subunit) nitration compared to the control group. The mechanism behind this is reduced reactive nitrogen species in the BECs that would eventually result in restored regulation of the apoptotic pathway and alleviation of I/R injury [143].

Arterial baroreflex pathway is one of the few important pathways for stroke prevention [144] and its dysfunction has been reported to be an important risk factor for the development of stroke [39]. In fact, the  $\alpha$ 7nAChR pathway, a ligand gated ion channel that is highly expressed in the macrophages of different tissues in the brain, is demonstrated to be the downstream of arterial baroreflex pathway and involved in neuroprotection against ischemic cerebral injury [145,146]. The activation of this receptor inhibits the production of inflammatory cytokines and thus reduces local inflammatory response. Studies have shown that metformin reduces chronic inflammation by inhibiting proinflammatory cytokines levels, in both animal and human, labelling it as a promising anti-inflammatory agent [39,147]. Additionally, metformin regulates the action of vagal nervous, one arm of arterial baroreflex in the CNS,

which in turn is mediated mainly by  $\alpha 7$ nACh receptor [148,149]. Moreover, metformin increases the life span of stroke-prone spontaneously hypersensitive rats, reducing the middle cerebral artery occlusion (MCAO) induced infarct size in the brain and upregulating the expression of  $\alpha 7$ nACh receptors. This resulted in downregulation of pro-inflammatory cytokines in serum and peri-infarct area of ischemic brain. However, this effect of metformin was markedly attenuated by deactivating the arterial baroreflex by sinoaortic denervation in rats. This further confirms the involvement of the pathway in metformin mediated protection against stroke.

#### 4.4.5. Dose, duration and timing of metformin treatment in ischemic stroke

Investigators have conducted a study based on dose, duration and timing of metformin administration in male C57BL/6 mice that were subjected to both transient and permanent MCAO surgery [150]. A 7-day treatment of 10 mg/kg prior to permanent MCAO, showed significant improvement as compared to shorter duration treatments. Therefore, pretreatment time window is an essential factor in metformin administration. In contrast, in transient MCAOs, mice did not show any significant improvement suggesting that metformin may not be beneficial in cases of blood reperfusion.

A different study treated Wistar rats with metformin at 200 mg/kg orally for 7 days and subjected to I/R for the next 7 days [151]. In the same way, the study also conducted both post and continuous (pre-post) administration and compared results with pre-administration. The results showed significant reduction in malonaldehyde level, an oxidative stress marker, in metformin pre-treatment group as compared to continuous group. However, studies have also shown that treatment with 200 mg/kg metformin for a longer duration of 14 days after transient MCAO (tMCAO) reduced the brain atrophy volume [30,122]. Below we have summarized the results of various studies based on the timing and duration of metformin treatment in experimental stroke models (Table 2).

### 5. Metformin's role in ischemic stroke comorbidities

T2D and smoking are common risk factors, as well as coexisting conditions, that can aggravate ischemic stroke prognosis and recovery [163–165]. People with diabetes have higher risk of developing ischemic stroke and myocardial infarction because of increased atherosclerosis and endothelial cell dysfunction, concomitantly causing slower recovery after cerebral I/R injury [166]. A diabetic situation can promote higher bleeding following an ischemic event slowing down the brain recovery process [167]. In addition, T2D has been shown to impair post stroke reparative neovascularization and impede the recovery due to vascular regression in the brain [161]. One study suggests that metformin reduces post stroke nitrotyrosine levels in the brain parenchyma and cerebrovasculature in a diabetic rat model [143]. Furthermore, these diabetic rats were observed to have increased caspase-3 activation with I/R injury induction and treatment with metformin reduced caspase-3 cleavage causing reduced apoptotic cell death. Interestingly, a recent study has suggested exacerbation of ischemia in chronic kidney disease-induced female mice and reports that chronic pre-treatment with metformin is beneficial in stroke recovery. The underlying mechanism is AMPK phosphorylation and activation of canonical NF- $\kappa$ B pathway, as shown by decreased expression of microglia/macrophages M1 signature genes within the ischemic lesions of CKD-induced mice treated with metformin [129].

Tobacco smoking (TS) is considered as a contributing etiology in some NDs and stroke. Moreover, TS promotes glucose intolerance and increases risk of developing T2D [168]. Several studies have shown that TS is associated with decreased vascular endothelial function in a causative and dose dependent manner [169,170] which is primarily related to ROS content of tobacco smoke, nicotine and oxidative stress driven inflammation [171,172]. This suggests that TS shares common pathogenic traits similar to that of T2D in stroke [173]. Furthermore,

**Table 2**

Therapeutic effects and underlying mechanism(s) of action of metformin in experimental stroke models based on timing/duration of metformin treatment.

Mechanism of metformin	Outcome	Timing/duration of metformin treatment	References
AMPK activation and AMPK-dependent M2 polarization of microglial cells	- Improved angiogenesis and neurogenesis	Post-stroke chronic (30 days)	[152]
AMPK activation, promoted eNOS phosphorylation	- Functional recovery	treatment	
	- Reduced ischemia-induced brain atrophy volume	Post-stroke (2 weeks)	[121]
	- Promoted focal angiogenesis and neurogenesis	treatment	
AMPK activation, modulating inflammatory and antioxidant pathways	- Attenuated cellular levels of NF- $\kappa$ B, TNF alpha and cyclooxygenase-2,	Pre-treatment	[39]
	- Increased levels of Nrf2 and heme oxygenase-1		
	- Enhanced levels of glutathione and catalase activities		
AMPK activation	Chronic:	- Pre/Post-stroke chronic (3 weeks)	[153]
	- Improved stroke-induced lactate generation,	treatment	
	- Imeliorated stroke-induced activation of AMPK	- Pre-stroke acute (3 days)	
	Acute:	treatment	
	- Increased infarct volume		
	- Increased pAMPK levels		
AMPK activation	- Improved stroke-induced behavioral deficits	- Post-stroke chronic (3 weeks)	[140]
	- Enhanced angiogenesis	treatment	
AMPK phosphorylation, NF- $\kappa$ B inhibition, down-regulation of cytokines and ICAM-1 expression	- Reduced infarct volume	Post-stroke (2 weeks)	[154]
	- Improved neurobehavioral outcomes	treatment	
	- Decreased BBB permeability		
Brain NF- $\kappa$ B suppression, reduction of pro-inflammatory cytokines and iNOS,	- Reduced infarct volume and improved neurological deficits	Pre-treatment (3 weeks)	[147]
	- Ameliorated microgliosis and astrogliosis		
AMPK-dependent autophagy	- Improved sensory motor signs	Pre-treatment (2 weeks)	[155]
	- Improved anxiolytic behavior and locomotion		
	- Decreased autophagy factors		
improved the arterial baroreflex function, enhanced cholinergic anti-inflammatory pathway	- Up-regulation of vesicular acetylcholine transporter (VACHT) and $\alpha 7$ nAChR	Pre-treatment (3 weeks)	[156]
	- Down-regulated levels of pro-inflammatory cytokines		
AMPK activation	- Enhanced learning and memory	Pre-treatment (2 weeks)	[157]

(continued on next page)



Table 2 (continued)

Mechanism of metformin	Outcome	Timing/duration of metformin treatment	References
AMPK activation	<ul style="list-style-type: none"> <li>- Improved neurological outcomes</li> <li>- Attenuated apoptotic cell death</li> <li>- Induced mitochondrial biogenesis proteins</li> </ul>	Pre-treatment (2 weeks)	[158]
Suppression of nitrotyrosine formation and nitration, improving Akt phosphorylation in endothelial cells, direct antioxidative effect	<ul style="list-style-type: none"> <li>- Attenuated stroke-induced nitrate signaling</li> <li>- Improved angiogenesis</li> <li>- Prevented vasoregression</li> <li>- Improved functional recovery</li> </ul>	Post-stroke (2 weeks) treatment	[143]
Activation of Akt1, reducing phosphorylation of JNK3 and c-Jun, elevation of cleaved caspase-3	<ul style="list-style-type: none"> <li>- Reduced cell apoptosis</li> <li>- Attenuated the deficits of hippocampal related behaviors</li> </ul>	Post-stroke (1 week) treatment	[142]
Pre-activation of AMPK-dependent autophagy	<ul style="list-style-type: none"> <li>- Reduced infarct volume</li> <li>- Reduced neurological deficits</li> <li>- Reduced cell apoptosis</li> </ul>	Pre-treatment (single dose)	[159]
Activation of peripheral AMPK	<ul style="list-style-type: none"> <li>- Reduced ischemic neuronal damage</li> <li>- Decreased development of post-ischemic glucose intolerance</li> </ul>	Post-stroke (1–3 days) treatment	[160]
Glycemic intervention	<ul style="list-style-type: none"> <li>- Improved neurovascular repair</li> <li>- Improved functional outcome</li> </ul>	Post-stroke (2 weeks) treatment	[161]
Glycemic intervention, AMPK activation and antioxidative effects	<ul style="list-style-type: none"> <li>- Reduced vascular remodeling</li> <li>- Reduced severity of hemorrhagic transformation</li> <li>- Decreased edema</li> <li>- Improved functional recovery</li> </ul>	Post-stroke (4 weeks) treatment	[162]
Decreased expressions of total and phosphorylated AMPK	<ul style="list-style-type: none"> <li>- Ameliorated brain infarct</li> <li>- Improved neurological scores</li> <li>- Reduced cell apoptosis</li> </ul>	Pre-treatment (1 week)	[150]

studies show that the release pattern of angiogenic, oxidative and inflammatory factors of BECs in response to hyperglycemia and TS-induced stroke conditions are similar, which supports common pathogenic involvements in BBB impairment [39]. A study investigated effects of nicotine exposure on neuronal glucose utilization in an in-vitro stroke model and found out that it caused decreased neuronal GLUT-1 uptake, up-regulated  $\alpha 7$  nicotinic acetylcholine receptor and eventually led to decreased glycolysis. This caused a state of glucose deprivation at the NVU in the brain and could possibly lead to enhanced ischemic brain injury and/or stroke risk [164] [174]. Furthermore, studies from our group show that metformin also activates the Nrf2 pathway, independent of AMPK phosphorylation, in both in-vitro and in-vivo cigarette smoke-induced cerebrovascular models [175]. Metformin induced renormalization of tight junction proteins, BBB integrity, inflammation and oxidative stress markers, and expression of Glut-1 and

thrombomodulin.

Electronic Cigarettes (E-Cigs), also known as Vapes, Blues or Juuls, have gained popularity among young adults, as they are marketed as a safer alternative to TS. It is also believed to be a useful tool for smoking cessation, however information on nicotine dependence and various cerebrovascular toxicities from e-cigs remain less known [176,177]. A recent Surgeon General's Report showed that it is challenging to make conclusions about the efficacy of e-Cigs for cessation based on clinical trials, mainly due to the heterogeneous group of products [178]. In addition, there are limited studies on effects of e-cigs on stroke outcome in comparison to that of TS. Investigations from our lab have attempted to decipher the brain effects of nicotine in both adult and adolescent rodent models. We have shown that exposure to nicotine and e-Cig vapor in adult mice causes downregulation of brain GLUT1 and GLUT3 expression and leads to decreased brain glucose uptake under both normoxic and ischemic conditions [164]. Another study compared effects of e-cigs versus that of TS in brain and found out that oxidative stress promoted by e-cigs extract was not dissimilar from that induced by extracts from traditional ones on BECs in a mouse model. In fact, they found out that both of their exposures worsen stroke outcomes in mice that underwent tMCAO by downregulating Nrf2 and thrombomodulin. Moreover, animals that received daily doses of metformin with either TS or e-cigs exposure, exhibited better stroke outcome as compared to the untreated counterparts. This was demonstrated by reduced infarct size and better neurological scores in animals receiving metformin. Also, the Nrf2 levels in brain of these animals were significantly higher compared to untreated group [130]. Additional studies in our lab have shown that prenatal e-Cig exposure increases the sensitivity of offspring to neonatal hypoxia. This caused motor and cognitive deficits and enhanced edema in adolescent offspring after neonatal hypoxia. Interestingly, prenatal e-Cig exposure decreased brain GLUTs expression in offspring after neonatal hypoxia [179]. Future studies should investigate the protective effects of metformin in the developing as well as aged brain, with respect to NDs and ischemic stroke.

## 6. Conclusions and future directions

Despite significant advances in the development of therapeutics for symptomatic treatment of neurodegenerative diseases, there is still a critical demand for novel therapeutic agents that can modify diseases and target the contributing pathways. Numerous studies mentioned in this review suggest metformin as a potential new treatment for neurodegenerative diseases and ischemic stroke because it has been demonstrated to improve functional recovery through affecting the injury mechanisms in experimental animal models. It has also been shown that metformin has promising effects on cognition and could be effective in counteracting the deleterious effects of aging which is due to its ability to affect several biological pathways including energy production. Metformin easily crosses the BBB and distributes in several brain regions. Therefore, safety, pharmacokinetic profile and multi-functional properties of metformin, make it a promising neurotherapeutic agent. However, it is still unclear what brain levels are necessary at what time to exert the neuroprotective effects and what type of regional brain distribution is necessary for protective efficacy. Hence, brain pharmacokinetic, biodistribution, time window selection and focused molecular studies are warranted in diseased animal models to further develop metformin as a neurotherapeutic to treat NDs.

## Abbreviations

NDS	neurodegenerative diseases
NVU	neurovascular unit
BBB	blood brain barrier
CNS	central nervous system
FDA	Food and Drug Administration
T2D	type 2 diabetes mellitus

Vd	volume of distribution
AMPK	AMP-activated protein kinase
mTOR	mechanistic target of rapamycin
IGF-1	insulin like growth factor-1
BECs	brain endothelial cells
MMPs	matrix metalloproteinases
VCAM-1	vascular cell adhesion molecule-1
SAMP8	senescence-accelerated prone mouse strain 8
ROS	reactive oxygen species
VEGF	vascular endothelial growth factor
AD	Alzheimer's disease
A $\beta$	amyloid- $\beta$
PD	Parkinson's disease
PGC-1 $\alpha$	peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$
LPS	lipopolysaccharide
MAPK	mitogen activated protein kinase
OPCs	oligodendrocytes progenitor cells
I/R	ischemia/reperfusion
OGD/R	oxygen-glucose deprivation and reperfusion
ICAM-1	intercellular adhesion molecule-1
MCAO	middle cerebral artery occlusion
eNOS	endothelial nitric oxide synthetase
GFAP	glial fibrillary acidic protein
PI3K	phosphatidylinositol 3 Kinase
JNK-3	c-Jun N- terminal kinase-3
CKD	Chronic Kidney Disease
TS	Tobacco smoking
E-Cigs	Electronic Cigarettes

## Funding

This work was supported by NINDS R01 NS#117906 to TJA.

## Authors' contribution

Conceptualization, S.S., S.N.; investigation, S.S., S.N., B.V., TJA.; original draft preparation, S.S., S.N.; review and editing, S.S., S.N., B.V., TJA.; funding acquisition, TJA.

## Declaration of competing interest

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

- [1] C.-W. Hung, et al., Ageing and neurodegenerative diseases, *Ageing Res. Rev.* 9 (2010) S36–S46.
- [2] Association, A.s., 2019 *Alzheimer's disease facts and figures*, *Alzheimers Dement.* 15 (3) (2019) 321–387.
- [3] E. Leray, et al., Epidemiology of multiple sclerosis, *Rev. Neurol.* 172 (1) (2016) 3–13.
- [4] C. Lill, C. Klein, Epidemiology and causes of Parkinson's disease, *Nervenarzt* 88 (4) (2017) 345–355.
- [5] P. Maher, The potential of flavonoids for the treatment of neurodegenerative diseases, *Int. J. Mol. Sci.* 20 (12) (2019) 3056.
- [6] J.J. Young, et al., Frontotemporal dementia: latest evidence and clinical implications, *Therapeutic advances in psychopharmacology* 8 (1) (2018) 33–48.
- [7] W.R. Brown, C.R. Thore, Cerebral microvascular pathology in ageing and neurodegeneration, *Neuropathol. Appl. Neurobiol.* 37 (1) (2011) 56–74.
- [8] Z. Zhong, et al., ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration, *Nat. Neurosci.* 11 (4) (2008) 420–422.
- [9] B.V. Zlokovic, Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders, *Nat. Rev. Neurosci.* 12 (12) (2011) 723–738.
- [10] T. Wyss-Coray, Ageing, neurodegeneration and brain rejuvenation, *Nature* 539 (7628) (2016) 180–186.
- [11] A.D. Gitler, P. Dhillon, J. Shorter, *Neurodegenerative Disease: Models, Mechanisms, and a New Hope*, The Company of Biologists Ltd, 2017.
- [12] E.J. Benjamin, et al., Heart disease and stroke statistics-2017 update: a report from the American Heart Association, *Circulation* 135 (10) (2017) e146–e603.
- [13] H. Brzica, et al., Role of transporters in central nervous system drug delivery and blood-brain barrier protection: relevance to treatment of stroke, *Journal of central nervous system disease* 9 (2017) (p. 1179573517693802).
- [14] S. Nozohouri, et al., Novel approaches for the delivery of therapeutics in ischemic stroke, *Drug Discov. Today* 25 (3) (2020) 535–551.
- [15] H. Dowden, J. Munro, Trends in clinical success rates and therapeutic focus, *Nat. Rev. Drug Discov.* 18 (7) (2019) 495–497.
- [16] S. Nozohouri, B. Vaidya, T.J. Abbruscato, Exosomes in ischemic stroke, *Curr. Pharm. Des.* 26 (2020) 5533–5545.
- [17] A.E. Sifat, B. Vaidya, T.J. Abbruscato, Blood-brain barrier protection as a therapeutic strategy for acute ischemic stroke, *AAPS J.* 19 (4) (2017) 957–972.
- [18] S.E. Swalley, Expanding therapeutic opportunities for neurodegenerative diseases: a perspective on the important role of phenotypic screening, *Bioorg. Med. Chem.* 28 (3) (2020) 115239.
- [19] F.F. Alamri, et al., Delayed atomoxetine or fluoxetine treatment coupled with limited voluntary running promotes motor recovery in mice after ischemic stroke, *Neural Regen. Res.* 16 (7) (2021) 1244.
- [20] J.T. Dudley, T. Deshpande, A.J. Butte, Exploiting drug-disease relationships for computational drug repositioning, *Brief. Bioinform.* 12 (4) (2011) 303–311.
- [21] V. Mallikarjun, J. Swift, Therapeutic manipulation of ageing: repurposing old dogs and discovering new tricks, *EBioMedicine* 14 (2016) 24–31.
- [22] A. Moskalev, et al., Geroprotectors. org: a new, structured and curated database of current therapeutic interventions in aging and age-related disease, *Aging (Albany NY)* 7 (9) (2015) 616.
- [23] M.O. Goodarzi, M. Bryer-Ash, Metformin revisited: re-evaluation of its properties and role in the pharmacopoeia of modern antidiabetic agents, *Diabetes. Obes. Metab.* 7 (6) (2005) 654–665.
- [24] C.J. Bailey, C. Day, Metformin: its botanical background, *Practical Diabetes International* 21 (3) (2004) 115–117.
- [25] D. Hadden, Goat's rue-French lilac-Italian fitch-Spanish sainfoin: gallega officinalis and metformin: the Edinburgh connection, *JOURNAL-ROYAL COLLEGE OF PHYSICIANS OF EDINBURGH* 35 (3) (2005) 258.
- [26] D.M. Nathan, et al., Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of diabetes, *Diabetes Care* 32 (1) (2009) 193–203.
- [27] M.P. Wróbel, et al., Metformin—a new old drug, *Endokrynologia Polska* 68 (4) (2017) 482–496.
- [28] M.J. Crowley, et al., Clinical outcomes of metformin use in populations with chronic kidney disease, congestive heart failure, or chronic liver disease: a systematic review, *Ann. Intern. Med.* 166 (3) (2017) 191–200.
- [29] J. Flory, K. Lipska, Metformin in 2019, *Jama* 321 (19) (2019) 1926–1927.
- [30] J.W. Calvert, et al., Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-mediated signaling, *Diabetes* 57 (3) (2008) 696–705.
- [31] A. Martin-Montalvo, et al., Metformin improves healthspan and lifespan in mice, *Nat. Commun.* 4 (2013) 2192.
- [32] A. Scheen, N. Esser, N. Paquot, Antidiabetic agents: potential anti-inflammatory activity beyond glucose control, *Diabetes Metab.* 41 (3) (2015) 183–194.
- [33] M. Foretz, et al., Metformin: from mechanisms of action to therapies, *Cell Metab.* 20 (6) (2014) 953–966.
- [34] G.G. Graham, et al., Clinical pharmacokinetics of metformin, *Clin. Pharmacokinet.* 50 (2) (2011) 81–98.
- [35] M. Markowicz-Piasecka, et al., Is metformin a perfect drug? Updates in pharmacokinetics and pharmacodynamics, *Curr. Pharm. Des.* 23 (17) (2017) 2532–2550.
- [36] T. Minamii, M. Nogami, W. Ogawa, Mechanisms of metformin action: in and out of the gut, *Journal of diabetes investigation* 9 (4) (2018) 701–703.
- [37] K. Isoda, et al., Metformin inhibits proinflammatory responses and nuclear factor- $\kappa$ B in human vascular wall cells, *Arterioscler. Thromb. Vasc. Biol.* 26 (3) (2006) 611–617.
- [38] Y. Liu, et al., Metformin attenuates blood-brain barrier disruption in mice following middle cerebral artery occlusion, *J. Neuroinflammation* 11 (1) (2014) 177.
- [39] G. Ashabi, et al., Pre-treatment with metformin activates Nrf2 antioxidant pathways and inhibits inflammatory responses through induction of AMPK after transient global cerebral ischemia, *Metab. Brain Dis.* 30 (3) (2015) 747–754.
- [40] G. Rena, D.G. Hardie, E.R. Pearson, The mechanisms of action of metformin, *Diabetologia* 60 (9) (2017) 1577–1585.
- [41] V. Piskovatska, et al., Metformin as a geroprotector: experimental and clinical evidence, *Biogerontology* 20 (1) (2019) 33–48.
- [42] M.D. Fullerton, et al., Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin, *Nat. Med.* 19 (12) (2013) 1649.
- [43] M. Johanns, et al., AMPK antagonizes hepatic glucagon-stimulated cyclic AMP signalling via phosphorylation-induced activation of cyclic nucleotide phosphodiesterase 4B, *Nat. Commun.* 7 (1) (2016) 1–12.
- [44] A.K. Madiraju, et al., Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase, *Nature* 510 (7506) (2014) 542–546.
- [45] A.S. Adler, et al., Motif module map reveals enforcement of aging by continual NF- $\kappa$ B activity, *Genes Dev.* 21 (24) (2007) 3244–3257.
- [46] J.S. Tilstra, et al., NF- $\kappa$ B inhibition delays DNA damage-induced senescence and aging in mice, *J. Clin. Invest.* 122 (7) (2012) 2601–2612.

- [47] G. Kanigur Sultuybek, T. Soydas, G. Yenmis, NF- $\kappa$ B as the mediator of metformin's effect on ageing and ageing-related diseases, *Clin. Exp. Pharmacol. Physiol.* 46 (5) (2019) 413–422.
- [48] O. Moiseeva, et al., Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF- $\kappa$ B activation, *Aging Cell* 12 (3) (2013) 489–498.
- [49] S.C. Johnson, P.S. Rabinovitch, M. Kaeberlein, mTOR is a key modulator of ageing and age-related disease, *Nature* 493 (7432) (2013) 338–345.
- [50] A.F. Cătoi, et al., Metformin modulates the mechanisms of ageing, in: *Metformin*, IntechOpen, 2019.
- [51] S. Nozohouri, et al., Estimating Brain Permeability Using in Vitro Blood-Brain Barrier Models, 2020.
- [52] N.J. Abbott, et al., Structure and function of the blood–brain barrier, *Neurobiol. Dis.* 37 (1) (2010) 13–25.
- [53] D. Bonkowski, et al., The CNS microvascular pericyte: pericyte-astrocyte crosstalk in the regulation of tissue survival, *Fluids and Barriers of the CNS* 8 (1) (2011) 1–12.
- [54] D.H. Jo, et al., Interaction between pericytes and endothelial cells leads to formation of tight junction in hyaloid vessels, *Molecules and cells* 36 (5) (2013) 465–471.
- [55] A. Armulik, et al., Pericytes regulate the blood–brain barrier, *Nature* 468 (7323) (2010) 557–561.
- [56] V. Muoio, P. Persson, M. Sendeski, The neurovascular unit—concept review, *Acta Physiol.* 210 (4) (2014) 790–798.
- [57] G. Jia, et al., Endothelial cell senescence in aging-related vascular dysfunction, *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1865 (7) (2019) 1802–1809.
- [58] M. Elahy, et al., Blood–brain barrier dysfunction developed during normal aging is associated with inflammation and loss of tight junctions but not with leukocyte recruitment, *Immun. Ageing* 12 (1) (2015) 2.
- [59] M.A. Erickson, W.A. Banks, Age-associated changes in the immune system and blood–brain barrier functions, *Int. J. Mol. Sci.* 20 (7) (2019) 1632.
- [60] Y. Yamazaki, et al., Vascular cell senescence contributes to blood–brain barrier breakdown, *Stroke* 47 (4) (2016) 1068–1077.
- [61] S.Y. Jeong, S. David, Age-related changes in iron homeostasis and cell death in the cerebellum of ceruloplasmin-deficient mice, *J. Neurosci.* 26 (38) (2006) 9810–9819.
- [62] S.I. Graves, D.J. Baker, Implicating endothelial cell senescence to dysfunction in the ageing and diseased brain, *Basic & Clinical Pharmacology & Toxicology* 127 (2) (2020) 102–110.
- [63] B.O. Popescu, et al., Blood-brain barrier alterations in ageing and dementia, *J. Neurol. Sci.* 283 (1–2) (2009) 99–106.
- [64] C. Pelegrí, et al., Increased permeability of blood–brain barrier on the hippocampus of a murine model of senescence, *Mech. Ageing Dev.* 128 (9) (2007) 522–528.
- [65] W.A. Banks, A. Moinuddin, J.E. Morley, Regional transport of TNF- $\alpha$  across the blood–brain barrier in young ICR and young and aged SAMP8 mice, *Neurobiol. Aging* 22 (4) (2001) 671–676.
- [66] A. Vorbrodt, et al., Immunogold study of regional differences in the distribution of glucose transporter (GLUT-1) in mouse brain associated with physiological and accelerated aging and scrapie infection, *J. Neurocytol.* 28 (9) (1999) 711–719.
- [67] R. Toornvliet, et al., Effect of age on functional P-glycoprotein in the blood-brain barrier measured by use of (R)-[<sup>11</sup>C] verapamil and positron emission tomography, *Clinical Pharmacology & Therapeutics* 79 (6) (2006) 540–548.
- [68] J.J. Lochhead, et al., Erratum: oxidative stress increases blood-brain barrier permeability and induces alterations in occludin during hypoxia-reoxygenation (*Journal of Cerebral Blood Flow and Metabolism* (2010) 30 (1625–1636)), *J. Cereb. Blood Flow Metab.* 31 (2) (2011) 790–791.
- [69] B.O. Popescu, Triggers and effectors of oxidative stress at blood-brain barrier level: relevance for brain ageing and neurodegeneration, *Oxidative Med. Cell. Longev.* 2013 (2013).
- [70] R. Beckmann, Biguanide (Experimenteller Teil), *Oral wirksame Antidiabetika* 29 (1971) 439–596.
- [71] Y. Chen, et al., Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides by up-regulating BACE1 transcription, *Proc. Natl. Acad. Sci.* 106 (10) (2009) 3907–3912.
- [72] D. Liang, et al., Cytotoxic edema: mechanisms of pathological cell swelling, *Neurosurg. Focus* 22 (5) (2007) 1–9.
- [73] N. Nath, et al., Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis, *J. Immunol.* 182 (12) (2009) 8005–8014.
- [74] K. Łabuzek, et al., Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide, *Pharmacol. Rep.* 62 (5) (2010) 956–965.
- [75] M de la Monte, S. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease, *Curr. Alzheimer Res.* 9 (1) (2012) 35–66.
- [76] C.-C. Hsu, et al., Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin, *J. Alzheimers Dis.* 24 (3) (2011) 485–493.
- [77] J. Li, et al., Metformin attenuates Alzheimer's disease-like neuropathology in obese, leptin-resistant mice, *Pharmacol. Biochem. Behav.* 101 (4) (2012) 564–574.
- [78] K. Alagiakrishnan, et al., Montreal cognitive assessment is superior to standardized mini-mental status exam in detecting mild cognitive impairment in the middle-aged and elderly patients with type 2 diabetes mellitus, *Biomed. Res. Int.* 2013 (2013).
- [79] S. Ahmed, et al., Effect of metformin on adult hippocampal neurogenesis: comparison with donepezil and links to cognition, *J. Mol. Neurosci.* 62 (1) (2017) 88–98.
- [80] H. Lian, et al., Astrocyte-microglia cross talk through complement activation modulates amyloid pathology in mouse models of Alzheimer's disease, *J. Neurosci.* 36 (2) (2016) 577–589.
- [81] B. De Strooper, E. Karran, The cellular phase of Alzheimer's disease, *Cell* 164 (4) (2016) 603–615.
- [82] Z. Ou, et al., Metformin treatment prevents amyloid plaque deposition and memory impairment in APP/PS1 mice, *Brain Behav. Immun.* 69 (2018) 351–363.
- [83] M. Ries, M. Sastre, Mechanisms of A $\beta$  clearance and degradation by glial cells, *Front. Aging Neurosci.* 8 (2016) 160.
- [84] C. Patrono, C. Baigent, Coxibs, traditional NSAIDs, and cardiovascular safety post-precision: what we thought we knew then and what we think we know now, *Clinical Pharmacology & Therapeutics* 102 (2) (2017) 238–245.
- [85] X.-Y. Lu, et al., Metformin ameliorates A $\beta$  pathology by insulin-degrading enzyme in a transgenic mouse model of Alzheimer's disease, *Oxidative Med. Cell. Longev.* 2020 (2020).
- [86] P. Picone, et al., Metformin increases APP expression and processing via oxidative stress, mitochondrial dysfunction and NF- $\kappa$ B activation: use of insulin to attenuate metformin's effect, *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* 1853 (5) (2015) 1046–1059.
- [87] R. Ishii, et al., Decrease in plasma levels of  $\alpha$ -synuclein is evident in patients with Parkinson's disease after elimination of heterophilic antibody interference, *PLoS One* 10 (4) (2015).
- [88] J.M. Savitt, V.L. Dawson, T.M. Dawson, Diagnosis and treatment of Parkinson disease: molecules to medicine, *J. Clin. Invest.* 116 (7) (2006) 1744–1754.
- [89] S. Ghavami, et al., Autophagy and apoptosis dysfunction in neurodegenerative disorders, *Prog. Neurobiol.* 112 (2014) 24–49.
- [90] L.D. Osellame, M.R. Duchen, Defective quality control mechanisms and accumulation of damaged mitochondria link Gaucher and Parkinson diseases, *Autophagy* 9 (10) (2013) 1633–1635.
- [91] M. Lu, et al., Metformin prevents dopaminergic neuron death in MPTP/P-induced mouse model of Parkinson's disease via autophagy and mitochondrial ROS clearance, *Int. J. Neuropsychopharmacol.* 19 (9) (2016) (p. pyw047).
- [92] H. Kang, et al., Activation of the ATF2/CREB-PGC-1 $\alpha$  pathway by metformin leads to dopaminergic neuroprotection, *Oncotarget* 8 (30) (2017) 48603.
- [93] S. Patil, et al., Neuroprotective effect of metformin in MPTP-induced Parkinson's disease in mice, *Neuroscience* 277 (2014) 747–754.
- [94] J. Jankovic, Parkinson's disease: clinical features and diagnosis, *J. Neurol. Neurosurg. Psychiatry* 79 (4) (2008) 368–376.
- [95] K. Tayara, et al., Divergent effects of metformin on an inflammatory model of Parkinson's disease, *Front. Cell. Neurosci.* 12 (2018) 440.
- [96] R.J. Franklin, J.M. Edgar, K.J. Smith, Neuroprotection and repair in multiple sclerosis, *Nat. Rev. Neurol.* 8 (11) (2012) 624–634.
- [97] J. Oh, Y.D. Lee, A.J. Wagers, Stem cell aging: mechanisms, regulators and therapeutic opportunities, *Nat. Med.* 20 (8) (2014) 870.
- [98] F.J. Sim, et al., The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation, *J. Neurosci.* 22 (7) (2002) 2451–2459.
- [99] L. Cantuti-Castelvetri, et al., Defective cholesterol clearance limits remyelination in the aged central nervous system, *Science* 359 (6376) (2018) 684–688.
- [100] S. Shen, et al., Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency, *Nat. Neurosci.* 11 (9) (2008) 1024.
- [101] R.H. Woodruff, et al., Platelet-derived growth factor regulates oligodendrocyte progenitor numbers in adult CNS and their response following CNS demyelination, *Mol. Cell. Neurosci.* 25 (2) (2004) 252–262.
- [102] A. Boyd, H. Zhang, A. Williams, Insufficient OPC migration into demyelinated lesions is a cause of poor remyelination in MS and mouse models, *Acta Neuropathol.* 125 (6) (2013) 841–859.
- [103] B. Neumann, et al., Metformin restores CNS remyelination capacity by rejuvenating aged stem cells, *Cell Stem Cell* 25 (4) (2019) 473–485 (e8).
- [104] A.S. Paintlia, et al., AMP-activated protein kinase signaling protects oligodendrocytes that restore central nervous system functions in an experimental autoimmune encephalomyelitis model, *Am. J. Pathol.* 183 (2) (2013) 526–541.
- [105] A. Gupta, B. Bisht, C.S. Dey, Peripheral insulin-sensitizer drug metformin ameliorates neuronal insulin resistance and Alzheimer's-like changes, *Neuropharmacology* 60 (6) (2011) 910–920.
- [106] F. Chen, et al., Antidiabetic drugs restore abnormal transport of amyloid- $\beta$  across the blood–brain barrier and memory impairment in db/db mice, *Neuropharmacology* 101 (2016) 123–136.
- [107] E. Kickstein, et al., Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling, *Proc. Natl. Acad. Sci.* 107 (50) (2010) 21830–21835.
- [108] M.-C. Chiang, et al., Metformin activation of AMPK-dependent pathways is neuroprotective in human neural stem cells against amyloid-beta-induced mitochondrial dysfunction, *Exp. Cell Res.* 347 (2) (2016) 322–331.
- [109] N. Katila, et al., Metformin lowers  $\alpha$ -synuclein phosphorylation and upregulates neurotrophic factor in the MPTP mouse model of Parkinson's disease, *Neuropharmacology* 125 (2017) 396–407.
- [110] N. Sanadgol, et al., Metformin accelerates myelin recovery and ameliorates behavioral deficits in the animal model of multiple sclerosis via adjustment of AMPK/Nrf2/mTOR signaling and maintenance of endogenous oligodendrogenesis during brain self-repairing period, *Pharmacol. Rep.* 72 (3) (2020) 641–658.



- [111] S.H.H. Largani, et al., Oligoprotective effect of metformin through the AMPK-dependent on restoration of mitochondrial hemostasis in the cuprizone-induced multiple sclerosis model, *J. Mol. Histol.* 50 (3) (2019) 263–271.
- [112] Y. Sun, et al., Metformin ameliorates the development of experimental autoimmune encephalomyelitis by regulating T helper 17 and regulatory T cells in mice, *J. Neuroimmunol.* 292 (2016) 58–67.
- [113] J. Houlton, D. Barwick, A.N. Clarkson, Frontal cortex stroke-induced impairment in spatial working memory on the trial-unique nonmatching-to-location task in mice, *Neurobiology of Learning and Memory* 177 (2021) 107355.
- [114] L. Lin, X. Wang, Z. Yu, Ischemia-reperfusion injury in the brain: mechanisms and potential therapeutic strategies, *Biochemistry & pharmacology: open access* 5 (4) (2016).
- [115] S.E. Lakhani, A. Kirchgeßner, M. Hofer, Inflammatory mechanisms in ischemic stroke: therapeutic approaches, *J. Transl. Med.* 7 (1) (2009) 97.
- [116] Y. Ni, et al., RIPK1 contributes to neuronal and astrocytic cell death in ischemic stroke via activating autophagic-lysosomal pathway, *Neuroscience* 371 (2018) 60–74.
- [117] Y. Wang, et al., White matter injury in ischemic stroke, *Prog. Neurobiol.* 141 (2016) 45–60.
- [118] M. Angels Font, A. Arboix, J. Krupinski, Angiogenesis, neurogenesis and neuroplasticity in ischemic stroke, *Curr. Cardiol. Rev.* 6 (3) (2010) 238–244.
- [119] P.M. George, G.K. Steinberg, Novel stroke therapeutics: unraveling stroke pathophysiology and its impact on clinical treatments, *Neuron* 87 (2) (2015) 297–309.
- [120] Q. Jin, et al., Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke, *Brain Behav. Immun.* 40 (2014) 131–142.
- [121] Y. Liu, et al., Metformin promotes focal angiogenesis and neurogenesis in mice following middle cerebral artery occlusion, *Neurosci. Lett.* 579 (2014) 46–51.
- [122] Y. Hattori, et al., Metformin inhibits cytokine-induced nuclear factor  $\kappa$ B activation via AMP-activated protein kinase activation in vascular endothelial cells, *Hypertension* 47 (6) (2006) 1183–1188.
- [123] M. Zhao, et al., Neuro-protective role of metformin in patients with acute stroke and type 2 diabetes mellitus via AMPK/mammalian target of rapamycin (mTOR) signaling pathway and oxidative stress, *Medical science monitor: international medical journal of experimental and clinical research* 25 (2019) 2186.
- [124] B. Gabryel, S. Liber, Metformin limits apoptosis in primary rat cortical astrocytes subjected to oxygen and glucose deprivation, *Folia Neuropathol.* 56 (4) (2018) 328–336.
- [125] D.G. Hardie, F.A. Ross, S.A. Hawley, AMPK: a nutrient and energy sensor that maintains energy homeostasis, *Nat. Rev. Mol. Cell Biol.* 13 (4) (2012) 251–262.
- [126] J. Li, et al., Neuroprotective effects of adenosine monophosphate-activated protein kinase inhibition and gene deletion in stroke, *Stroke* 38 (11) (2007) 2992–2999.
- [127] D.G. Hardie, B.G. Frenguelli, A neural protection racket: AMPK and the GABAB receptor, *Neuron* 53 (2) (2007) 159–162.
- [128] T. Leech, N. Chattipakorn, S.C. Chattipakorn, The beneficial roles of metformin on the brain with cerebral ischaemia/reperfusion injury, *Pharmacol. Res.* (2019) 104261.
- [129] M. Grissi, et al., FO081 metformin alleviates stroke severity in female mice with chronic kidney disease through AMPK activation and subsequent decrease of microglia/macrophages M1 polarization, *Nephrology Dialysis Transplantation* 34 (Supplement\_1) (2019) (p. gfg096, FO081).
- [130] M.A. Kaisar, et al., Offsetting the impact of smoking and e-cigarette vaping on the cerebrovascular system and stroke injury: is metformin a viable countermeasure? *Redox Biol.* 13 (2017) 353–362.
- [131] D.J. DeGracia, Towards a dynamical network view of brain ischemia and reperfusion. Part I: background and preliminaries, *Journal of experimental stroke & translational medicine* 3 (1) (2010) 59.
- [132] P. Surinkaew, et al., Role of microglia under cardiac and cerebral ischemia/reperfusion (I/R) injury, *Metab. Brain Dis.* 33 (4) (2018) 1019–1030.
- [133] J. Zeng, et al., Metformin protects against oxidative stress injury induced by ischemia/reperfusion via regulation of the lncRNA-H19/miR-148a-3p/Rock2 Axis, *Oxidative Med. Cell. Longev.* 2019 (2019).
- [134] X. Hu, et al., Microglial and macrophage polarization—new prospects for brain repair, *Nat. Rev. Neurol.* 11 (1) (2015) 56.
- [135] M.A. Moskowitz, E.H. Lo, C. Iadecola, The science of stroke: mechanisms in search of treatments, *Neuron* 67 (2) (2010) 181–198.
- [136] M. Fang, et al., Metformin treatment after the hypoxia-ischemia attenuates brain injury in newborn rats, *Oncotarget* 8 (43) (2017) 75308.
- [137] R. Yuan, et al., Metformin reduces neuronal damage and promotes neuroblast proliferation and differentiation in a cerebral ischemia/reperfusion rat model, *NeuroReport* 30 (3) (2019) 232–240.
- [138] L. Huang, et al., Glial scar formation occurs in the human brain after ischemic stroke, *Int. J. Med. Sci.* 11 (4) (2014) 344.
- [139] M. Pekny, U. Wilhelmsson, M. Pekna, The dual role of astrocyte activation and reactive gliosis, *Neurosci. Lett.* 565 (2014) 30–38.
- [140] V.R. Venna, et al., Chronic metformin treatment improves post-stroke angiogenesis and recovery after experimental stroke, *Eur. J. Neurosci.* 39 (12) (2014) 2129–2138.
- [141] R. Klocke, et al., Granulocyte colony-stimulating factor (G-CSF) for cardio- and cerebrovascular regenerative applications, *Curr. Med. Chem.* 15 (10) (2008) 968–977.
- [142] X.-H. Ge, et al., Metformin protects the brain against ischemia/reperfusion injury through PI3K/Akt1/JNK3 signaling pathways in rats, *Physiol. Behav.* 170 (2017) 115–123.
- [143] M. Abdelsaid, et al., Metformin treatment in the period after stroke prevents nitrate stress and restores angiogenic signaling in the brain in diabetes, *Diabetes* 64 (5) (2015) 1804–1817.
- [144] D. Bałczewska, P. Ptaszyński, I. Cygankiewicz, Baroreflex sensitivity: measurement and clinical aspects, *Przegląd lekarski* 72 (11) (2015) 682–689.
- [145] B. Lu, et al.,  $\alpha$ 7 nicotinic acetylcholine receptor signaling inhibits inflammasome activation by preventing mitochondrial DNA release, *Mol. Med.* 20 (1) (2014) 350–358.
- [146] E. Meroni, et al., Intestinal macrophages and their interaction with the enteric nervous system in health and inflammatory bowel disease, *Acta Physiol.* 225 (3) (2019) e13163.
- [147] X.-C. Zhu, et al., Chronic metformin preconditioning provides neuroprotection via suppression of NF- $\kappa$ B-mediated inflammatory pathway in rats with permanent cerebral ischemia, *Mol. Neurobiol.* 52 (1) (2015) 375–385.
- [148] D.-J. Li, et al., Dysfunction of the cholinergic anti-inflammatory pathway mediates organ damage in hypertension, *Hypertension* 57 (2) (2011) 298–307.
- [149] J.S. Petersen, G.F. DiBona, Effects of central metformin administration on responses to air-jet stress and on arterial baroreflex function in spontaneously hypertensive rats, *J. Hypertens.* 15 (3) (1997) 285–291.
- [150] T. Deng, et al., Pre-stroke metformin treatment is neuroprotective involving AMPK reduction, *Neurochem. Res.* 41 (10) (2016) 2719–2727.
- [151] M. Karimipour, et al., Pre-treatment with metformin in comparison with post-treatment reduces cerebral ischemia reperfusion induced injuries in rats, *Bulletin of Emergency & Trauma* 6 (2) (2018) 115.
- [152] P.-F. Wang, et al., Polyinosinic-Polycytidylic Acid Has Therapeutic Effects against Cerebral Ischemia/Reperfusion Injury through the Downregulation of TLR4 Signaling Via TLR3 vol. 192(10), 2014, pp. 4783–4794.
- [153] J. Li, et al., Effects of metformin in experimental stroke, *Stroke* 41 (11) (2010) 2645–2652.
- [154] Y. Farbood, et al., Targeting adenosine monophosphate-activated protein kinase by metformin adjusts post-ischemic hyperemia and extracellular neuronal discharge in transient global cerebral ischemia, *Microcirculation* 22 (7) (2015) 534–541.
- [155] A. Sarkaki, et al., Metformin improves anxiety-like behaviors through AMPK-dependent regulation of autophagy following transient forebrain ischemia, *Metab. Brain Dis.* 30 (5) (2015) 1139–1150.
- [156] J.-M. Guo, et al., Involvement of arterial baroreflex and nicotinic acetylcholine receptor  $\alpha$ 7 subunit pathway in the protection of metformin against stroke in stroke-prone spontaneously hypertensive rats, *Eur. J. Pharmacol.* 798 (2017) 1–8.
- [157] N. Ghadermezhad, et al., Metformin pretreatment enhanced learning and memory in cerebral forebrain ischaemia: the role of the AMPK/BDNF/P70S6 signalling pathway, *Pharm. Biol.* 54 (10) (2016) 2211–2219.
- [158] G. Ashabi, et al., Activation of AMP-activated protein kinase by metformin protects against global cerebral ischemia in male rats: interference of AMPK/PGC-1 $\alpha$  pathway, *Metab. Brain Dis.* 29 (1) (2014) 47–58.
- [159] T. Jiang, et al., Acute metformin preconditioning confers neuroprotection against focal cerebral ischaemia by pre-activation of AMPK-dependent autophagy, *Br. J. Pharmacol.* 171 (13) (2014) 3146–3157.
- [160] S. Harada, W. Fujita-Hamabe, S. Tokuyama, The importance of regulation of blood glucose levels through activation of peripheral 5'-AMP-activated protein kinase on ischemic neuronal damage, *Brain Res.* 1351 (2010) 254–263.
- [161] R. Prakash, et al., Vascularization pattern after ischemic stroke is different in control versus diabetic rats: relevance to stroke recovery, *Stroke* 44 (10) (2013) 2875–2882.
- [162] M.M. Elgebaly, et al., Vascular protection in diabetic stroke: role of matrix metalloproteinase-dependent vascular remodeling, *J. Cereb. Blood Flow Metab.* 30 (12) (2010) 1928–1938.
- [163] H.H. Kyu, et al., Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the global burden of disease study 2013, *bmj* 354 (2016) (p. i3857).
- [164] A.E. Sifat, et al., Nicotine and electronic cigarette (E-Cig) exposure decreases brain glucose utilization in ischemic stroke, *J. Neurochem.* 147 (2) (2018) 204–221.
- [165] A.E. Sifat, et al., Neurovascular unit transport responses to ischemia and common coexisting conditions: smoking and diabetes, *Am. J. Phys. Cell Phys.* 316 (1) (2019) C2–C15.
- [166] W. Huynh, et al., The effect of diabetes on cortical function in stroke: implications for poststroke plasticity, *Diabetes* 66 (6) (2017) 1661–1670.
- [167] A. Ergul, et al., Increased hemorrhagic transformation and altered infarct size and localization after experimental stroke in a rat model type 2 diabetes, *BMC Neurol.* 7 (1) (2007) 33.
- [168] C. Willi, et al., Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis, *Jama* 298 (22) (2007) 2654–2664.
- [169] J.S. Gill, et al., Cigarette smoking: a risk factor for hemorrhagic and nonhemorrhagic stroke, *Arch. Intern. Med.* 149 (9) (1989) 2053–2057.
- [170] P. Naik, et al., Oxidative and pro-inflammatory impact of regular and denicotinized cigarettes on blood brain barrier endothelial cells: is smoking reduced or nicotine-free products really safe? *BMC Neurosci.* 15 (1) (2014) 51.
- [171] S. Das, et al., Oxidative stress in the brain of nicotine-induced toxicity: protective role of *Andropogon paniculata* Nees and vitamin E, *Appl. Physiol. Nutr. Metab.* 34 (2) (2009) 124–135.



- [172] J.R. Paulson, et al., Nicotine exacerbates brain edema during in vitro and in vivo focal ischemic conditions, *J. Pharmacol. Exp. Ther.* 332 (2) (2010) 371–379.
- [173] F. Giacco, M. Brownlee, Oxidative stress and diabetic complications, *Circ. Res.* 107 (9) (2010) 1058–1070.
- [174] K.K. Shah, P.R. Boreddy, T.J. Abbruscato, Nicotine pre-exposure reduces stroke-induced glucose transporter-1 activity at the blood–brain barrier in mice, *Fluids and Barriers of the CNS* 12 (1) (2015) 1–9.
- [175] S. Prasad, et al., Role of Nrf2 and protective effects of metformin against tobacco smoke-induced cerebrovascular toxicity, *Redox Biol.* 12 (2017) 58–69.
- [176] K.W. Bold, et al., Reasons for trying e-cigarettes and risk of continued use, *Pediatrics* 138 (3) (2016) (p. e20160895).
- [177] Health, U.D.o, H. Services, E-Cigarette Use among Youth and Young Adults. A Report of the Surgeon General. Atlanta, GA, 2016.
- [178] J.M. Adams, Smoking cessation—progress, barriers, and new opportunities: the surgeon general’s report on smoking cessation, *Jama* 323 (24) (2020) 2470–2471.
- [179] A.E. Sifat, et al., Prenatal electronic cigarette exposure decreases brain glucose utilization and worsens outcome in offspring hypoxic–ischemic brain injury, *J. Neurochem.* 153 (1) (2020) 63–79.