

Review Article

A comprehensive approach to investigate the contradictory effects of metformin therapy in cerebral ischemic injury

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Abstract

Ischemic brain injury involves a complex sequence of excitotoxic and oxidative events. Metformin is proposed as one of the potential candidates for returning the body to its basic homeostasis in ischemic situations. Metformin can either protect or damage cells by activating AMP-activated protein kinase (AMPK) and its downstream factors; so, it has a dual role in the cerebral ischemia context, but more investigations are needed to define its exact underlying mechanism. Herein, we classify the controversial results of metformin therapy in the experimental models of brain ischemia; central and peripheral injection of metformin, chronic and acute treatment, pre- and post-treatment with metformin, tissue-specific role of metformin, dose-specific effect of metformin, age-dependent aspects of metformin therapy. Categorizing different types of cerebral ischemia is important in investigating the dual role of metformin. Due to the variations in metformin therapy, it can be used for chronic treatment, but the patients must be informed about its harmful effects. Although the mechanisms in which AMPK protects/degenerates neurons against ischemic stress situation are still unknown.

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Introduction

Worldwide, stroke is responsible for more than 4.4 million deaths (Modi et al., 2006). As life expectancy increases, the incidence of stroke continues to rise, while improvements in medical care increases survival rate (Towfighi and Saver, 2011). There are different types of ischemia with/involving different cellular processes that have been introduced in the clinical

studies (Brathwaite and Macdonald, 2013; Ostergaard et al., 2013), so understanding how to treat subjects by pharmacological or herbal agents would be useful (Liu et al., 2013; Wei et al., 2013; Yenari and Han, 2013). Focal ischemia which is also called brain stroke and global ischemia happens in some certain disorders/diseases such as cardiac arrest, coronary artery bypass surgery and cardio-respiratory injury (Dirnagl et al., 1999; Moler et al., 2009). There are at least 3 models of global cerebral ischemia: 2-vessel occlusion (2-VO), 4-VO and 8-VO. In the 2-VO model,

the common carotid arteries are occluded, in 4-VO both vertebral and common carotid arteries are clamped. The level of cerebral blood flow is reduced to 15% (Furlow, 1982; He et al., 2005). The 8-VO model is more severe /serious than others in which vertebral and ala foramen and the common carotid and external carotid arteries are occluded and cerebral blood flow is diminished to 7% (Wellons et al., 2000; He et al., 2002). Ischemic brain injury involves a complex sequence of excitotoxic and oxidative events, including: cellular energy depletion, disrupted protein synthesis, membrane depolarization, acidosis, free radical production, DNA damage, apoptosis, and necrosis (Snider et al., 1999; Love, 2003). There are so many compensatory processes that are activated to repair the damaged parts and restore normal cell functions in ischemia (Szabo and Dawson, 1998; Ha and Snyder, 2000). However, all of these processes are energy-consuming and deplete Adenosine three phosphates (ATP) storages; so, over-activation of these pathways might be deleterious to cells (Chan, 2001; Du et al., 2003; Ying et al., 2003). Therefore, investigators focus on the novel therapeutic drugs against the toxicity of ischemia.

Even though many research efforts focus on the neuronal damage after stroke; it is noteworthy that vascular disorders have also an important role both in the occurrence and the recovery from ischemic brain injury (Li et al., 2009). Using agents like statins, and antiplatelets in several studies is reported to be useful for primary and secondary prevention of stroke, better functional outcomes, reduction in the severity of stroke and mortality after stroke (Sanossian et al., 2006; Athyros et al., 2008; Ni Chroinin et al., 2013; Tziomalos et al., 2013). Up to now, many studies have investigated the effects of different antidiabetic agents like sulfonylurease and metformin on the ischemia-induced neuronal cell injuries.

Metformin (Figure. 1) is the first line treatment for

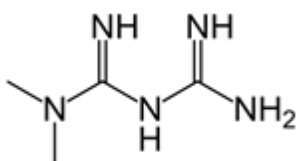


Fig. 1. Chemical structure of N-1,1-dimethylbiguanide (Metformin).

hyperglycemia which improves insulin sensitivity in diabetic patients (Nathan et al., 2009). Metformin is associated with fewer hypoglycemic attacks than insulin and sulphonylureas, indicating that using metformin instead of insulin had better results (Holman et al., 2008). Irregularity in blood glucose levels and intolerance to glucose are some post-stroke symptoms. It is proposed that metformin is one of the potential candidates for restoring basic homeostatic functions. The beneficial effects of metformin on blood glucose levels appears to be a result of complex multi-factorial mechanisms: i) metformin activates AMP-activated protein kinase (AMPK) and decreases hepatic gluconeogenesis consequently (He et al., 2009); ii) it increases the uptake of glucose by skeletal muscles and white adipocytes (Bailey and Turner, 1996); and iii) metformin improves metabolic activity profile along with additional weight reduction. (Rojas et al., 2011). Interestingly, metformin can increase cell survival cells by activating AMPK and its downstream factors. For example, metformin induces neurogenesis, mitochondrial biogenesis, antioxidant pathways, autophagy, and increases angiogenesis in various cells especially in brain (Kuramoto et al., 2007; Kristensen et al., 2013; Takata et al., 2013; Vytla and Ochs, 2013; Ashabi et al., 2014; Jiang et al., 2014; Venna et al., 2014). In several stressful situations, when cellular ATP levels falls down, the AMPK is phosphorylated and activates its related proteins (Mukherjee et al., 2008). Under the ischemic conditions, Thr172 in the catalytic subunit of AMPK protein is phosphorylated, then the phosphorylated AMPK increases glucose uptake and enhances cellular energy for better metabolism (Atherton et al., 2005; Horman et al., 2006).

While the beneficial role of metformin is controversial, some researchers have indicated that inducing AMPK phosphorylation by metformin disrupts cell survival and increases cell death. They all agree that during ischemia the level of AMPK rises up and leads to apoptosis and necrosis after ischemia. The role of metformin and other AMPK activators in bone metabolism, with increased incidence of fractures in women, and increased incidence of myocardial infarction and cardiovascular-related death, confirms the negative impacts of metformin (Smiley and Umpierrez, 2007). So, here is the question: why

metformin has a dual effect in the cerebral ischemia. Our studies reported that metformin pretreatment enhances cell viability, decreases apoptosis and enhances cellular protection, which involves mitochondrial biogenesis, and antioxidant pathways (El Messaoudi et al., 2011; Fujita-Hamabe et al., 2011; Brathwaite and Macdonald, 2013). The protective/destructive effects of metformin are influenced by variouselements. For example ,the phase in which the treatment is started, the method of administrating, dosage of treatment and types of ischemia could all play a role in the impact of metformin in the cerebral ischemia (El Messaoudi et al., 2011; Fujita-Hamabe et al., 2011; Brathwaite and Macdonald, 2013; Farbood *et al.*, 2015b). In this review, we classify the controversial results of metformin therapy in brain ischemia experimental models.

Central and Peripheral administration of metformin have different effects on neurons

Central (intracerebroventricular or i.c.v) and peripheral (intraperitoneal or i.p) administration of metformin might have dual effects on cerebral ischemia. Peripheral activation of AMPK after ischemic stress might suppress ischemic neuronal damage by inhibiting post-ischemic glucose intolerance. On the other hand, during metabolically stressful situations like cerebral ischemia, AMPK expression in brain (central AMPK) is reported to be initiated by ATP depletion accompanied by an increased AMP/ATP ratio (Ronnett et al., 2009). Furthermore, central AMPK activation by metformin injection after ischemic stress is known to induce neuronal damage (Li et al., 2007). The exact mechanisms of these two administrating types are not clear. Evidence indicates that central administration of metformin increases blood glucose after ischemia while systemic metformin injection decreases blood glucose level (Harada et al., 2010). Hyperglycemia is one of the plausible reasons for the amplification of ischemia toxicity in the brain (Dietrich et al., 1993). It is suggested that systemic administration of metformin increases AMPK phosphorylation and AMPK initiates some downstream pathways that protect cells against

ischemic conditions (Ashabi et al., 2014). Central activation of AMPK by i.c.v. administration of metformin enhances cell death via unknown mechanisms (Harada et al., 2010). Also, there are no reports to suggest i.c.v. injection of metformin could protect cells against stroke. Such variations between central and peripheral administration of AMPK may partially depend on the ability of cells to utilize metformin.

Types of metformin administration affect its mechanism

Unlike the proposal that metformin exerts its effect mainly through activation of AMPK in systemic administration (Takata et al., 2013), there are some AMPK independent pathways. For example, metformin increases tyrosine phosphorylation in the insulin receptors and enhances cell viability in oxygen-glucose deprived cells (Mielke et al., 2006). Neurons cannot use metformin to increase glucose transporters (GLUTs) expression. That is one point where its mechanism of function differs from how it behaves in peripheral tissues.

Metformin has a dual effect as pre and/or post –treatment

Post ischemic glucose intolerance

It seems that both pre and post-treatment with metformin have neuroprotective effects. But there are limited data as to whether prior treatment with antidiabetic agents is beneficial in diabetic patients who suffer from stroke. Metformin penetrates Blood-brain-barrier (BBB) rapidly and acts immediately after injection (Labuzek et al., 2010; Yeung et al., 2011). It has been shown that after ischemic stress insulin sensitivity is impaired which results in hyperglycemia. collectively called “post ischemic glucose intolerance” events , they may aggravate/cause neuronal damage and develop/increase the risk of diabetes incidence (Gray et al., 2004; Kernan et al., 2005; Vancheri et al., 2005). If we can find a way to inhibit this ischemic-induced hyperglycemia with drugs, neuronal damage could be suppressed (Harada et al., 2009). With this in mind, it has been reported that post-ischemia treatment

with metformin has attenuated ischemic injury (Harada et al., 2010; Harada et al., 2011). Post ischemic administration of metformin increases angiogenesis and enhances vascular endothelial growth factor expression in neurons (Liu et al., 2014b; Venna et al., 2014). Abdelsaid and colleagues in a 2015 paper declared/claimed that post-treatment using metformin reduced infarct size and oxidative stress in ischemic diabetic rats (Abdelsaid et al., 2015). Prakash et al., 2013 findings were in agreement with Abdelsaid findings that metformin post-treatment reduced vascular injury in diabetic rats (Prakash et al., 2013). They claimed that this protective mechanism of metformin post-treatment action is independent of its hypoglycemic effect. In fact, post-treatment of metformin in ischemic diabetic rats increased antioxidative capacity and hence defended/protected the neurons (Rosen and Wiernsperger, 2006; Faure et al., 2008).

Post-treatment and lactic acidosis

Our results suggested that post treatment of metformin leads to lactic acidosis (Yeung et al., 2011). Metformin therapy declines/decreases the pyruvate dehydrogenase activity and thus enhances anaerobic metabolism. Shifting to an anaerobic metabolism increases the production of tricarboxylic acid cycle precursors (McGuinness and Talbert, 1993). Inhibition of pyruvate dehydrogenase leads to a reduced ability to channel /transfer these precursors into the aerobic metabolism which then causes an upgrade in the metabolism/conversion of pyruvate to lactate and subsequently increases lactic acid level (McGuinness and Talbert, 1993; Price, 2003).

Pre- or post-treatment with metformin and AMPK activation

Pre-treatment with the drug before any oxidative stress initiates the signaling pathways and gives the knowledge /information about its cellular mechanism. Metformin needs time to elevate AMPK level, and then it activates different pathways such as mitochondrial biogenesis, Mammalian Target of Rapamycin and GLUT expression. Hence, chronic pretreatment with metformin may be helpful in patients who are high risk

for stroke (Ashabi et al., 2014). Our studies demonstrated that metformin pretreatment increases mitochondrial biogenesis, antioxidant enzymes, Nrf2 pathway and reduces inflammation in the brains of ischemic rats (Ashabi et al., 2014; Ashabi et al., 2015; Sarkaki et al., 2015). Additionally, we evaluated some electrophysiological studies which indicated that metformin could enhance neuronal extracellular discharge in ischemic rats (Farbood et al., 2015b). Our data is in agreement with other published papers that either pre- or post-treatment with metformin can decrease the level of inflammation in ischemic neurons and consequently, Pre-administration of metformin may increase BBB recovery following brain stroke (Ashabi et al., 2014; Liu et al., 2014a; Liu et al., 2014b; Farbood et al., 2015a). Altogether, these studies indicate that both pre/post treatment with metformin protect cells against ischemic injury. Metformin pre-treatment in experimental models initiates AMPK related neuroprotective pathways earlier than ischemic insult (Li et al., 2010) but post-treatment protocols ameliorates/modulates glucose regulation in brain and then protects cells (Ferrannini and DeFronzo, 2015). However, the precise mechanisms underlying protection in post-treatment is still unknown. Future studies are needed to compare the effects of pre- and post-treatment of metformin in ischemic models to indicate which one would be more protective.

Chronic treatment versus acute treatment by metformin in cerebral ischemia

In clinical studies, it has been revealed that chronic metformin treatment can decrease the risk of stroke and attenuate cardiac disease (Petticrew, 1998). Besides, Li et al., 2010 demonstrated that acute treatment with metformin is neurotoxic/has neurotoxic effects and that chronic pretreatment metformin has neuroprotective effects in focal ischemia experimental models (Li et al., 2010). These results show that chronic metformin treatment is like a preconditioning situation. Li et al. have suggested that chronic metformin pretreatment decreases AMPK phosphorylation which is responsible for stroke injury. On the other hand, acute metformin treatment induces

AMPK. Elevated level of AMPK increases detrimental/destructive pathways such as autophagy, glucose production and accumulation, increased lactate and nitric oxide (NO) level which exacerbated the injury (Zou et al., 2003; Galardo et al., 2007; Hou et al., 2008; Li and McCullough, 2009; Jiang et al., 2014). In a recent study Jiang et al have indicated that acute metformin pretreatment increase autophagy and protects neurons against cerebral focal ischemia (Jiang et al., 2014). Also acute metformin treatment increased neurovascular injury in diabetic rats which were ischemic and similarly it has a deteriorating effect in healthy rats which indicates the same mechanism of acute metformin therapy applies and stressful conditions (Li et al., 2010; Li et al., 2013). Chronic metformin administration has lasting effect sin both diabetic and normal rats under ischemia (Elgebaly et al., 2010). Our studies revealed that metformin can increase autophagy in the rats model of cerebral global ischemia (Sarkaki et al., 2015). Some studies suggest that the detrimental effects of acute metformin therapy are mediated by activation of neuronal NO synthase (nNOS) (Turnley et al., 1999; Li et al., 2010). In contrast, Harada et al., 2010 have shown that either acute or chronic metformin treatment decreases infract size and regulates blood glucose level after stroke (Harada et al., 2010). In this study, enhanced level of AMPK in the cortex protects neurons against ischemic injury (Harada et al., 2010). Our studies indicated that chronic metformin treatment protects neurons and attenuates cell death via activation of AMPK (Ashabi et al., 2014). In this regard, our results point out that AMPK increases mitochondrial biogenesis (Ashabi et al., 2014) and enhances Nrf2 antioxidant pathway in cerebral ischemia model (Ashabi et al., 2015). It seems that metformin treatment induces AMPK activation and subsequently AMPK initiates protective pathways. In chronic treatment and in some other cases, the level of AMPK increases immediately, but in the long term it decreases. The protective pathways are triggered by AMPK in the neurons, but acute metformin treatment may be toxic because the animals or patients are not habituated to this condition and the enhancement in the level of AMPK can initiate detrimental/harmful effects. Refer to Table 1 (Pre- or post-treatment and chronic or acute administration was classified in the Table. 1).

Metformin's tissue-specific behavior is an important factor in therapeutic approaches

Obviously, metformin behaves differently in each special organ under ischemic conditions (Cheung et al., 2000; Kim and Tian, 2011; Seo-Mayer et al., 2011; Soraya et al., 2012). These effects of metformin are observable in the neuronal context and especially in brain ischemia. Each part of the brain shows a different response to metformin therapy (Harada et al., 2010; Ashabi et al., 2014). The role of metformin in brain is based on activation/inhibition of AMPK. The effect of AMPK activation is cell-type specific. For example i.p. administration of metformin increases AMPK in cortical cells while it has no significant effect in other regions such as hippocampus and striatum in the ischemic experimental models (Harada et al., 2010). It is suggested that according to the cell types and various stressful conditions, at first a single region of brain is damaged because of ischemia and then AMPK increases and is distributed to the other regions of brain (McCullough et al., 2005). In global cerebral ischemia, mostly hippocampus is under stress (Ashabi et al., 2014)Therefore undergoes cell death, but in focal cerebral ischemia the striatum and cortex are mostly damaged (Li et al., 2010). During metabolic stress situations, the level of AMPK rises but its protective/degenerative role is under investigation. We can hypothesis that the effect of metformin and activation of AMPK in the brain is related to the type of ischemia, but its exact downstream pathways are unclear.

Response to metformin depend on age-related changes

Age is the most important independent risk factor for stroke (Rosamond et al., 2008; Slemmer et al., 2008). The aged brain undergoes numerous neurochemical and physiological changes (Dorce and Palermo-Neto, 1994; Anyanwu, 2007). Unfortunately, most experimental stroke studies are performed on young animals, and therefore, they may not fully replicate the effects of ischemia on neural tissue in aged subjects. Aged male mice have less histological damages after focal ischemia despite increased functional deficits and

Table 1: Classification of published articles in role of metformin in the cerebral ischemia.

Kinds of metformin treatment	Authors	Kind of Stress or contexts	Protective?	Focal or global ischemia	Pre or post treatment	Central or peripheral administration
Acute treatment	Petticrew, 1998	Diabetes	Yes	Cardiac arrest (global)	Pre	Peripheral
	Li et al., 2010	Normal	No	Focal ischemia	Pre	Peripheral
	Jiang et al., 2014	Normal	Yes	Focal ischemia	Pre	Peripheral
	Li et al., 2013	Normal	No	Focal ischemia		Peripheral
	Harada et al., 2010	Normal	Yes	Focal ischemia	Post	Peripheral and central
Chronic treatment	Li et al., 2010	Normal	Yes	Focal ischemia	Pre	Peripheral
	Ashabi et al., 2014	Normal	Yes	Global ischemia	Pre	Peripheral
	Elgebaly et al., 2010	Normal and diabetes	Yes	Focal ischemia	Pre	Peripheral
	Liu et al., 2014a and 2014b	Normal	Yes	Focal ischemia	Pre	Peripheral
	Abdelsaid et al., 2015	Diabetes	Yes	Focal ischemia	Post	Peripheral
	Prakash et al., 2013	Diabetes	Yes	Focal ischemia	Post	Peripheral
	Venna et al., 2014	Normal	Yes	Focal ischemia	Post	Peripheral

mortality, the underlying mechanism of which is still mysterious (Liu et al., 2009). Recent studies demonstrated that aging is accompanied by an increase in AMP/ATP ratio in multiple tissues (Hardie and Hawley, 2001; Petersen et al., 2003; Wang et al., 2003), yet AMPK levels are surprisingly lower in aged muscle (Qiang et al., 2007; Reznick et al., 2007). Hypoxia is unable to induce AMPK activity in aged hepatocytes, suggesting that AMPK signaling is decreased by age (Mulligan et al., 2005) and may contribute to the inability of aged animals to mount an effective response in stressful conditions. Liu et al 2011 demonstrated that aging leads to a baseline up-regulation of phosphorylated AMPK expression in the brain, but similar to peripheral tissues, AMPK signaling becomes less responsive to injury (Liu et al., 2011). Therefore, the age of animals or patients should be considered for planning a research project.

High dosage of metformin can be neurotoxic

it has been revealed that higher doses of metformin are neurotoxic in ischemia. Our study indicated that high doses of metformin may increase cell death in the ischemic cells; the dose of 400mg/kg has no protective effect. The dose of 400mg/kg metformin increases cell death compared to the control animals (Ashabi et al., 2014). Researchers have suggested that one reason could be due to overstimulation of AMPK mediated energy-producing cascades such as glycolysis, GLUT, and autophagy; all of which could further cause much stress to the ischemic brain and exacerbate the metabolic failure (Wang et al., 2003; Tuerk et al., 2007; Du et al., 2009; Li and McCullough, 2009; Wang and Guan, 2009). Further studies are needed to determine the exact dosage of metformin for an appropriate clinical therapy in stroke.

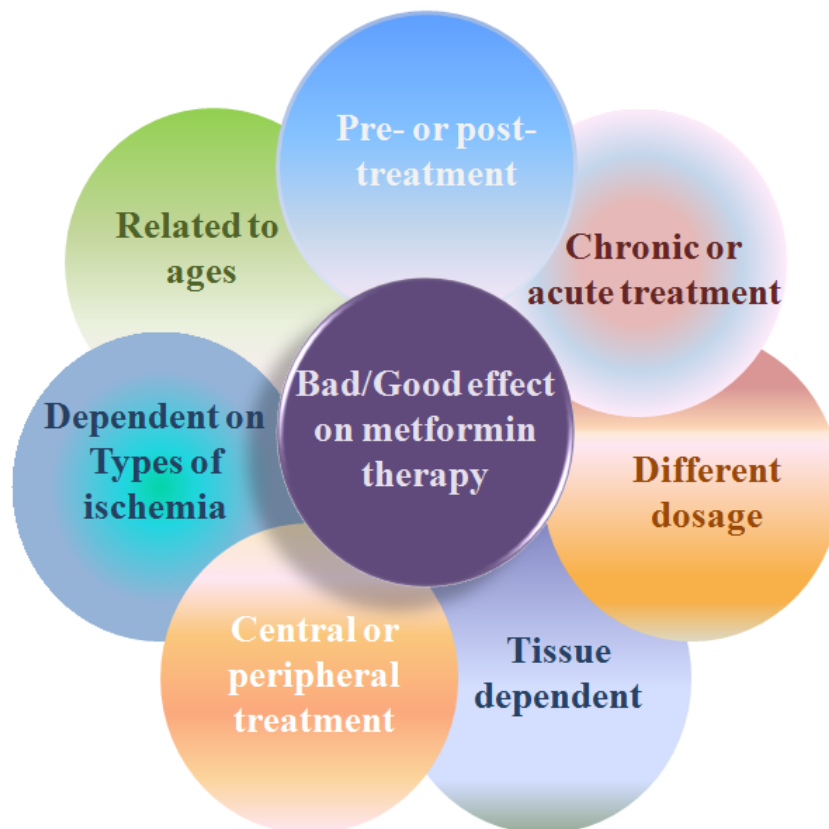


Fig. 2. A diagram of most important factors involved in metformin therapy in cerebral ischemia.

Focal and global brain ischemia are differently affected by metformin therapy

In addition to the above issues, types of brain ischemia is another factor which should be considered in metformin therapy. Global cerebral ischemia (4VO and 2VO) and focal brain ischemia are important factors to detect the exact role of metformin therapy in the experimental models. In each type of ischemia AMPK acts differently. For example, researchers have declared that AMPK inhibition has neuroprotective effect in focal cerebral ischemia (McCullough et al., 2005; Li et al., 2007) while AMPK inhibition in global cerebral ischemia damaged neurons in the brain (Li and McCullough, 2010; Ashabi et al., 2015). Since, AMPK is an energy sensor kinase and its activation or over activation depends on the type of metabolic stress (Seo-Mayer et al., 2011). In our recent study, we claim that cerebral global ischemia decreased neuron firing rates and inhibited BBB disruption in the brain. In contrast, pretreatment by metformin elevated the neuronal excitability in ischemic rats (Farbood et al.,

2015b). In focal ischemia models blood flow reduction happens only in the striatum and frontal cortex region. However, there are dissimilarities in different kinds of global ischemia models (2VO, 4VO and 8VO). 2VO ischemia model has more blood flow in the brain compared to 4VO (Furlow, 1982; Pomfy and Franko, 1999; Wellons et al., 2000; Sanderson and Wider, 2013). So, blood perfusion level is one of the main factors in the phosphorylation of AMPK and the activation of its downstream pathways.

Conclusion

Metformin is one of the therapeutic drugs used for diabetes type II. Among the antidiabetic agents metformin has lesser/milder side effects, it can also control blood glucose, reduce stroke and cardiovascular mortality by 26% compared with other antidiabetic drugs (Petticrew, 1998)., it is also used to remedy some other disorders such as decreasing plasma free fatty acids and low-density lipoproteins (Smiley and Umpierrez, 2007) and the diminished glucose tolerance in the cells (den Hertog et al.,

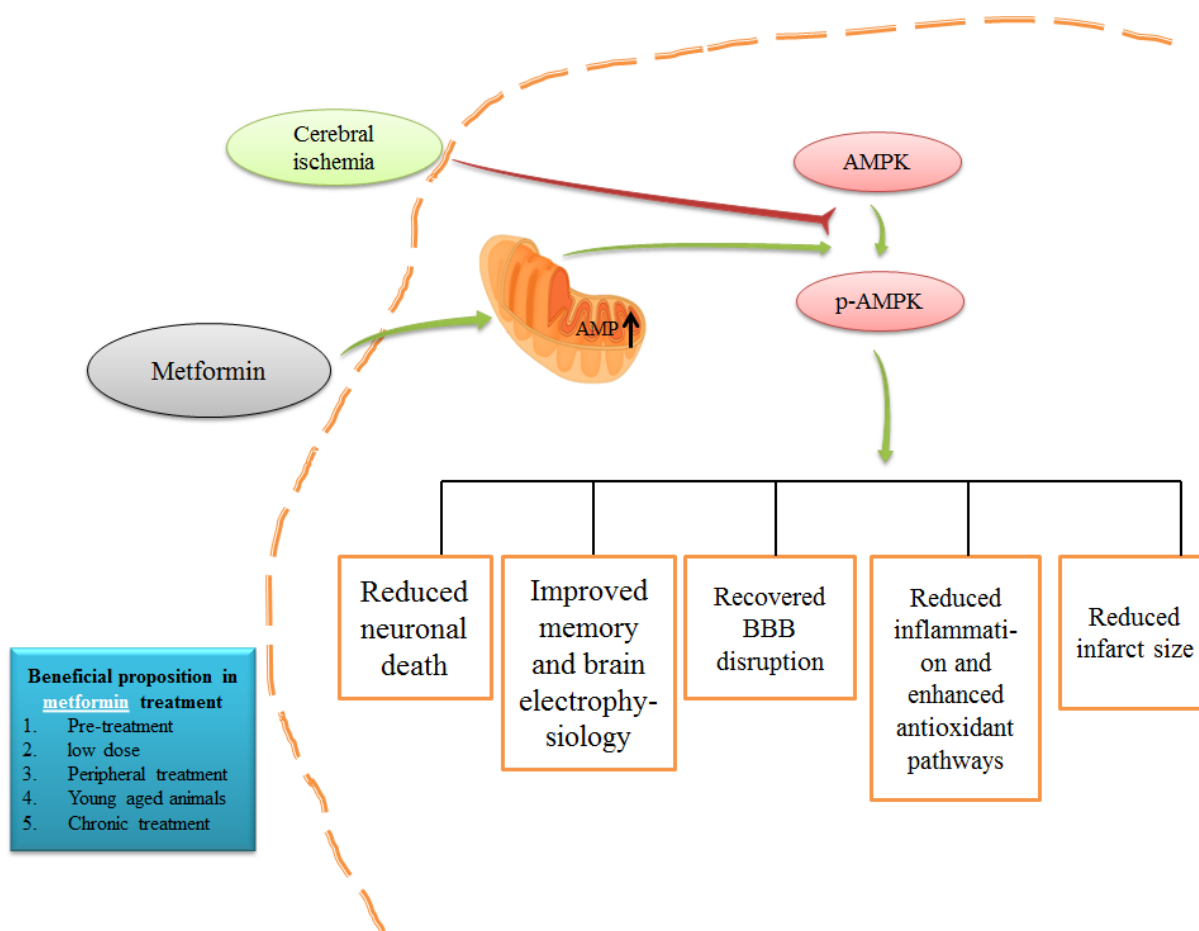


Fig. 3. Schematic diagram of metformin treatment in five selected condition which proposed to had beneficial effect against global cerebral ischemia model. Metformin treatment in each 5 condition causes AMP enhancement in the mitochondria and subsequently leads to phosphorylated AMPK (p-AMPK) elevation (green arrow). Global cerebral ischemia inhibited the AMPK phosphorylation in the present of metformin (red arrow). According to our results and previous reports it has been claimed that increased level of p-AMPK increased cell viability, reduced cell death, enhanced memory and brain electrophysiology, reduced BBB disruption, increased antioxidant level and inhibited neuro-inflammation and decreased infarct size in the brain.

2013). It is suggested that metformin can be used for chronic treatment, but patients must be informed about its harmful features (Smiley and Umpierrez, 2007; Ronnett and Aja, 2008). Pretreatment by metformin can be more effective than its post-treatment, but there are potential problems in treating normal population and post-treatment is more preferred (Figure. 2). In Addition, we suggest using metformin inpatient at high risk for developing ischemia, such as diabetic people or patients suffering from cardiovascular disorders. In figure. 2 all possible states of metformin therapy is categorized/illustrated (Figure. 2) and figure. 3 shows the schematic diagram which declared most conceivable /common conditions in metformin therapy (Figure. 3). AMPK activation by metformin has protective \properties in the neuronal context, so we

illustrated the possible situations in which AMPK is activated and classified them (Figure. 3). Taken Together, this condition better illustrates the protective role of metformin: chronic pretreatment by metformin in young animals (with low doses) might diminish the injuries in the hippocampal tissue. Also, we suggested that peripheral administration of metformin is more useful than its central injection. We claimed that metformin therapy has more beneficial effects in the young people and chronic metformin pretreatment is more recommended in the patients who have a/are at high risk for cerebral stroke. Although future studies are required to clearly identify the mechanisms in which metformin protects/degenerates neurons against ischemic stress conditions. Herein, a major issue that needs much clarification is exploring the difference

between the effect of pre- and post-treatment metformin in ischemic models in order to help us indicating which would be more protective. Other subjects that would be considered in potential projects could determine the optimum dosage for metformin therapy, its tissue specific actions (mechanism of action), and planning the best protocol considering the age and types of ischemia.

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Conflict of interest

All authors declared that there is no conflict of interest.

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