Chapter 1

Introduction

1 Introduction

Stroke is a cerebrovascular disease caused by the sudden interruption of the blood flow to the brain that persists beyond 24 hours or until death (Aho et al., 1980). Stroke is an emerging cause of death and mortality around the globe. Stroke may be ischemic or hemorrhagic. Ischemic stroke is caused due to the blockage of blood vessels that supply blood to the brain, resulting in the deprivation of oxygen and nutrients to the brain area (Powers et al., 2019). Ischemic stroke is classified as either global or focal, depending on which part of the brain is affected. The blood flow to the entire brain was severely diminished in a global ischemic stroke. Due to blockages in the arteries that carry blood to the brain, the blood supply to a particular area of the brain is blocked, resulting in focal ischemic stroke. The stroke symptoms include facial drooping, arm weakness, and speech difficulties (Hui, Tadi, & Patti, 2018).

1.1 Epidemiology

Stroke is the second leading cause of death globally. It affects 13.7 million people and kills around 5.5 million annually. Approximately 87% of stroke cases are ischemic infarctions, a prevalence that increased substantially between 1990 and 2016, attributed to decreased mortality and improved clinical interventions (Johnson et al., 2019). The incidence of stroke increases with age and doubles after 55 years. However, in an alarming trend, stroke cases in people aged 20–54 years increased from 12.9% to 18.6% of all cases globally between 1990 and 2016. Nevertheless, age-standardized attributable death rates decreased by 36.2% over the same period (Boehme, Esenwa, & Elkind, 2017; Johnson et al., 2019; Kelly-Hayes, 2010). The occurrence of stroke in men and women also depends on age. It is higher at younger ages in women, whereas incidence increases slightly with older age in men. The higher risk for stroke in women is due to

factors related to pregnancy, such as preeclampsia, contraceptive use, hormonal therapy, and migraine with aura (Johnson et al., 2019).

1.2 Causes of cerebral stroke

A risk factor is a characteristic of an individual that increases the risk for the disorder compared to someone without that characteristic. Two types of risk factors are involved in the development of stroke, controllable and uncontrollable factors.

Uncontrollable	**Controllable**
Age	Hypertension
Sex	Smoking, alcohol, drug abuse
Race/ethnicity	Physical inactivity
Family history of transient ischemic attacks (TIAs)	Diet, Hyperlipidemia
Genetics	Diabetes mellitus
	Atrial fibrillation

Table 1.1: Risk factors of stroke.

Uncontrollable factors include age, sex, race, and history of family strokes (Cruz-Flores et al., 2011; Kapral et al., 2005) and controllable factors include hypertension, alcohol, smoking, metabolic disease, lifestyle, and genetics (J. Cheng & Hurn, 2010; Kuriakose & Xiao, 2020). Considering the effect of the above risk factors on the clinical variability of stroke, the stroke Therapy Academic Industry Roundtable (STAIR) recommendations include that experimental evaluation of agents intended for the potential treatment of cerebral ischemia or stroke should be carried out not only in young, healthy male animals but also in aged and female animals and animals with co-morbid conditions such as diabetes, hypertension, etc. (Fisher & Bastan, 2008).

1.3 Ischemic stroke

Ischemic strokes occur due to the blockade of a blood vessel. Ischemic strokes are of two types, embolic and thrombotic strokes. In an embolic stroke, a blood clot (embolus) forms somewhere in the body (usually in the heart) and travels through the bloodstream to the brain. The clot lodges there, blocking the blood vessel and causing an embolic stroke. In the case of a thrombus stroke, blood flow is impaired because of a blockage to one or more arteries supplying blood to the brain (Hui et al., 2018).

1.4 Clinical diagnosis of stroke

After a stroke, every minute, 1.9 million neurons are lost irreversibly. Therefore, a quick stroke diagnosis and early treatment can prevent neuron damage. Thus, an acronym is given for the stroke symptom, i.e., FAST (**F:** Face drooping, **A**: Arm numbness, **S**: Speech abnormality, **T**: Time to call ambulance). Computed tomography (CT) and magnetic resonance imaging (MRI) is widely used to assess ischemic strokes (Muir, Buchan, von Kummer, Rother, & Baron, 2006). The imaging modality chosen for initial acute ischemic stroke (AIS) evaluation is mainly driven by the 24 h × 7 days of immediate equipment availability and the possibility of providing the critical information required for the different possible treatments. As a result, CT is the readily available and most commonly used imaging technique employed for acute stroke diagnosis (Latchaw et al., 2009).

1.5 Animal models of focal ischemia

Understanding the pathophysiology of ischemic injury to develop novel therapeutic strategies in preclinical research adds value to stroke treatment (Sicard & Fisher, 2009). Several animal models have been developed to study ischemic injury in pre-clinical studies. Stroke can be induced by intraluminal middle cerebral artery occlusion (MCAO), thromboembolic model, nonclot embolus models, direct MCAO surgery model, photochemically induced thrombosis,

endothelin induced MCAO, ferric chloride ($FeCl_3$) induced distal MCAO (Sicard & Fisher, 2009). Intraluminal MCAO is widely used in focal ischemic models due to its resemblance to human cerebral anatomy and physiology. Intraluminal MCAO has good reproducibility and is suitable for transient and permanent ischemia without craniotomy (Macrae, 2011). This model mimics the human stroke conditions in animal studies, including several advantages, it reduces cerebral blood flow, alters cerebral metabolism, consistently produces cerebral infarction, causes embolism, leading to sensory and motor, cognition, and other dysfunctions (Longa, Weinstein, Carlson, & Cummins, 1989).

1.6 Platelet contribution to ischemic stroke

Thrombotic diseases are gradually escalating with high mortality and morbidity (Del Brutto, Chaturvedi, Diener, Romano, & Sacco, 2019; Powers et al., 2018; Virani et al., 2020). Platelets are the major contributors to thrombotic diseases, including stroke, atherosclerosis, ischemic heart diseases, deep vein thrombosis, etc.(Picker, 2013; Powers et al., 2019). Platelets are derived from megakaryocytes and circulated in the blood. The primary goal of platelets is to prevent bleeding by the formation of the hemostatic plug, which eventually causes clot retraction (Palta, Saroa, & Palta, 2014) at the site of injury. Platelet adhesion, activation, and platelet aggregation are crucial for thrombus generation. Platelets interacted with several receptors and platelet aggregators to form a thrombus. Activated platelets release several platelets aggregating agents, including collagen, adenosine diphosphate (ADP), thrombin, serotonin, and arachidonic acid (AA) metabolites, such as thromboxane A2 (TXA2), and prostaglandin E2 (PGE2). Apart from these agents, several other factors also contribute the platelet aggregation and thrombus formation, including reactive oxygen species (ROS) and inflammatory mediators. Eventually, extrinsic and intrinsic pathways are involved in thrombus growth after platelet aggregation. The extrinsic pathway is involved in the plasma-driven hemostasis by tissue factor (TF) (Lasne, Jude,

& Susen, 2006). Under physiological environments, the vascular endothelium maintains the contact between TF and plasma procoagulants. However, TF binds to factor VII and calcium after vascular damage, thereby converting factor X to Xa (Owens III & Mackman, 2010). The intrinsic pathway promotes the activation of factor X to Xa through a series of events that involve the factors XII, XI, IX, and VIII (Hall & Hall, 2020; V. Kumar, Abbas, Fausto, & Aster, 2014). Activated factor Xa converts the prothrombin to thrombin. Thrombin further converts the fibrinogen to fibrin to form stable clots. Platelets are involved in developing focal cerebral ischemic injury due to the formation of thrombus or emboli in cerebral arteries (Del Zoppo, 1998). Platelets adhere to vascular injury and contribute to 80% of ischemic strokes (Durukan, Strbian, & Tatlisumak, 2008). Several platelet-derived compounds interacted with platelet receptors and formed the thrombus. ADP (purinergic receptors, P2Y1 and P2Y12), collagen (glycoprotein receptors, GPIb and GPVI), TXA2 (TP), and thrombin (protease-activated receptors, PAR-1 and PAR-4) interacted with their receptors on platelets, thereby causing platelet aggregation and thrombosis.

1.7 Treatment and prevention of stroke

Pharmacotherapy of stroke involves primary and secondary treatment. Primary treatment consists of restoring blood flow by thrombolytic, thrombectomy, antiplatelet, and antithrombotics. Neuroprotective agents are used in secondary treatment (Bansal, Sangha, & Khatri, 2013; Matei, Camara, & Zhang, 2021). Recombinant tissue plasminogen activator (rtPA), a thrombolytic agent, is the only approved drug for the treatment of ischemic stroke by the food and drug administration (FDA) (Roth, 2011). rtPA administration within 4.5 hrs after stroke is beneficial for stroke treatment. Antiplatelet and antithrombotic drugs, aspirin, clopidogrel, and heparin are administered within 48 hrs after stroke. Neuroprotective agents include edaravone, fasudil, ginkgo mihuan, lumbricus rubellus extract, cerebrolysin, citicoline, and kallidinogenase

approved for the management of stroke (X. Chen & Wang, 2016). After ischemic stroke, it is important to prevent the neuronal damage in the penumbra region to minimize the stroke induced short-term and long-term diablities. Therefore, adminsitration of neuroprotectvie agents may minimize the damage in penumbra region which will eventually reduce the severity of stroke (Baron, 2018).

1.8 Limitations of current treatments of stroke

Intravenous injection of rtPA (alteplase) for eligible patients administered within 4.5 hours after onset of stroke symptoms. Due to the time factor, alteplase is not beneficial for all stroke patients. Therefore, it is challenging to treat stroke with rtPA treatment. Importantly, rtPA-eligible patients immediately receive alteplase without delay, even if mechanical thrombectomy is being considered (Campbell et al., 2015). Mechanical thrombectomy is indicated for patients within 6 hours (10%) and 24 hours (9 %) after stroke (Chia, Leyden, Newbury, Jannes, & Kleinig, 2016; Cohen, Kearney, Griffiths, Nadesalingam, & Bathula, 2015). However, very few hospitals have sufficient resources and expertise to deliver mechanical thrombectomy (Josephson & Kamel, 2018). In these circumstances, the eligible patients will be treated with rtPA. Asprin (ASP), and clopidogrel (CLOP), the antipaltelet agents are the prime line preventive agent for noncardioembolic stroke. ASP and CLOP cause gastric bleeding. Dual antiplatelet therapy of ASP and CLOP is not usually recommended after a stroke because of the ineffectiveness of the combination or is no more effective for preventing another stroke than either clopidogrel or aspirin alone. Moreover, this combination can produce heart attacks or acute coronary syndromes (Caplan, 2013).

Onset of stroke	**Recommendation**	**Limitations**
< 4.5 hrs	rtPA	i) Low therapeutic index ii) Risk of intracerebral hemorrhage iii) Low efficacy in recanalization of larger intracranial arteries (internal carotid artery (ICA) or proximal MCA) iv) Due to time dependence, all may not be benefited
6-16 hrs	Mechanical thrombectomy	i) Low access to neurointreventionlists ii) Vasospasm iii) Trauma to other blood vessels
24 hrs	Antiplatelets (ASP/CLOP)	**ASP** i) Risk of GI bleeding, ulcers, and dyspepsia ii) Combination with other anticoagulants causes intracerebral hemorrhage iii) Not suitable for asthma, rhinitis, bronchospasm, angioedema, or urticaria patients iv) Aspirin resistance **CLOP** **i)** Risk of GI bleeding **ii)** Induced intracerebral hemorrhage when administered with other anticoagulants iii) Breastfeeding and liver damage patients are not suitable for CLOP treatment **ASP+CLOP** i) Monotherapy is beneficial ii) Ineffective **iii)** Risk of heart attack or acute coronary syndrome

Table 1.2: Limitations of current therapy in stroke.

1.9 Mitochondrial dysfunction in ischemic stroke

Mitochondria, the powerhouse of the cells, maintain the cell energy homeostasis and regulate neuronal death after ischemic stroke. Energy balance disrupted due to reduction in blood supply alters the adenosine triphosphate (ATP) synthesis (Vosler, Graham, Wechsler, & Chen, 2009). Neurons are highly dependent on ATP compared to other brain cells. But their energy reserves are inadequate. ATP depletion due to the ischemic event following reperfusion injury (I/R injury) triggers the influx and efflux of ions and water resulting in membrane depolarization (Dharmasaroja, 2016). Due to the loss of transmembrane ionic gradient and homeostasis after ischemic stroke, neurons undergo severe pathophysiological events, such as mitochondrial damage and oxidative stress (He, Ning, Zhou, Khoshnam, & Farzaneh, 2020). Further, mitochondrial damage accelerates the ischemic stroke by elevation of ROS, calcium accumulation, defective mitochondrial biogenesis, triggering cell death by induction of the mitochondrial permeability transition pore (MPTP), and activation of apoptosis (Lesnefsky, Chen, Tandler, & Hoppel, 2017; Lin & Beal, 2006; Sanderson, Reynolds, Kumar, Przyklenk, & Hüttemann, 2013). MPTP opening causes a decrease in mitochondrial membrane potential (MMP), resulting in the depolarization of mitochondria, swelling and the translocation of the mitochondrial substances to the cytoplasm (Rasola & Bernardi, 2007). These events eventually cause necrotic and apoptotic cell death after I/R injury. Ischemia, following reperfusion, resupplies the nutrients and oxygen, re-activates the mitochondrial aerobic respiration and leads to the generation of ROS (Sanderson et al., 2013). ROS generation was significantly raised after mitochondrial damage, which caused the subsequent opening of MPTP and a decrease in MMP (Zhou, Yazdi, Menu, & Tschopp, 2011). ROS causes blood-brain barrier (BBB) disruption (Qu, Chen, Hu, & Feng, 2016), aggravates brain edema (Bao et al., 2018), deoxyribonucleic acid

(DNA) destruction (Beal, 2009), and decreases antioxidant enzymes (Holley, Bakthavatchalu, Velez-Roman, & St Clair, 2011; Milanlioglu et al., 2016) after stroke.

1.10 Mitochondrial Biogenesis

Deprivation of energy after ischemic stroke places the mitochondria under detrimental circumstances (Sims & Muyderman, 2010). However, mitochondria strive to maintain redox homeostasis and energy balance. Under exaggerated conditions, mitochondrial death resulted in a reduced mitochondrial number. Depleting mitochondrial numbers indicates decreased energy supply and redox homeostasis, which keeps cells in a stressful environment. Hence, the escalation of mitochondrial numbers could help the cell to maintain normal homeostasis (Scarpulla, 2002). Mitochondrial biogenesis (MB) is described as the generation of mitochondria from pre-existing mitochondria. MB is stimulated by low temperature, caloric restriction, exercise, oxidative stress, and cell division. Peroxisome proliferative activated receptor-γ (PPARγ) co-activator 1α (PGC1-α) is the key regulator of mitochondrial biogenesis activated under these conditions and promotes cell survival (Aharoni-Simon, Hann-Obercyger, Pen, Madar, & Tirosh, 2011). Further (PGC-1α) activates the downstream mitochondrial transcription factors such as nuclear respiratory factors (NRF1 and NRF2) and mitochondrial transcriptional factor A (tFAM) (Gureev, Shaforostova, & Popov, 2019), which accelerates mitochondrial biogenesis. NRF2 triggers the upregulation of antioxidant enzymes (Gureev et al., 2019). This series of events eventually increase the mitochondrial number by activation of PGC-1α, which alleviates oxidative stress, mitochondrial dysfunction, and apoptosis (Escrivá, Rodríguez-Peña, & Vallejo, 1999). Recent studies indicate that the pharmacological activation of MB offers a novel neuroprotective approach to stroke management (L. Li et al., 2016; Mehta, Kumari, Mendelev, & Li, 2012).

1.11 Indole-3-carbinol

Cruciferous vegetables such as broccoli, cabbage, brussels sprouts, green peas, cauliflower, turnips, swedes, collard, and kale are rich sources of nutrients. Among them, indole-3-carbinol (I3C), a breakdown product from cruciferous vegetables, is reported to have numerous pharmacological activities (Singh, Patil, Kang, Niyonizigiye, & Kim, 2021). I3C inhibits a wide range of cancers, including breast, prostate, liver, and lung, etc. (Aggarwal & Ichikawa, 2005). I3C inhibits collagen and ADP-induced platelet aggregation by inhibiting TXA2, PGE2, and GP IIb/IIIa receptors (Park, 2008; Ramakrishna & Krishnamurthy, 2022). I3C shows antioxidant and anti-inflammatory (Y. Choi, Abdelmegeed, Mohamed A, Song, Byoung-Joon, 2018) (Youngshim Choi, Kim, Park, Lee, & Park, 2012), neuroprotective (El-Naga, Ahmed, & Al Haleem, 2014), anti-nephrotoxic (El-Naga & Mahran, 2016), anti-obesity (H.-S. Choi, Jeon, Lee, & Lee, 2014; Youngshim Choi et al., 2012; Y Choi, Um, & Park, 2013; Poornima & Mirunalini, 2014), glucose regulation (Jayakumar & Sankaran), cardioprotection (Adwas, Elkhoely, Kabel, Abdel-Rahman, & Eissa, 2016; Akkiraju et al., 2021; W. Deng et al., 2014). I3C improves the neurobehavioral out companies in MCAO rats by improving the mitochondrial antioxidant enzymes (P. Paliwal et al., 2018). Despite pharmacological activities, I3C rapidly undergoes acid condensation and about 50 % of I3C is converted into diindolylmethane (DIM) (Bradlow & Zeligs, 2010). I3C intake through oral route causes rapid conversion into its metabolites such as 2-(indol-3-ylmethyl)-3,3-diindolylmethane (DIM), [2-(indol-3-ylmethyl)-indol-3-yl]indol-3-ylmethane (LTr1), indolyl-carbazole (ICZ), and 1-(3-hydroxymethyl)-indolyl-3-indolylmethane (H1-1M). I3C and its metabolites have been identified in the plasma, liver, heart, lungs, kidney, and brain (Anderton et al., 2003; Anderton et al., 2004) of mice.

1.12 Diindolylmethane

Diindolylmethane (DIM) shows a variety of pharmacological activities such as anticancer (S. M. Kim, 2016), antioxidant, anti-inflammatory (López-Vázquez et al., 2017), antiarthritic (Du et al., 2019), antiobesity (H. Yang et al., 2017), antidiabetic (Jayakumar, Pugalendi, & Sankaran, 2014), hepatoprotective (Munakarmi, Chand, Shin, Jang, & Jeong, 2020), cardioprotective (Zong et al., 2013), and neuroprotective (Rzemieniec, Wnuk, Lasoń, Bilecki, & Kajta, 2019). However, DIM role in the inhibition of platelet aggregation, thrombosis, and protection against ischemic stroke is not yet identified.

1.13 Rationale

Following ischemic stroke, stroke patients have to be treated immediately to avoid mortality and morbidity. Thrombectomy is a surgical procedure used to remove clots and is also widely used to treat stroke patients. The primary therapy for stroke patients is thrombolytic therapy with intravenous administration of rtPA. If the stroke patients are not suitable for either of these treatment approaches, antiplatelet and antithrombotic agents are widely used to treat ischemic stroke. Indeed, if ischemic strokes are treated with thrombolytic agents or clots removed surgically, at the end, antiplatelet and antithrombotic drugs are used to prevent the recurrence of strokes. Hence, antiplatelet and antithrombotic treatments are an essential part of stroke treatment. However, antiplatelet and antithrombotic agents like ASP and CLOP exhibit several adverse effects, including gastric bleeding, intracerebral hemorrhage, and increased risk of heart attack or acute coronary syndrome (Caplan, 2013). Therefore, new chemicals are being sought for treating ischemic strokes with no adverse events.

I3C has been reported to improve neurological functions in stroke animals due to its antiplatelet and antithrombotic activities (P. Paliwal et al., 2018). Previous reports indicate that I3C is rapidly eliminated from the body (Anderton et al., 2004). Therefore, multiple doses of I3C

need to be administered. Moreover there is no data of the phramcokinetic profile of I3C in ischemci stroke. Thus, understanding the pharmacokinetics of I3C would help fix its dosage regimens. I3C oral administration generates high amounts of DIM and is considered to involve in the mediation of pharmacological activities of I3C (Bradlow & Zeligs, 2010). As a result, simultaneous quantification of I3C and DIM in body tissues can distinguish the therapeutic potential of I3C. Moreover, I3C pharmacological activities have been reported after oral administration (Aggarwal & Ichikawa, 2005). Antiplatelets and antithrombotics are administered orally and intravenously to treat stroke patients. Moreover, comatic stroke patients are treated through the intravenous route (Bansal et al., 2013). Therefore, finding a beneficial route to treat stroke patients is essential. Oral treatment of I3C has been reported to protect the brain from ischemic stroke (P. Paliwal et al., 2018). However, the potential of intravenous administration of I3C for the treatment of stroke has to be evaluated. Therefore, the therapeutic potential of I3C after the oral or intravenous route has to be identified. Hence, we have planned to evaluate the pharmacokinetics and pharmacodynamics of I3C in stroke animals.

Additionally, it is crucial to distinguish between the pharmacological effects of I3C and DIM when choosing a possible stroke treatment chemical. Previous reports indicate that I3C ameliorated platelet aggregation (ADP, collagen, thrombin, and AA) (Ramakrishna & Krishnamurthy, 2022) and protected the brain from ischemic stroke (P. Paliwal et al., 2018). DIM was believed to mediate pharmacological activities (Aggarwal & Ichikawa, 2005). Therefore, peripherally, we have planned to investigate the antiplatelet, antithrombotic, and thrombolytic activities of DIM. Platelets adhere to damaged endothelium and recruit more platelets, leading to platelet aggregation. Further, activated platelets release platelet aggregating agents like ADP, thrombin, and arachidonic acid, eventually leading to thrombus generation. The

formation of thrombi causes oxygen and nutrient deprivation leading to cell death. Further, we will compare the antiplatelet and antithrombotic activities of DIM and I3C to identify the potential compound. Peripherally, antiplatelet and antithrombotic drugs inhibit platelet aggregation and thrombus generation. Therefore, we have evaluated the efficacy of DIM in ameliorating platelet aggregation, thrombus generation, inflammation, and oxidative stress in rats after MCAO injury.

Centrally, many neurons are damaged beacsue of MCAO and ischemic reperfusion injury (I/R) and need to be repaired instantly to prevent further neuronal damage. Mitochondria regulate energy balance, redox balance, and cell death. Mitochondrial dysfunction is an initial event in the pathogenesis of stroke. During or after MCAO, there is a demand for energy due to blockade of blood flow or reperfusion. Being damaged by MCAO blockade or reperfusion injury, mitochondria cannot produce enough amounts of ATP for cell function. In these circumstances, the mitochondria number has to be increased to meet the energy demand. Moreover, impaired bioenergetics and altered mitochondrial structure play a central role in activating death signalling. Therefore, protecting the mitochondria could be a promising target for mitigating neuronal damage after ischemic stroke. Pharmacological preservation of mitochondrial function has been reported as neuroprotection in cerebral ischemia in preclinical studies (F. Liu, Lu, Manaenko, Tang, & Hu, 2018). However, there is no mitochondrial protective drug available in the clinics to treat ischemic stroke. Therefore, there is interest in finding mitochondrial protective agents for stroke management. Adenosine monophosphate (AMP)-activated protein kinase (AMPK) regulates cellular energy homeostasis by promoting mitochondrial biogenesis through the activation of PGC-1α. Further, PGC-1α activates the NRF1 and NRF2. Activated NRF1 stimulates the tFAM, while activated NRF2 promotes the antioxidant enzyme levels. The tFAM

then promotes mitochondrial biogenesis leading to an increase in mitochondrial number. Therefore, stimulation of mitochondrial biogenesis can rescue the cell from an energy-deficient state. Moreover, AMPK activation is also associated with decreased oxidative stress and inflammation in the ischemic stroke brain. I3C exhibits mitochondrial protective activity. However, the underlying mechanisms of the mitochondrial protective role of I3C have to be evaluated. Therefore we have considered the mitochondrial protective mechanisms of I3C in focal cerebral ischemic rats. Based on the above facts, we have formulated the hypothesis on the potential cerebropreotective effects of I3C and DIM, as depicted in figure 1.1.

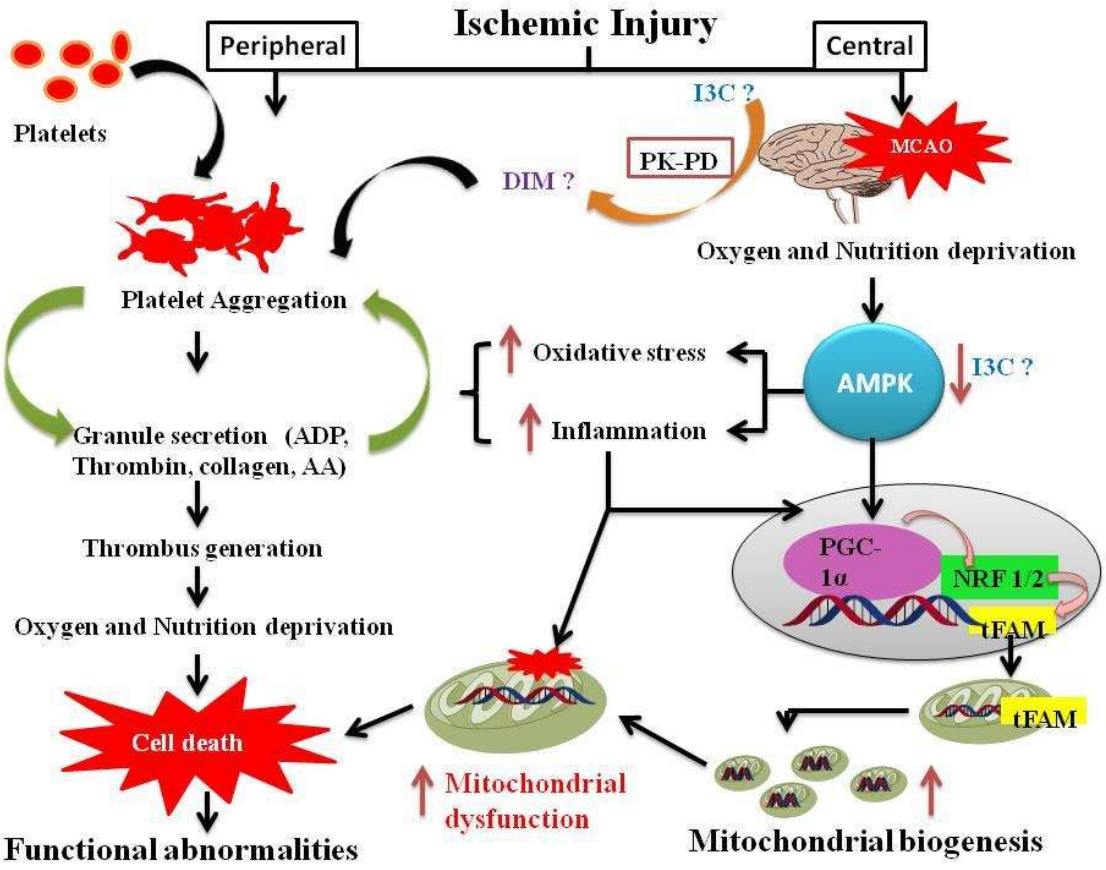

Figure 1.1. Proposed hypothesis. The proposed hypothesis is based on the application of I3C and its major metabolite DIM against MCAO-induced ischemic reperfusion injury. I3C has been reported to improve neurobehavioral outcomes in stroke animals due to its antiplatelet and

antithrombotic activities. Previous reports indicate that I3C is rapidly eliminated from the body (Anderton et al., 2004), indicating that I3C has to be administered multiple times. Thus, understanding the pharmacokinetics of I3C would help fix its dosage regimens. Hence, we have considered the pharmacokinetics and pharmacodynamics of I3C and also the beneficial route of I3C to treat ischemic stroke (Objective 1). Peripherally, MCAO causes platelet aggregation. DIM has been reported to mediate the pharmacological activities of I3C. Therefore, we hypothesized that DIM would inhibit platelet aggregation and thrombus formation (objective 2) and improve brain function, followed by MCAO-induced ischemic reperfusion injury in the rats (objective 3). Centrally, MCAO injury causes oxygen and nutrition deprivation leading to a decrease in AMPK levels, thereby mitochondrial biogenesis, oxidative stress, and inflammation pathways upregulated. We have proposed that I3C further enhance the mitochondrial biogenesis in MCAO rats by upregulating PGC-1α, NRF1, and tFAM and ameliorates inflammation and oxidative stress (objective 4). To validate the above proposed mechanisms, we here set a specific objectives as given below.

1.14 Objectives

Objective I: Evaluation of pharmacokinetic and pharmacodynamic properties of I3C in MCA occluded rats

Objective II: Evaluation of the antiplatelet and antithrombotic activity of Diindolylmethane

Objective III: Pharmacological effects of Diindolylmethane in MCA occluded rats

Objective IV: Evaluation of mitochondrial based mechanism by I3C in MCA occluded rats