Chapter 1

General Introduction & Aims of the thesis
INTRODUCTION

Stroke

Definition & type(s) of stroke

Stroke is a pathological entity wherein the abrupt cessation of blood supply to a part of the central nervous system (brain, spinal cord or retina) leads to tissue damage and neurological dysfunction. According to the updated definition, stroke broadly includes the following: CNS infarction, ischemic stroke, silent CNS infarction, intracerebral haemorrhage, subarachnoid haemorrhage and cerebral venous thrombosis (1). As per the new definition, ischemic stroke includes only focal ischemia, excluding global ischemia due to the differences in their pathology and mechanisms. In focal ischemia, stenosis or occlusion of an artery impedes the perfusion to the respective territory leading to cell death. In contrast, in global ischemia, a decrease in cerebral perfusion resulting from a reduction in blood pressure (e.g. cardiac arrest) or severely increased intracranial pressure (e.g. severe head trauma) leads to selective neuronal cell death in vulnerable areas such as hippocampus, neocortex, thalamus and basal ganglia (1). Besides, survivors of global ischemia always experience reperfusion of the ischemic cerebral tissue, which will additionally contribute to the endured neuronal damage, whereas in focal ischemia reperfusion may either be absent or present. The treatment modalities of focal and global ischemia differ also. Focal ischemia is acutely treated with reperfusion strategies to re-establish blood flow in an artery. In contrast, correction of the systemic disorder underlying the hypoperfusion is the mainstay in global ischemia.

Stroke can also be broadly classified into ischemic (80-85%) and hemorrhagic stroke (15-20%). Ischemic stroke is further sub-classified into thrombotic and embolic stroke. Whereas thrombotic stroke develops by a gradually progressing clot formation within a cerebral artery, embolic stroke is caused by dislodging of a blood clot or atheromatous plaque from an extracranial site. Hemorrhagic stroke results from a rupture of a blood vessel in the brain parenchyma or subarachnoid space not only hampering the blood flow, but also leading to a rise in the intracranial pressure.

Disease burden

Stroke is the second most common cause of death and leading cause of adult disability, accounting for 11.13 % of total deaths worldwide. The prevalence of stroke was 33 million in 2010, with 16.9 million people having a first stroke worldwide (2). The major global burden of stroke resides in middle-income and low-income nations, where incidence of
stroke is the highest (3). Moreover, due to an increasingly aging population, the number of stroke patients is expected to more than double over the next 40 years (3). Stroke has a major impact on the quality of life of survivors and their caretakers and results in a significant economic impact due to health care costs and loss of productivity (4). The global burden from stroke is predicted to rise from around 38 million DALYs (disability adjusted life years) in 1990 to 61 million DALYs in 2020.

Risk factors

Several factors increase the risk of stroke. Hypertension contributes to about 54% of all stroke cases (5). Apart from hypertension, atrial fibrillation, diabetes, resident lifestyle and smoking account for two-thirds of all first-time stroke cases (2,6).

PATHOPHYSIOLOGY

Cerebral Ischemia & Reperfusion

Brain function and viability require continuous utilization of metabolic energy in form of ATP, which is derived from the oxidative phosphorylation of glucose delivered via the arterial circulation. A complex biochemical cascade of events is initiated upon acute blood flow cessation ultimately resulting in the depletion of energy rich stores (ATP). At this stage, the brain is unable to meet its high energy demands (7). The critical reduction of cerebral perfusion eventually leads to ischemic infarction with a central core of irreversible neuronal damage (necrosis) in areas where blood flow decreases below the threshold of cell survival. In the central core, shortage of ATP causes failure of plasma membrane ionic pumps resulting in the cell being unable to maintain ionic gradients causing increased passive transport of water (cytotoxic edema) secondary to the increase in intracellular Na⁺ and release of K⁺ into the extracellular space (7) (Fig. 1). Brain regions outside the central core, i.e. those with collateral blood supply, undergo a less severe insult, but do show dysfunctional restoration of ion gradients in turn leading to waves of depolarization (peri-infarct depolarizations, PIDs or spreading depressions, SDs) and excessive release of neurotransmitters from presynaptic nerve terminals. Together, the increased release of neurotransmitters such as glutamate and the extracellular released K⁺ ions initiate further generation of depolarization waves, thus initiating a vicious cycle. Such a region is termed the 'ischemic penumbra' (at-risk tissue), and constitutes predominantly a rim of brain tissue with its blood supply restrained between two critical thresholds, i.e., the threshold of electrical failure and the threshold of electrical failure and the threshold of energy and ionic pump failure (8). This penumbral tissue is biochemically dynamic, with
constrained perfusion but partially preserved energy state and still salvageable, if blood flow is rapidly restored. If blood flow is however not restored in a due time, the infarct core progresses into the penumbra.

Excitotoxicity

The increased extracellular concentrations of glutamate, due to an impaired glial re-uptake of neurotransmitters, induce an excessive activation of glutamate receptors (excitotoxicity) at the postsynaptic membranes. These receptors primarily include NMDA
and AMPA receptor types which mediate Ca\(^{2+}\) influx into the cells, resulting in an intracellular calcium overload (9) (Fig. 1). This excitotoxicity and imbalance of ions lead to activation of a variety of cellular signal transduction cascades, including protein kinase C, phospholipase A2, phospholipase C, cyclooxygenase, calcium-dependent nitric oxide synthase, calpain, various proteases, and endonucleases.

**Mitochondrial dysfunction**

The Ca\(^{2+}\) overload also elicits mitochondrial dysfunction, thus impairing its oxidative phosphorylation, increasing free radical formation and triggering the release of apoptogenic molecules (10). As a result of free-radical generation, irreversible mitochondrial damage, inflammation, and both necrotic and apoptotic cell death are initiated (11,12). The resulting formation of mitochondrial permeability transition pores causes the mitochondrial membrane to become leaky leading to a burst of free radicals and release of cytochrome c, a key mediator of apoptosis (13). Free radicals can react irreversibly with several cellular constituents such as proteins, double bonds of phospholipids, and nuclear DNA. Further, in conjunction with a weakened scavenger system, free radicals cause lipid peroxidation, membrane damage and dysregulation of cellular processes (Fig. 1). In the brain’s normal condition of high energy demand and adequate oxygen supply, increased mitochondrial calcium levels drive an increase in ATP production via oxidative phosphorylation. However, when oxygen is not available in adequate amounts to accept electrons from NADH, these electrons form superoxide radicals from the residual oxygen. Hydrogen sulfide (H\(_2\)S) during such a condition can act as an electron donor to replenish the reduced ATP stores (14).

**Reperfusion injury**

Reperfusion is the phase wherein the blood flow in a previously occluded vessel is re-established in order to perfuse relevant region(s) of the organ. Re-establishing the blood flow to the ischemic territory helps to restore aerobic energy metabolism (15,16), protein synthesis, neuronal electrical activity (17), and to mitigate tissue damage (15,18,19). However, sudden reperfusion into the brain tissue after hypoxia causes a series of adverse events, including oxygen overload, postischemic hyperperfusion, leukocyte infiltration, platelet and complement activation and BBB disruption which may lead to secondary oxidative tissue injury (reperfusion injury) (7). In fact, reperfusion may cause additional harm to the ischemic tissue, as the ischemia sets the stage for oxygen to generate free radicals rather than to contribute to cellular energy production (20).
NADPH oxidase (NOX), a major source of reactive oxygen species (ROS) reacts with newly generated oxygen radicals during the reperfusion phase to produce superoxide radicals (21). These radicals react with iron-sulfur containing proteins, releasing free iron which results in hydroxyl radical formation. Nitric oxide in the mitochondria reacts with superoxide radicals readily and more efficiently than superoxide dismutase (SOD) (22). This reaction forms the potent free radical peroxynitrite, which irreversibly inactivates SOD, including complexes I and II of the mitochondrial respiratory chain. Damage to endothelial cells as well as platelets, leucocytes and other blood cell types is also evident upon reperfusion. Activated neutrophils produce superoxide radicals which can form into hydrogen peroxide. Subsequently, neutrophil myeloperoxidase converts hydrogen peroxide to hypochlorous acid, which can produce hydroxyl radicals upon reacting with superoxide. Eicosanoids generated from arachidonic acid on the other hand increase the adhesion of platelets and leucocytes to capillary walls, leading to micro-thrombi. Superoxide radicals and ICAM-1 (released from endothelial cells and leucocyte membranes during reperfusion) can potentiate the leucocyte adhesion process. Moreover, in some acute ischemic stroke patients, thrombolysis can lead to symptomatic intracranial bleeds possibly resulting from increased levels of matrix metalloproteinases which have deleterious effects on blood-brain barrier (BBB).

**Blood-brain barrier disruption**

Free radicals generated in detrimental amounts during the process affect cell membranes and other cellular components. Such damage to the endothelial lining of brain vessels impairs the BBB. The BBB is a dynamic interface consisting of endothelial cell, astrocyte, pericyte, and the adjacent neurons (23) which regulates the flow of substances into and out of the brain thereby maintaining the cerebral homeostasis. The endothelial damage results in an increased BBB permeability to plasma proteins and a fluid shift towards the extracellular space, leading to vasogenic edema (24). All these events cumulatively result in vasogenic edema, which in turn impairs blood flow and worsens the tissue ischemia, triggering another vicious cycle. Massive brain edema, as occurs in a subset of ischemic stroke cases, elevates intracranial pressure (ICP) and may progress to brain herniation, which is a major cause of early mortality.

**Neuroinflammation**

It has been increasingly recognized that inflammation is a key contributor to the pathophysiology of cerebral ischemia (9). Inflammation seems to exert a dual role, as it acutely worsens the ischemic injury yet in the long-term proves beneficial through contributing to tissue remodelling (25). Elements of the immune system are substantially
involved in the ischemic cascade ranging from the initial acute vascular events to ultimate brain damage and subsequent tissue repair that occurs at later time points (9). Pro-inflammatory signals can be generated within minutes after ischemic events (9). Moreover, free radicals generated activate microglia which induce peripheral immune cell infiltration into the ischemic brain (7). With the progression of the ischemic cascade, cell death ensues, leading to another stage of inflammation caused by the release of danger signals from those cells. Subsequent activation of purinergic receptors on microglia and macrophages in turn results in the production of pro-inflammatory cytokines (9). Also damage-associated molecular pattern molecules (DAMPs) are released by ischemic cells, which activate toll-like receptors (TLRs) and subsequently up-regulate pro-inflammatory gene expression (9).

Taken together, knowledge of the dynamics of the pathophysiological processes occurring during and after ischemia is crucial to successfully design therapies and new medication. However, the dynamics of the interplay of these processes is largely unknown. Unfortunately, development of effective therapeutic strategies is confronted with a lack of efficient pre-clinical models mimicking one or more of the mechanisms underlying ischemic stroke.

**MODELLING FOCAL CEREBRAL ISCHEMIA**

Several endovascular as well as surgical-based techniques have been used in the last decades to model ischemic stroke (see Table), commonly in rodents (24,26,27). While the surgical approach mainly involves a craniectomy-based occlusion of relevant cerebral arteries, it may alter intracranial pressure, hemodynamics and local brain temperature thus possibly affecting the pathophysiological sequelae of ischemic damage. Moreover, specific non-craniectomy based techniques such as macrosphere injection result in a highly variable infarction size (28). The studies carried out in this thesis have focused on the endovascular mode of occlusion of a major cerebral artery using a nylon-based (filament approach) suture as described below.

Middle cerebral artery (MCA) is one of the most commonly involved vessels in ischemic stroke patients. As rodent cerebrovascular anatomy to a large extent resembles the human counterpart (Fig. 2), MCA occlusion is mimicked in animal models to result in either a transient or permanent MCA stroke. In creating such models, the filament based approach is advantageous mainly for 2 reasons: i) it avoids the surgical exposure of the skull in order to gain access to a cerebral artery, and ii) the filament can be withdrawn at any time point to (re)-establish blood flow to the ischemic brain region (‘controlled’ reperfusion) or left in situ to create a permanent occlusion. Nevertheless, surgical
experience and mortality rate are decisive in reproducibility of the ischemic lesion and establishing such a model.

Table. Modalities to induce focal cerebral ischemia

<table>
<thead>
<tr>
<th>MODE OF OCCLUSION</th>
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<tr>
<td>Nylon suture</td>
<td>Aspey et al., 1998; Belayev, Alonso, Busto, Zhao, &amp; Ginsberg, 1996, Longa, et al., 1989; Spratt et al., 2006; Zhao et al., 2008 (29-44)</td>
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<td>Autologous blood clots</td>
<td>Overgaard et al., 2010 (34); Dinapoli, et al., 2006 (35); Jin et al., 2014 (36)</td>
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<td>Polyvinyl siloxane</td>
<td>Yang et al., 2002 (37)</td>
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<td>Microspheres</td>
<td>Mayzel-Oreg et al., 2004 (38); Demura, et al., 1993 (39)</td>
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<tr>
<td>Macrospheres</td>
<td>Gerriets et al., 2003 (40)</td>
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<td>Photothrombosis</td>
<td>Watson et al., 1985 (41); Cai et al., 1998 (42); Li et al., 2014 (43)</td>
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<tr>
<td>Peri-arterial injection of endothelin-1</td>
<td>Nikolova et al., 2009 (44)</td>
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Endovascular approach for middle cerebral artery occlusion (MCAO) in rodents

In this thesis, stroke was produced in rats by occluding the middle cerebral artery using an endovascular approach using nylon filaments with a silicon tip. To familiarize the reader with the details of the procedure’s subsequent steps are outlined in detail below.

Anesthetic induction & laser Doppler flow (LDF) set up

MCAO is induced under general anesthesia. We employed the inhalational anesthetic isoflurane (1.5-5%) in a mixture of oxygen and air (30:70%) delivered via a nose cone in spontaneously breathing rat. A temperature probe is inserted into the rectum to ensure constant body temperature in the physiological range by a feed-back control of a heating pad in the operation table. With the animal resting in a prone position and head fixed using a stereotactic frame, a midline incision is made over the skull and a small shallow burr-hole generated in the right temporal bone using a drill. The position of the hole is 5mm lateral and 2mm caudal to the bregma overlying the territory supplied by the MCA (Fig. 3A). Care is taken not to break the inner bone layer. A laser Doppler flow (LDF) fiber is then fixed to the hole for subsequent control of ischemia induction. The LDF fiber is coupled to a PeriFlux 5000 monitor (Perimed Instruments, Jarfalla, Sweden) and connected to a LabView-based multimodal monitoring program.
Figure 2: Vascular anatomy of the rodent and human brain. Left (upper and lower) panel depicts the 3 major arterial supply territories (anterior, middle and posterior) as shown on dorsal surface of rodent and human brain. MCA supplies the major brain regions in rodent as well as in human brain. At the base of the brains, a well-developed arterial collateral pathway between the 3 territories exists, denoted as the circle of Willis, which connects the anterior circulation by anterior communicating artery (AComA; indicated by the asterisk) and the posterior circulation by the posterior communicating artery (PcomA), as exemplified schematically in the right panel. ACA, anterior cerebral artery; BA, basilar artery; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; PComA, posterior communicating artery; SCA, superior cerebellar artery; VA, vertebral artery.

Carotid access and filament insertion

After positioning the LDF fiber, the animals are turned to a recumbent (supine) position and receive a subcutaneous injection of buprenorphine (0.05mg/kg) to reduce post-operative pain. For induction of focal cerebral ischemia, we use the intraluminal MCA
occlusion approach described by Longa and co-workers (1989) (31) with some modifications. Briefly, the right common carotid artery (CCA) is exposed via a midline incision in the neck and dissected between the sternocleidomastoid and the sternohyoid muscles parallel to the trachea. The CCA is then carefully freed from its adventitial sheath and the vagal nerve (Fig. 3B). The carotid bifurcation is exposed, and internal carotid artery (ICA) dissected free from the adjacent tissue. After transiently ligating the CCA and ICA, the external carotid artery (ECA) is permanently ligated distally to the origin of the superior thyroid artery. ECA is then excised and the resulting stump (Fig. 3C) aligned in parallel to the CCA to facilitate

![Figure 3: Outline of the surgical approach of filament-induced MCAO in rat. (A) Position of the Laser- Doppler flow probe overlying the MCA supplied territory at the skull bone (5 mm lateral and 2 mm posterior of the bregma). (B) Carotid vasculature along with vagus nerve coursing along the lateral aspect of CCA. (C) Creation of ECA stump for subsequent filament insertion after electrocoagulation of STA and Oc.A. (D) Progression of the filament along the ICA via the ECA stump. The filament is cautiously inserted to prevent its entry into PPA instead advancing it medially into the intracranial ICA coursing through the carotid canal (E). Before the filament is fully inserted until it reaches the MCA origin, the ligature over CCA is removed to avoid hypoperfusion and ECA stump is tightly fixed along with the filament. ECA, external carotid artery; ICA, internal carotid artery; Oc. A, occipital artery; PPA, posterior parietal artery; R-CCA, right common carotid artery; STA, superior thyroid artery.]
insertion of the occluding filament (prepared using a 4-0 nylon suture with silicon-coated tip) into the ICA (Fig. 3D,E). The ligatures around the CCA and ICA are then removed and the occluding filament advanced until a sharp drop of the LDF signal indicated occlusion of the MCA (Fig. 4A). After ensuring the absence of intracranial bleeding, the occluder is tightly fixed to the ECA. Thereafter, the neck incision is closed, as is the incision over the skull after removal of the LDF fiber. The isoflurane is withdrawn, and after recovery from anesthesia, the animal is transferred to its home cage with free access to water and moistened food pellets. For a transient MCA occlusion (tMCAO), the occluder will be withdrawn after a relevant time period after re-anesthetizing the animal under isoflurane anesthesia. In the permanent MCA occlusion (pMCAO), the occluder will be left in place for the desired follow-up time. A schematic representation of a filament occluding the MCA origin at the base of the brain is shown in figure 4B.

Figure 4: Laser-Doppler flow (LDF) confirmation of the correct filament position for MCA occlusion (MCAO). (A) Blood flow pattern as recorded perioperatively during MCAO induction in a rat. The initial drop in the stable baseline is caused by temporary occlusion of the CCA during filament insertion and reverts back to its initial level. The second and larger abrupt decline in the blood flow originates from occlusion at the MCA origin (MCAO). (B) Schematic representation of the final position of the filament with its silicon coated tip occluding the MCA origin (bold black arrow). LDF, laser Doppler flow; ACA, anterior cerebral artery; BA, basilar artery; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; PComA, posterior communicating artery; SCA, superior cerebellar artery; VA, vertebral artery.
The filament-induced MCAO, since its inception in late 1980s, has underwent several modifications to result in a reproducible amount of ischemic damage and avoid adverse effects such as accidental subarachnoid hemorrhage and hyperthermia. Consequently, it has evolved into a reliable tool to study the pathophysiology of stroke and therapeutics.

CURRENT TREATMENT STRATEGIES

The inception of thrombolysis in the mid 1990s played a crucial role in advancing the management of acute ischemic stroke (AIS). The aim of systemic or local application of thrombolytic agents or endovascular techniques is the lysis/disruption of the thrombus/emboli occluding brain vessels. The thrombolytic technique used initially, i.e. systemic (intravenous) thrombolysis, may however pose major issues with respect to efficacy, safety and applicability. Not only do contraindications such as history of intracranial haemorrhage and recent surgery (45) preclude its use, the occurrence of hemorrhagic transformation of the infarcted area may aggravate ischemic damage and can even result in worse functional outcome (46,47). Besides, intravenous thrombolysis is incapable of relieving proximal occlusions of major intracranial arteries, which comprise one-third of cases of anterior circulation stroke. Hence, the intra-arterial thrombolytic approach is currently regarded more appropriate in this regard (48). Intra-arterial therapy basically consists of chemical dissolution of clots, either with locally delivered thrombolytic agents or clot retrieval or thrombectomy using mechanical devices. A recent Dutch trial (MR CLEAN) employed endovascular therapy in patients with AIS (within 6 h of symptom onset) in addition to routine IV thrombolysis, consisting of arterial catheterization to the level of occlusion to deliver a thrombolytic agent or perform thrombectomy, or both. This endovascular treatment in conjunction with routine systemic thrombolysis produced superior outcomes (improved recanalization and functional outcome at 90 days) compared to the standard thrombolytic treatment, yet with a comparable mortality (48). Such result is compatible with the opinion that endovascular therapy has the potential of becoming the first-line treatment in near future (49,50). However, other studies comparing endovascular treatment to the current thrombolysis regimen (IMS III, MR RESCUE and SYNTHESIS Expansion) have failed to show superiority of endovascular treatment (51,50). Thus, currently, intravenous thrombolysis with rtPA is the only specific pharmacological reperfusion therapy for AIS proven to be effective and approved by the FDA (52). Its 3 hour therapeutic window has recently been extended in Europe to 4.5 hours after the beneficial results provided by the ECASS III trial (53), which were confirmed in several other centers (54). Studies are now aiming a further extension of the time window beyond 4.5 h (55,56). Nevertheless, despite its clinical effectiveness, only a minority of patients is eligible for intravenous thrombolysis due to the limited therapeutic
window and several contraindications (57,58). To extend the therapeutic window for thrombolysis, hypothermia has been explored as a potential treatment in several pre-clinical models of stroke. Although hypothermic treatment has yielded promising results in mitigating ischemic damage both in tMCAO and pMCAO models (59), addition of thrombolysis on the outcome on ischemic damage has been debated (60,61,62) as hypothermia may influence the activity of fibrinolytic enzymes. Clinically, targeted temperature management (TTM) is gaining significance in the management of acute brain injuries, including ischemia and haemorrhage, as evident from recent trials. However, results of TTM are of a mixed nature. In this context, the ICTuS-L safety trial (intravenous thrombolysis plus hypothermia for acute treatment of ischemic stroke – longer tPA window) revealed no significant neurological improvement, mortality nor was able to extend the time window in patients of ischemic stroke arriving within 6 h from symptom onset (63). Based on these results, further studies are warranted to ascertain the efficacy of TTM in addition to rt-PA treatment. Besides, adverse effects associated with hypothermia and/or rewarming need to be tackled effectively, as these may potentially aggravate the brain insult.

Over 1,000 neuroprotective agents have been proposed and several showed success in animal studies. However, none of the more than 100 agents that made it to clinical trials ultimately proved successful in human patients (64). Possible reasons for the failure to translate these treatments from animal to patient studies may include differences in lesion size, composition of brain tissue or timing of drug delivery (64). Because of the interplay of the many individual processes involved discussed above, the effect of a therapeutic intervention is hard to predict. Hence stroke is often considered as ‘mechanisms in search of a treatment’ and several pre-clinical models have been developed and modified in the last 3 decades to mimic the clinical situation to enable development of effective therapeutics and overcome the existing translational blockade.
AIMS OF THIS THESIS

The aim of the thesis is to model focal cerebral ischemia with or without reperfusion in adult rats to mimic features of clinical ischemic stroke and test novel therapeutic agents for their neuroprotective properties. Because of the lack of a permanent focal cerebral ischemia model with long-term survival, we optimized the endovascular filament-based approach. In chapter 2, we set out to model malignant brain infarction in the rat, characterized by severe infarction accompanied with brain swelling, delayed growth of the ischemic area, yet displaying a low mortality rate. To this end, we employed the intravascular filament approach to occlude the proximal origin of MCA permanently. The rationale behind the study was to modify and develop a filament with a bowling pin-shaped silicon coated tip, which would result in an improved collateral blood flow in posterior cerebral circulation in contrast to occlusion using conventional filaments. Having established this goal, it is of interest to longitudinally study the ischemic brain tissue, e.g. by assessing brain ionic imbalances through the measurement of potassium levels. Therefore, in chapter 3, a MR-based feasibility study was performed utilizing a cryogenic copper-based radio frequency surface resonator cooled down to 77 K. The study assessed in vivo potassium ($^{39}$K) levels in healthy and stroke-induced rat brain. Therapeutic agents targeting the impaired BBB observed in stroke may crucially mitigate the subsequent secondary brain damage. Neurotrophins apart from being involved in neuronal survival and synaptic plasticity, have shown to mitigate brain ischemia by limiting BBB permeability increase. Chapter 4 explored the role of neurotrophins (pigment epithelial-derived factor, PEDF and epidermal growth factor, EGF) in rats subjected to 1h of MCA occlusion followed by reperfusion. Effect of intravenous infusion of either EGF, PEDF or saline initiated 3 h post reperfusion were studied on infarct evolution and blood-brain permeability parameters using MRI at 1, 2, 4 and 7 days post reperfusion.

SUL121, a novel chromanol-based compound (Sulfateq BV, Groningen, NL), has been shown to mitigate oxidative stress and inflammation in various in vitro and in vivo models including forced hypothermia-rewarming injury in rats by maintaining the level of H$_2$S synthesizing enzymes (Dugbartey et al. unpublished). In addition, SUL121 improved systemic vascular function. We therefore aimed in chapter 5, to explore the putative effects of SUL121 on cerebral ischemic damage and its modulation on aortic function in tMCAO in rat. Finally, we explored hypothermia injury to cultured microglia, as targeted temperature management (TTM) has been shown to have promising results in mitigating brain damage in pre-clinical models and is now undergoing clinical trials in various centers worldwide. However, hypothermia-mediated neuroprotection carries a risk of re war ming-induced adverse effects akin to reperfusion injury due to excess oxidative stress. Hence,
targeting these effects need to be addressed and relevant therapeutic measures adopted to prevent such injury. Previous studies have shown dopamine to act in mitigating oxidative stress upon hypothermia-rewarming-induced cellular and tissue (non-neural) injury, mediated via the H₂S signaling cascade (65,66). In this regard, we employed a murine microglial cell line in chapter 6 to investigate protective properties and the underlying mechanisms of exogenously administered dopamine in hypothermia-rewarming injury.

REFERENCES

(18) Gu WG, Jiang W, Brannstrom T, Wester P. Long-term cortical CBF recording by laser-Doppler


(60) Tang XN, Liu L, Koike MA, Yenari MA. Mild hypothermia reduces tissue plasminogen activator-related hemorrhage and blood brain


