Experimental focal cerebral ischemia
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Chapter 7

Summary & General Discussion
SUMMARY & GENERAL DISCUSSION

Stroke is the leading cause of adult disability worldwide and the burden is predicted to rise from 38 million DALYs (disability adjusted life years) in 1990 to 61 million DALYs in 2020. Ischemic stroke accounts for around 80% of all stroke cases. In chapter 1, we outline the burden and recent terminology of stroke in general. Moreover, pathophysiological mechanisms underlying ischemic stroke and current treatment strategies are reviewed. In order to mimic some of the underlying mechanisms, pre-clinical modeling of ischemic stroke, especially in rodents, has been established for over 30 years. Current models have been focusing on induction of transient and/or permanent cerebral ischemia employing various approaches such as blood clot/micro-macrosphere injection, silicon coated tip filaments and craniectomy-based electrocoagulation of relevant brain arteries. In chapter 1, these methods are outlined with a prime focus on filament-based approach and its relevant modifications. However, despite substantial basic and clinical research efforts, to date the only effective therapeutic measures are early revascularization [(mostly by systemic thrombolysis using recombinant tissue plasminogen activator (rtPA)] and supportive care aimed at reducing acute complications (stroke unit concept, decompressive craniectomy in case of malignant stroke) (1). While a large body of literature has demonstrated substantial therapeutic success of many additional interventions in experimental models, the translation of these basic research findings has led to discouraging results in clinical trials (2,3). Hence, developing appropriate pre-clinical models and their optimization is needed to ensure better translation.

The studies in the current thesis can be broadly divided into two parts. Firstly, in chapters 2 and 3, we introduced a rodent focal cerebral ischemia model of occlusion of the middle cerebral artery (MCA) as a tool to study novel therapeutics and employed a cryogenic MRI coil (optimized and developed at the Department of Computer Assisted Clinical Medicine, Mannheim) to measure tissue potassium (K⁺) level in this model. In chapter 2, we modified the current permanent focal cerebral ischemia model using an intravascular filament approach in rat with the aim to substantially improve survival. To achieve this, we re-designed the silicon coating to create a bowling pin-shaped tip filament. We demonstrate this tip to still result in a severe infarction with accompanying brain swelling, which in contrast to previous approaches is much better confined to the region supplied by the MCA while maintaining collateral blood flow across the posterior cerebral artery (PCA) supplied territory. The modification of the tip not only resulted in gross improvement of survival, the resulting infarction also displayed delayed growth of infarction. These features thus mimic those of malignant MCA stroke as encountered in the clinical setting, thereby creating a model with an acceptable mortality rate to study mechanisms underlying delayed cell death and examine effectiveness of novel
experimental drugs and cell-based therapeutics. In chapter 3, we successfully used the new occluding tip device to study electrolyte changes in the early phase of focal ischemia. We employed a cryogenic copper-based surface resonator cooled down by liquid nitrogen to 77 °K (-196 °C) using a custom built cryostat housing to measure tissue $^{39}$K signal comparing healthy and stroke-affected rat brain with a 9.4 Tesla MRI. This cryogenic coil had an improved signal-to-noise ratio (SNR) in comparison to the measurement obtained at room temperature (by a factor of 2.7 ± 0.2). Moreover, the $^{39}$K signal in the infarcted hemisphere of the rat brain was reduced by about 75 % at 77 °K in comparison to 40 % reduction at 300 °K (27 °C) in comparison with the contralateral hemisphere signifying the improved signal detection at 77 °K. The improved assessment of ionic imbalance occurring in ischemic stroke may potentially be used to study progression of ischemic damage in combination with other X-nuclei imaging (such as sodium-MRI) for future studies.

In the second part of the thesis, we explored the neuroprotective properties of novel therapeutic agents in rodent models of ischemic stroke and in an in vitro model of microglial injury. In chapter 4, we evaluated the effects of pigment epithelial-derived growth factor (PEDF) on ischemic damage and brain swelling in a transient ischemic stroke model by longitudinal MRI assessment. PEDF was found to significantly reduce the ischemic lesion volume and edema formation, which is possibly related to its potent effect counteracting VEGF-induced hyperpermeability and stabilizing tight junction (TJ) proteins. In addition, in chapter 5, the novel chromanol-based compound SUL121 was observed to mitigate ischemic damage in a focal cerebral ischemia model, to increase the level of endogenous H$_2$S synthesizing enzymes especially CBS, and to revert the impaired NO-mediated relaxation in aorta. Finally, in chapter 6, we demonstrate that exogenously administered dopamine reduces hypothermia-rewarming induced injury in cultured murine microglial (BV2) cells, which appeared to depend on a dopamine D1 receptor-mediated action activating the cAMP cascade, and upregulation of the levels of the H$_2$S synthesizing enzymes, CBS and 3-MST.

Together, these studies provided an overview of the successful modelling of cerebral ischemia in vivo, developing a non-invasive imaging tool to assess the associated ionic imbalance (improved $^{39}$K signal using a cryogenic coil) and a novel occluding filament creating a successful model of permanent ischemia representing malignant stroke. Further, we investigated potential therapeutic agents (PEDF, SUL121) for their putative neuroprotective actions and disclosed a protective effect of dopamine in an in vitro model of hypothermia-rewarming injury in relation to its property to maintain expression of key H$_2$S synthesizing enzymes.
TOWARDS OPTIMIZED MODELS OF STROKE

With less than 10% of ischemic stroke patients undergoing thrombolytic therapy, modeling chronic cerebral ischemia resulting from a substantial permanent stroke is the need of the hour enabling testing of new therapeutics (as outlined in the Fig. 1 & Table). Importantly, many experimental stroke studies feature non-progressive infarction and/or short-term outcome measures. In contrast, key outcome parameters of interventions in human stroke constitute of long-term (3-month) survival and functional recovery, as measured by the modified Rankin scale or Barthel index, which represent the ability of a patient to perform activities of daily life or the ability to communicate (4,5). Thus, similar to clinical trials, long-term survival with ischemic damage significantly involving cortical as well as striatal regions should be used as important outcome measures in experimental studies. Furthermore, long-term outcome analyses should also enable one to evaluate the success of a novel therapeutic concept or pharmacological agent.

Improving the permanent stroke model

In chapter 2, the spectacular increase of post-stroke survival and delayed growth of the infarction mimicking malignant stroke represent key major advantages of the BP-tip approach (Table) in a pMCAO model compared to the conventional filament groups. Malignant stroke is a devastating condition resulting from a blockade of blood flow either at the distal part of internal carotid artery (ICA) or proximal origin of MCA. This results in a large hemispheric ischemia involving mainly the region supplied by MCA accompanied by a massive brain swelling (6,7) and high mortality, eventually due to a herniation of brain stem. With conservative treatment, up to 80% mortality has been observed (7) and therefore malignant stroke represents a neurosurgical emergency. The neurosurgical intervention consists of a decompressive craniectomy (DC), which provides space for the expanding brain tissue and relieves the ‘malignant’ edema (6). In most instances, this highly extensive brain exploration to tackle the severe progressing ischemic damage may also contribute to the ischemia-related impairment of psychosocial state of the patients including their quality of life in the long run. In this context, there is a lack of effective pre-clinical models of malignant stroke with an improved survival for pathophysiological studies to characterize relevant therapeutics to curtail the malignant expansion of ischemia and edema. Most of these studies used the filament based MCAO to induce so-called malignant MCA stroke (8). Doerfler et al (1996) reported in rats undergoing pMCAO-induced malignant stroke using an intravascular filament a mortality rate of 35% (evident between 24 – 48 h), mainly attributed to herniation of brain stem. Although not quantified, ischemic affliction to hypothalamic region was observed in this study. Indeed, infarction of the hypothalamus
PRE-CLINICAL ISCHEMIC STROKE MODELS

Figure 1. The need for chronic stroke models. I/R, ischemia/reperfusion; MCAO, middle cerebral artery occlusion; ICA, internal carotid artery.
Table. Salient features of conventional versus our modified filament-induced middle cerebral artery occlusion in rat

<table>
<thead>
<tr>
<th>Feature(s)</th>
<th>Modified filament (BP-tip)</th>
<th>Conventional filaments (L-TB, S-TB, DOC tip)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>Markedly reduced (~12 %) at 24 h</td>
<td>Severe (~50 %) at 24-36 h</td>
</tr>
<tr>
<td>Selective MCA blockade</td>
<td>Achieved</td>
<td>Apart from MCA, can interrupt blood flow across PCA supplied territory</td>
</tr>
<tr>
<td>Vascular distortion (at base of brain)</td>
<td>None</td>
<td>Present</td>
</tr>
<tr>
<td>due to filament tip</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collateral blood flow</td>
<td>Maintained</td>
<td>Interrupted (esp. posterior circulation)</td>
</tr>
<tr>
<td>Ischemic damage</td>
<td>Moderate-severe</td>
<td>Severe (rapid progressive)</td>
</tr>
<tr>
<td>Hypothalamic involvement</td>
<td>Negligible</td>
<td>Invariably seen</td>
</tr>
<tr>
<td>Brain swelling</td>
<td>Moderate-severe</td>
<td>Severe</td>
</tr>
<tr>
<td>Long-term studies</td>
<td>Possible</td>
<td>Not possible</td>
</tr>
<tr>
<td>Ischemic damage progression and relevant</td>
<td>Possible</td>
<td>Not possible</td>
</tr>
<tr>
<td>therapeutic studies</td>
<td></td>
<td></td>
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</tbody>
</table>

BP, bowling-pin shaped tip; L-TB, long tubing-based tip; S-TB, short tubing-based tip; DOC, Doccol tip; MCA, middle cerebral artery; PCA, posterior cerebral artery

Compromising important regulatory functions including thermoregulation, likely represents a key aspect of the high mortality rate. Except for data published in chapter 2, we have also been able to image the progression of ischemic damage and tissue remodeling in BP-tip occluded rats employing longitudinal MR imaging even up to 18 days, with the hypothalamus being completely spared from the damage (Fig. 2, unpublished data). Furthermore, the main alternative method to filament inclusion, i.e. craniectomy based pMCAO may result in alterations of intracranial pressure, hemodynamics and local brain temperature affecting the development of ischemic damage which localizes at a site supplied by the distal MCA branches compared to filament-based occlusion which blocks proximal branch of MCA. Moreover, specific techniques such as macrosphere injection result in a highly variable infarction size. Together these features restrict the suitability of such pMCAO models to mimic malignant MCA stroke. Thus, our model allows exploration of the potential of novel therapeutics mitigating the rapid progression in infarct size and brain swelling, such as those limiting metabolic and ionic derailment, tissue hypoxia, cortical spreading depression/peri-infarct depolarizations (CSD/PID), blood-brain barrier integrity or improving brain collateral pathways. Patients at-risk (with an absence of
secondary effects due to early reperfusion) for developing malignant stroke may be administered with agents acting against one or more of the above events to curtail the impending infarct growth.

![Figure 2. T2-weighted images depicting ischemic damage in a rat brain with a right-sided permanent middle cerebral artery occlusion using a BP-tip filament assessed longitudinally from day 1 to day 18. Note that the hypothalamic region (arrows) is spared from ischemic damage. BP, bowling pin-shaped tip.](image)

**Improving selection and follow-up by MRI monitoring of ionic imbalance**

In the past decades, several therapeutic agents with proven efficacy in pre-clinical transient MCAO models failed to improve outcome in permanent MCAO models. One factor explaining this discrepancy might be that interventions target reperfusion damage in tMCAO, which is intrinsically absent in pMCAO models. In the former model, mechanisms such as ionic dysfunction, blood-brain barrier (BBB) dysfunction, metabolic failure, tissue hypoxia could be of relevance. Ionic disbalance is one of the initial consequences of an ischemic insult. Ischemia, such as in acute stroke, causes abnormal or impaired energy metabolism, disturbing the Na\(^+\)-K\(^+\)-ATPase and interrupting the ionic transport across the cells (9), leading to an increase in the intracellular Na\(^+\) and extracellular K\(^+\) concentration.

In **chapter 3**, we were able to measure brain tissue K\(^+\) in healthy and ischemic rat brain using a custom built liquid nitrogen cooled cryogenic copper-based surface resonator coil designed for small animal imaging in a 9.4 Tesla MR scanner. Brain extracellular potassium (K\(^+\)), a key cation, is regulated by factors such as neuronal activity, energy production by
the cells, clearance by circulating blood, as well as redistribution of potassium in the extracellular space. These factors which control extracellular K⁺ in physiologically functioning brain are impaired during ischemia. The development of the described K⁺-based coil was preceded by the establishment of a triple resonant MR coil by the same group (10) for detection of Na⁺ and K⁺ along with acquisition of anatomical proton images (¹H) coupled in a single set up for rat head in vivo, although with a very low gain in ³⁹K signal detection. We showed for the first time that the cryogenic cooling of a surface resonator coil to significantly improve the signal-to-noise (SNR) ratio and measure ³⁹K signal in vivo. The coil seems apt to quantify progression of cerebral ischemia as observed in our modified pMCAO model previously described in chapter 2. Moreover, pathophysiological substrates involved in such a progression could lead to developing therapeutic targets, for instance in patients with malignant stroke. Several clinical studies have observed the occurrence of episodic cortical spreading depression (CSD) to play a significant role in progression of infarct size. Failure in brain ion homeostasis represents a key feature of CSD as evident by a marked increase in extracellular K⁺ with a reduction in Ca²⁺, Cl⁻ and Na⁺ shrinking the extracellular space due to water movement into neurons, in response to other pathophysiological features such as altering BBB permeability and a massive release of excitatory amino acid such as glutamate (9). This excess glutamate in turn interacts with its receptors, mainly NMDA receptor (NMDAR) to trigger K⁺ release causing further depolarization of neurons propagating to neighbouring regions and initiate further cyclical episodes. In patients with malignant stroke, occurrence of such numerous episodes of depolarizations around the infarct core (peri-infarct depolarizations, PID) can lead to infarct progression (11,12,13). The development of a non-invasive method to ascertain the viability of ischemic tissue by assessing its ionic imbalance in acute stroke can be of relevance in two major ways: a) it would allow the identification of patients that do not meet the “clinical window of opportunity” but that still have a significant volume of viable tissue eligible for treatment with rtPA, and b) it enables the possibility of longitudinal assessment of potential interventions. Although cryogenic resonator coils have been successfully developed for human imaging (14), adopting a similar set up specifically for brain imaging could be of immense value.

NOVEL DIRECTIVES IN ISCHEMIC STROKE THERAPEUTICS

Although there are multiple reasons for the translational failures, a key aspect in pre-clinical testing has primarily focused on the tMCAO model which is claimed to simulate up to 10% of the clinical cases where early recanalization is established. Rapid reperfusion as encountered after thrombolytic therapy, although desired, may contribute to a secondary
injury via a cascade of events including hemodynamic disturbances, inflammatory processes, free radical formation and breakdown of the BBB. On the other hand, the filament-induced MCAO in our study resulting in a permanent stroke (without reperfusion) led to a gradual development of the ischemic damage peaking up to 72 h post occlusion. Such a scenario represents patients who are ineligible for thrombolysis. In such an instance, lesion progression may depend on several factors such as near-complete lack of oxygen and nutrient supply for the regions distal to the occlusion, depolarization waves triggered from the infarct core expanding over time and a compromised collateral blood flow unable to feed the at-risk tissue. Although an intricate molecular mechanisms interplay underlie the pathophysiology of ischemic stroke, one should comprehend its complexity to choose the type of therapeutic agent, appropriate time window for its administration and potential influence on endogenous molecular pathways, which if affected, could lead to adverse effects affecting the ultimate neurological outcome.

**Potential therapeutic targets in tMCAO model**

We demonstrated in chapter 4, that application of pigment epithelial-derived factor (PEDF) between 24 hours and 1 week after reperfusion resulted in a significant reduction in lesion size along with alleviated BBB opening in a rat model of transient MCAO (90 min) as evident from longitudinal MR imaging. BBB permeability is a critical issue and poses a hurdle in the research and development of new therapies for acute ischemic stroke. Stroke has however been shown to compromise the barrier function of BBB (15). Therapeutic agents targeted in alleviating the increased BBB permeability could in turn mitigate the progression of delayed ischemic damage and secondary injury. In this context, several growth factors such as erythropoietin (EPO), G-CSF, BDNF, IGF-1 have shown protection in pre-clinical models of ischemic stroke including mitigating BBB permeability (16,17). PEDF has been shown to have conflicting results in mitigating damage in various pre-clinical ischemic stroke models. A recent study by Zille et al (2014) in a mouse tMCAO model failed to show any protection on ischemic damage, cell death or behavioural outcome upon a continuous infusion of PEDF via a cannula placed in lateral ventricle (18). On the other hand, Sanagi and co-workers (2008) demonstrated that PEDF overexpression was neuroprotective in a rat tMCAO (70 min) model and damage assessed 24 h after reperfusion (19). The authors injected an adenoviral vector containing the PEDF gene directly into the striatum. The variable results can be attributed to the different treatment approaches employed in these studies. A critical question whether administration of growth factors to mitigate BBB dysfunction also influences endogenous trophic factors or whether their treatment needs to be initiated only during the repair phase are to be addressed.
While exogenous growth factor therapy has been considered a promising avenue for treating ischemic brain injury, more studies are necessary to elucidate optimal timing, dosing and mode of administration, as well as possible combinations of growth factors and their effects when combined with current management strategies. Clinical studies in this regard have been unsuccessful. For instance, the phase II/III German Multicenter EPO Stroke Trial failed to show a beneficial effect on the neurological outcome in stroke patients when treated with either EPO or placebo within 6 h of symptom onset. Some of the factors attributed to this failure include overestimated efficacy of EPO in pre-clinical studies, lack of studies employing combinational use of rtPA with EPO and untoward side effects of EPO (20,21). The recent AXIS-2 trial, which tested 3 days i.v. infusion of granulocyte-colony stimulating factor (G-CSF) in patients with acute ischemic stroke assessing its efficacy 90 days after stroke, was unsuccessfully concluded after clinical Phase IIb trials (22), despite optimistic conclusions of a systematic review of the corresponding preclinical data (23) and successful completion of a clinical Phase I trial (24). Many of the growth factors share intracellular pathways and their exogenous administration can lead to altered expression of endogenously produced factors. In this regard, our modified stroke model can act as a tool in characterizing the potential use of growth factors, especially in sub-acute to chronic phase post occlusion.

In chapter 5, we administered SUL121 intravenously to rats undergoing cerebral ischemia-reperfusion injury to investigate its putative protective effects on brain damage as well as on systemic vascular function. We chose a pre-treatment regime as we wanted to investigate whether SUL121 infusion would have an effect at all in our model. Similar to a recent study (Dugbartey et al., unpublished), SUL121 treatment was shown to upregulate the level of CBS, while the infarction itself reduced the level of 3-MST. Moreover, SUL-treated animals improved the ACh-mediated aortic relaxation compared to saline-treated control animals, potentially acting via preserving NO-mediated vasorelaxation. In general, cerebral ischemia led to an impairment of NO-mediated vasorelaxation in rat aortas. Besides, the excess relaxation could be contributed by boosting relaxant prostanoids specifically by SUL121. In line with published studies showing debatable results with exogenous H\textsubscript{2}S donors (25,26,27), establishment of the role of boosting of CBS by SUL121 on brain ischemia and systemic vasculature needs further studies by employing relevant blockers of endogenous H\textsubscript{2}S production or knock-out models. Nevertheless, the action of H\textsubscript{2}S on cerebral ischemia remains controversial. Exogenously administered H\textsubscript{2}S (e.g. NaHS) particularly in rodent models of cerebral ischemia have been reported both to mitigate (26) as well as to aggravate the ischemic lesion (28) in a concentration-dependent manner. In line with the effects of exogenously administered H\textsubscript{2}S, inhibition of endogenous H\textsubscript{2}S production (AOAA, a non-specific inhibitor of H\textsubscript{2}S synthesizing enzymes) at a high dose (0.5 mmol/kg, intraperitoneally) aggravated the damage in rats subjected to
transient MCAO under chloral hydrate anesthesia, while a low dose (0.025, 0.05, 0.1 mmol/kg) significantly reduced the infarction (29). The endogenous H$_2$S under physiological conditions is known to act as a neuromodulator during NMDAR-mediated responses necessary for long-term potentiation involved in memory consolidation (30). Hence, either boosting endogenous H$_2$S or inhibiting its production to the extremes may induce deleterious effects in brain ischemia and may warrant optimal dosing to be efficacious.

Importantly, the experimental protocol in chapter 5 resulted in small brain infarct sizes both in control as well as in treated groups 24 h post MCAO. As discussed in the chapter, this mitigation of infarct size is likely attributed to the anesthetic regimen employed wherein the animals were exposed to isoflurane even during the occlusion period. Therefore, isoflurane itself may be used to mitigate ischemia development, as shown in various cerebral ischemia models (31). Particularly, pre- as well as post-conditioning effects of isoflurane are prime candidates that may have been involved in the apparent neuroprotective action seen in our study. Besides, it would be apt to explore whether isoflurane per se influences the endogenous level of H$_2$S synthesizing enzymes in brain. Thus, an interplay between prolonged exposure to isoflurane, the endogenous H$_2$S system, vascular NO regulation and the effects of SUL121 may exist and needs to be extensively studied.

**Potential therapeutic options in pMCAO model**

Permanent vascular blockade leads to cessation of blood supply resulting in tissue hypoxia. Several pre-clinical studies have been inconclusive in depicting a significant improvement using agents carrying oxygen (artificial oxygen carriers) when administered under normobaric oxygenation (NBO) conditions or when treated with NBO or hyperbaric oxygenation alone (32). While their clinical translation has not led to a satisfactory improvement in the neurological outcome, new generation oxygen carriers are currently being developed. On the other hand, inhibiting the depolarization waves using glutamate receptor (primarily NMDAR) antagonists has shown some promising results in pre-clinical models. However, the clinical translation of these antagonists has failed due to their psychotropic effects (33). Besides, NMDAR-mediated responses play a crucial role in post stroke recovery and hence, inhibition in the acute phase can worsen the outcome. Currently, novel antagonists (eg. PSD95 protein inhibitors) are being developed which circumvent adverse effects by blocking pathways such as free radical generation produced by the neuronal nitric oxide synthase (nNOS) without blocking NMDARs (34). PSD95 inhibitors have shown promising results in both tMCAO as well as pMCAO models (35) including in a non-human primate model of pMCAO (36).
Apart from targeting hypoxia and the generation of depolarization waves, gaseous anesthetics, particularly isoflurane show cerebroprotective effects as revealed by various pre-clinical studies of cerebral ischemia and hemorrhage. However, whether the protection is sustained is still controversial. A recent study by Kim et al (2015) revealed post treatment of isoflurane to mitigate the ischemic damage in a tMCAO model treated with rtPA upon reperfusion (37). Besides, rats subjected to tMCAO using ketamine/xylazine showed a significantly higher volume of ischemic damage compared to those undergoing occlusion under isoflurane (38). Potential actions of isoflurane include preconditioning effect leading to ischemia tolerance in rat brain by activating p38 MAPK (39), reduced glutamate release and enhancing GABA receptor-A mediated hyperpolarization (40), and inhibiting the activation of TLR 4 pathway (41). Moreover, due to a cerebral vasodilatory effect of isoflurane as evident from clinical studies (42,43), a possible role of inverse steal effect cannot be ruled out in an ischemic brain wherein the blood from the non-ischemic (contralateral) hemisphere is diverted to the ischemic side.

Although most the above mentioned studies have been performed in tMCAO models, exploring isoflurane effects in pMCAO models could be of relevance from a clinical perspective and warrants further exploration. For instance, the inverse steal effect of isoflurane could aid in improving collateral blood flow in ischemic hemisphere in cases of malignant stroke. An immediate possibility would be to administer isoflurane as a sedative or blood pressure lowering agent in an ICU setting under intense cardiac and respiratory monitoring, particularly in patients at-risk of developing malignant infarction. Furthermore, investigating the effects of isoflurane (only) treatment on the endogenous gasotransmitters (NO, CO and H$_2$S) in the CNS could be of major relevance to reveal potential interplay in such pathological states.

**Boosting H$_2$S – a potential factor to mitigate hypothermia-rewarming associated adverse effects**

At the end, in chapter 6, we observed that murine (BV2) microglial cells revealed a significant reduction in their viability when exposed to HR injury. Levels of CBS and 3-MST were significantly downregulated upon the insult. In this scenario of reduced viability and downregulated CBS and 3-MST levels, dopamine treatment was shown to rescue the cells and upregulate the enzyme levels. Furthermore, dopamine was shown to mediate this protection via a D1 receptor type-mediated action, but unaffected by its reuptake inhibition. This is in contrast to previous studies (44,45). The protective effect of dopamine in our study was primarily observed in brain microglial cells in contrast to the above studies, which employed non-neural cells. The interplay between the H$_2$S cascade –
hypothermia/rewarming – dopamine warrants further studies which could be of immense relevance when considering hypothermia as a therapeutic approach in CNS disorders.

Targeted temperature management (TTM) is currently gaining attention in the management of CNS disorders especially trauma and ischemia. Although mild to moderate degree of hypothermia is widely accepted in clinics, severe hypothermia in accidental cases (with a core body temperature reaching upto 15-20 °C) has been nevertheless reported worldwide. The latter can have deleterious effects especially on the CNS upon rewarming. In line with our preliminary in vitro results, boosting the endogenous levels of H₂S synthesizing enzymes in CNS, primarily CBS (abundantly expressed in brain), can be an alternative to mitigate such adverse effects and dopamine could prove to be a potential candidate.

With sparse literature on potential protective effects of dopamine to mitigate CNS injury, studies have revealed mixed results (46). One such study by Park and co-workers (2003) (47) observed dopamine infusion to prevent cerebral ischemia by maintaining adequate cerebral perfusion pressure while attenuating brain injury in a neonatal swine model of bacterial meningitis. Further, administration of levodopa (a dopamine precursor) initiated 2 days after tMCAO and continued for 12 consecutive days significantly improved sensorimotor function in rats undergoing tMCAO, however, without affecting the ischemic damage (48). Moreover, in a clinical study, a single dose levodopa and physiotherapy (49) improved motor recovery in thrombo-embolic stroke patients. In addition, levodopa treatment improved procedural motor learning in patients with chronic stroke (≥1 y after stroke) in comparison to placebo treated group (50). Nonetheless, further studies are warranted to investigate molecular pathways underlying ischemia progression spatio-temporally, especially in a permanent stroke model.

PARADIGM SHIFT IN STROKE RESEARCH – NEED OF THE HOUR

It has been more than 30 years since the inception of pre-clinical modelling of cerebral ischemia (51) to mimic features of clinical ischemic stroke. Although numerous studies have been able to delineate the underlying pathophysiological mechanisms and decipher novel targets, clinical translation for potential neuroprotectants (esp. blockade of incipient neuronal cell death) has failed until now except for thrombolytic intervention. The strive for an ideal pre-clinical stroke model still continues (51). Several guidelines have been put forth to improve pre-clinical models of ischemic stroke, bridge the gap between the pre-clinical research and human trials, and accelerate the evolution of stroke therapeutics.
including the design and implementation of stroke trials (52) with a focus on pre-clinical multi-centric ischemic stroke trials (53). Exploring avenues such as induced pluripotent stem cell (iPSC)-derived neuronal and glial cells from stroke patients for prognostic as well as for drug screening, gut-brain axis and hypoxia-tolerant species could pave the way to overcome the translational roadblock.

One major aspect of improving pre-clinical models encompasses the study of the pathophysiology of chronic ischemic stroke and the development of relevant therapeutics. Our pMCAO model induced by BP-tip filament can be of substantial relevance. Importantly, this model allows the delineation of mechanisms pertaining to ischemia alone without the interference of events related to reperfusion injury. Clinically, such a condition would be more akin to majority of patients who are unable to undergo thrombolysis. Our model can thus assist in testing novel therapeutics in such cases which are crucial in targeting specific molecular events evident in ischemia-only condition with the absence of reperfusion-mediated injury.

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