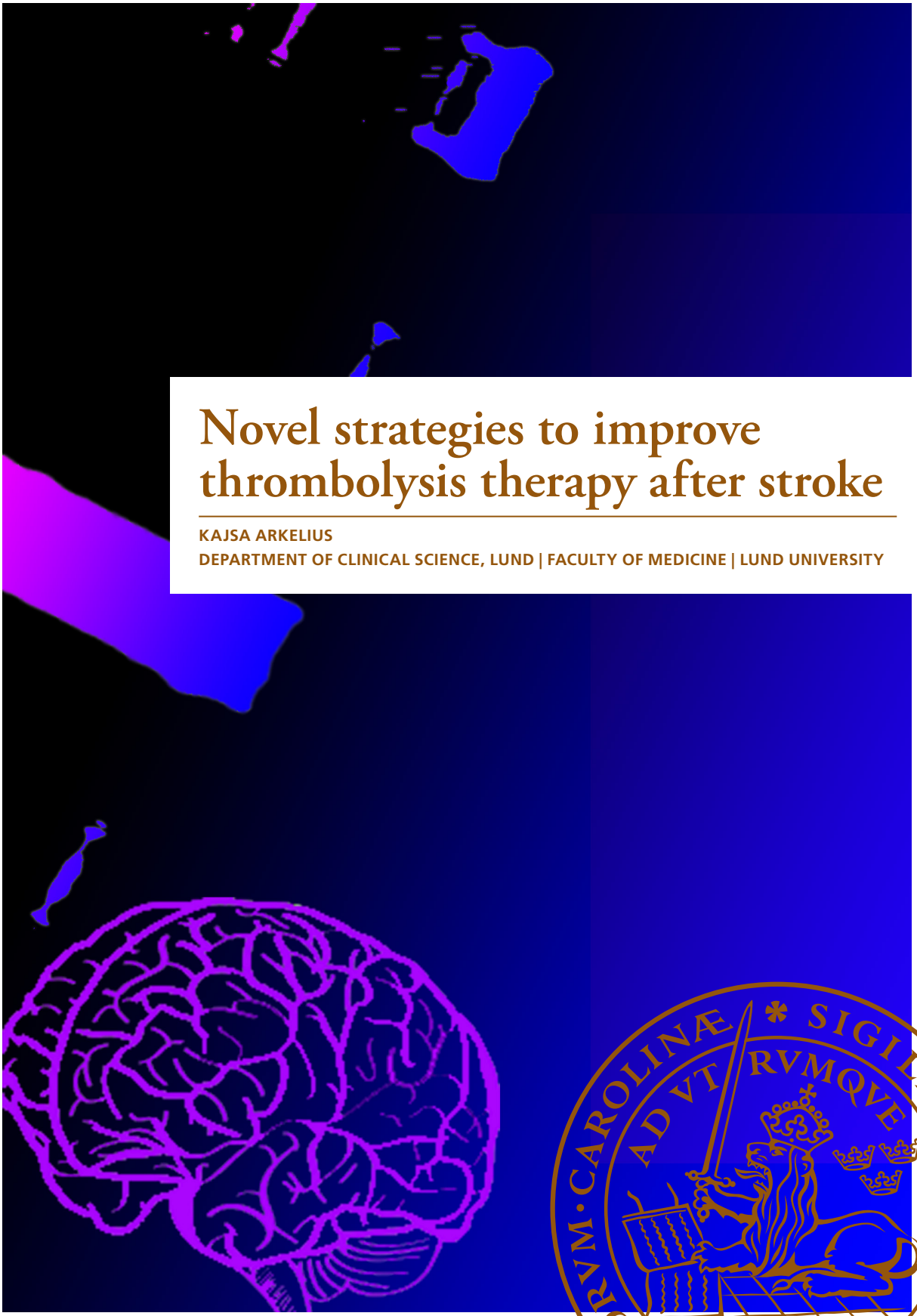
Novel strategies to improve thrombolysis therapy after stroke

Arkelius, Kajsa

2022

Document Version:
Publisher's PDF, also known as Version of record



Novel strategies to improve thrombolysis therapy after stroke

KAJSA ARKELIUS
DEPARTMENT OF CLINICAL SCIENCE, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

Novel strategies to improve thrombolysis therapy after stroke

Kajsa Arkelius

LUNDS
UNIVERSITET

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Segerfalkssalen, BMC, Lund, Sweden. Thursday 16th of June 2022 at 09.00 AM.

Faculty opponent
Professor Milos Pekny
Institute of Neuroscience and Physiology
University of Gothenburg, Sweden

Organization	Document name
LUND UNIVERSITY	DOCTORAL DISSERTATION
Author Kajsa M. Arkelius	Date of issue 2022-06-16

Title and subtitle
Novel strategies to improve thrombolysis therapy after stroke

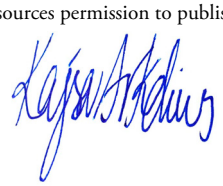
Abstract

Ischemic stroke is one of the leading causes of death and disability worldwide, with limited treatment options. The only available drug treatment for acute ischemic stroke is the recombinant tissue-Plasminogen Activator (rt-PA). Still, its use within the clinic is heavily restricted. Beyond the treatment's beneficial effects, administration of rt-PA increases the risk of hemorrhagic transformation. The incidence of a hemorrhagic event can cause worsened stroke outcomes and even death, and its occurrence correlates with increased matrix metalloproteinase 9 (MMP-9) levels. The protease can break down the blood-brain barrier (BBB), explaining the rt-PA's increased risk of hemorrhagic transformation. The MMP-9s expression can be triggered by the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, which is activated by the lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1).
Less than 10 % of all ischemic stroke patients receive treatment with rt-PA, and evidence demonstrates that reperfusion therapy in acute ischemic stroke can be beneficial up to 24 h after stroke onset. Therefore, aims this thesis to enhance rt-PA therapy and improve stroke outcomes in the acute phase of ischemic stroke.

Firstly, we established a thromboembolic stroke model in rats that closely resemble the human ischemic stroke and mimics the effects of rt-PA therapy in terms of infarct formation, neurological function, and hemorrhagic incidence. The clinical relevance of the model enables translational investigations of stroke pathophysiology and evaluation of new therapeutic methods to enhance the existing treatment.
Next, we showed that inhibiting ERK1/2 activation in combination with rt-PA therapy reduces the expression of MMP-9 and prevents the rt-PA-induced risk of hemorrhagic transformation in mice. While the ERK1/2 inhibitor did improve the safety of rt-PA treatment, it did not improve stroke outcomes in terms of infarct volume.
Finally, we evaluated two different MMP-9 inhibitors in combination with rt-PA therapy: a LOX-1 inhibitor that blocks the increase of MMP-9 expression and or a direct MMP-9 inhibitor that prevents its activation. Both inhibitors, combined with rt-PA treatment, demonstrated a protective effect against the hemorrhagic transformation of the stroke. Furthermore, treatment with the LOX-1 inhibitor conjugated with rt-PA also showed a significant improvement in neurological function.
This thesis introduces a novel way of improving thrombolysis therapy in acute ischemic stroke care.

Key words
Ischemic stroke, Thrombolysis, rt-PA, MMP-9, ERK1/2, LOX-1, BBB

Supplementary bibliographical information		**Language** English
ISSN and key title 1652-8220		**ISBN** 978-91-8021-262-5
Recipient's notes	**Number of pages** 75	Price
	Security classification	

Signature  Date 2022-05-09

Novel strategies to improve thrombolysis therapy after stroke

Kajsa Arkelius

LUNDS
UNIVERSITET

Cover photo by Kajsa Arkelius

Faculty of Medicine
Department of Clinical Science, Lund

Lund University, Faculty of Medicine Doctoral Dissertation Series 2022:102
ISBN 978-91-8021-262-5
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

"All we have to decide is what to do with the time that is given us."

— J.R.R. Tolkien

Table of contents

Abbreviations

ATP	Adenosine triphosphate
BBB	Blood-Brain Barrier
ERK1/2	Extracellular signal-regulated kinase 1/2
dsDNA	Double-stranded deoxyribonucleic acid
HBMECs	Human brain microvascular endothelial cells
HGD/R	Hypoxia + glucose deprivation-reperfusion
LOX-1	Lectin-like oxidized low-density lipoprotein receptor-1
MAPK	Mitogen-activated protein kinases
MCA	Middle cerebral artery
MEK1/2	Mitogen-activated protein kinase kinase 1/2
MERCI	Mechanical Embolus Removal in Cerebral Ischemia
MMP	Matrix metalloproteinase
MMP-9	Matrix metalloproteinase 9
MRI	Magnetic resonance imaging
NF-κB	Nuclear factor kappa B
oxLDL	Oxidized low-density lipoprotein
rt-PA	Recombinant tissue-Plasminogen Activator
tMCAO	Transient middle cerebral artery occlusion

List of Original Articles

This thesis is based on the following papers:

I. Validation of a stroke model in rat compatible with rt-PA-induced thrombolysis: new hope for successful translation to the clinic.

Kajsa Arkelius, Denis Vivien, Cyrille Orset, Saema Ansar

Scientific Reports 10, 12191 (2020).

II. Combination treatment with U0126 and rt-PA prevents adverse effects of the delayed rt-PA treatment after acute ischemic stroke

Cyrille Orset, **Kajsa Arkelius**, Antoine Anfray, Karin Warfvinge, Denis Vivien, Saema Ansar

Scientific Reports 11, 11993 (2021).

III. LOX-1 and MMP-9 inhibition attenuate the detrimental effects of delayed rt-PA therapy and improve outcomes after acute ischemic stroke

Kajsa Arkelius, Trevor S Wendt, Henrik Andersson, Anaële Arnou, Rayna J Gonzales, Saema Ansar

Manuscript

Summary

Ischemic stroke is one of the leading causes of death and disability worldwide, with limited treatment options. The only available drug treatment for acute ischemic stroke is the recombinant tissue-Plasminogen Activator (rt-PA). Still, its use within the clinic is heavily restricted. Beyond the treatment's beneficial effects, administration of rt-PA increases the risk of hemorrhagic transformation. The incidence of a hemorrhagic event can cause worsened stroke outcomes and even death, and its occurrence correlates with increased matrix metalloproteinase 9 (MMP-9) levels. The protease can break down the blood-brain barrier (BBB), explaining the rt-PA's increased risk of hemorrhagic transformation. The MMP-9s expression can be triggered by the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, which is activated by the lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1).

Less than 10 % of all ischemic stroke patients receive treatment with rt-PA, and evidence demonstrates that reperfusion therapy in acute ischemic stroke can be beneficial up to 24 h after stroke onset. Therefore, aims this thesis to enhance rt-PA therapy and improve stroke outcomes in the acute phase of ischemic stroke.

Firstly, we established a thromboembolic stroke model in rats that closely resemble the human ischemic stroke and mimics the effects of rt-PA therapy in terms of infarct formation, neurological function, and hemorrhagic incidence. The clinical relevance of the model enables translational investigations of stroke pathophysiology and evaluation of new therapeutic methods to enhance the existing treatment.

Next, we showed that inhibiting ERK1/2 activation in combination with rt-PA therapy reduces the expression of MMP-9 and prevents the rt-PA-induced risk of hemorrhagic transformation in mice. While the ERK1/2 inhibitor did improve the safety of rt-PA treatment, it did not improve stroke outcomes in terms of infarct volume.

Finally, we evaluated two different MMP-9 inhibitors in combination with rt-PA therapy: a LOX-1 inhibitor that blocks the increase of MMP-9 expression and or a direct MMP-9 inhibitor that prevents its activation. Both inhibitors, combined with rt-PA treatment, demonstrated a protective effect against the hemorrhagic transformation of the stroke. Furthermore, treatment with the LOX-1 inhibitor conjugated with rt-PA also showed a significant improvement in neurological function.

This thesis introduces a novel way of improving thrombolysis therapy in acute ischemic stroke care.

Popular science summary

Every year, fifteen million people suffer a stroke. In 80% of cases, the stroke is due to a blood clot getting stuck in one of the brain's blood vessels. The blood clot restricts blood flow to the whole or a part of the brain, and within minutes, brain cells will begin to die due to oxygen deficiency. The dying cells will give rise to a permanent brain injury, leading to loss of speech, movement, mental function, and even death. The only way to save the dying cells is to break down the blood clot and restore the blood flow within the vessel. Currently, only one available anticoagulant drug can be given when a blood clot arises that has the ability to prevent further brain injury. However, its use within healthcare is highly restricted.

Treatment with the anticoagulant may only be administered within 4.5 hours from stroke onset due to the risk of other complications. Beyond the drug treatment potential of restoring the blood flow to the brain, it can also cause deadly complications such as a brain bleed. The narrow time window of the treatment limits the number of patients who can receive treatment drastically, which today is less than 10 % of all stroke patients.

This thesis aims to enhance the anticoagulant therapy and improve stroke outcomes. It has been shown that the drug, in addition to its anticoagulant properties, also affects the body to produce a particular protein called matrix metalloproteinase 9 (MMP-9). This protein can break down the vessel walls in the brain, causing the cerebral hemorrhage seen with the anticoagulant treatment. By blocking MMP-9 combined with the anticoagulant, we want to examine if the risk of a brain bleed is reduced.

We started by establishing an animal model that mimics the pattern of a human getting a blood clot in the brain as closely as possible. In the animal model, we saw the same positive effect of the anticoagulant in humans, but we also saw its side effect, the increased risk of a brain bleed. Seeing these similarities between the human stroke and this animal model of stroke makes it possible for us to use this model to study and evaluate new treatment options that can improve the currently available therapies.

Using the animal model, we then investigated whether we could counteract the increased risk of a brain bleed after the anticoagulant treatment, and it turned out that it was possible. By giving an MMP-9 blocker just before treatment with the

anticoagulant, we saw an apparent reduction in the risk of a cerebral hemorrhage. In addition, we found a clear improvement in the behavior of the animals that received the combined treatment, indicating that the combined treatment helped improve the stroke outcome.

This thesis demonstrates a new way of improving the currently available drug treatment for when the blood flow within the brain is obstructed due to a blood clot.

Populärvetenskaplig sammanfattning

Varje år drabbas femton miljoner människor av en stroke. I 80 % av fallen beror stroken på att en blodpropp har fastnat i ett av hjärnans blodkärl. Blodproppen begränsar blodflödet till hela eller en del av hjärnan, och inom några minuter kommer hjärnceller att börja dö på grund av syrebrist. De döende cellerna kan leda till permanenta hjärnskador som leder till förlust av tal, rörelse, mental funktion och till och med död. Det enda sättet att rädda de döende cellerna är att bryta ner blodproppen och återställa blodflödet i kärlet. För närvarande finns det endast ett tillgängligt blodproppslösande läkemedel som kan ges vid en blodpropp och har förmågan att förhindra ytterligare hjärnskada. Dess användning inom sjukvården är dock mycket begränsad.

Behandling med blodproppslösande läkemedel får endast ges inom 4,5 timmar från strokedebut på grund av risken för andra komplikationer. Utöver dess egenskap att återställa blodflödet till hjärnan, kan läkemedlet också orsaka dödliga komplikationer som hjärnblödning. Behandlingens smala tidsfönster begränsar drastiskt antalet patienter som kan få behandling, vilket idag är mindre än 10 % av alla strokepatienter.

Syftet med denna avhandling är att förbättra säkerheten för blodproppslösande läkemedel och förbättra sjukdomsutfallet. Det har visat sig att läkemedlet, förutom dess blodproppslösande egenskaper, även påverkar kroppen att producera ett speciellt protein som kallas matrix metalloproteinas 9 (MMP-9). Detta protein kan bryta ner kärlväggarna i hjärnan, vilket orsakar hjärnblödningen som ses med den blodproppslösande behandlingen. Genom att blockera MMP-9 i kombination med det blodproppslösande läkemedlet vill vi se om risken för hjärnblödning minskar.

Vi började med att etablera en djurmodell som så nära som möjligt följer samma mönster som när en människa får en blodpropp i hjärnan. I djurmodellen såg vi samma positiva effekt av det blodproppslösande läkemedlet på människor, men vi såg också dess bieffekt, den ökade risken för en hjärnblödning. Att se dessa likheter mellan den mänskliga stroken och denna djurmodell av stroke, gör det möjligt för oss att använda denna modell för att studera och utvärdera nya behandlingsalternativ som kan förbättra de för närvarande tillgängliga läkemedlen.

Med hjälp av djurmodellen undersökte vi sedan om vi kunde motverka den ökade risken för hjärnblödning efter den propplösande behandlingen, och det visade sig att

det var möjligt. Genom att ge ett läkemedel som motverkar MMP-9 strax före behandling med det propplösande läkemedlet såg vi en tydlig minskning av risken för hjärnblödning. Dessutom fann vi en tydlig minskning av hjärnskador hos djuren som fick den kombinerade behandlingen, vilket tyder på att den bidrog till att förminska strokens påverkan.

Denna avhandling visar ett nytt sätt att förbättra den nuvarande tillgängliga läkemedelsbehandlingen vid en blodpropp i hjärnan.

Introduction

The brain is one of nature's most incredible inventions, and it lets us develop and accomplish extraordinary things daily. Every second, 86 billion neurons within the brain receive and send 5-50 signals, allowing us to see, think, move, and imagine[1, 2]. The constant working of the brain makes it the primary consumer of energy within our bodies, and when that energy stops coming, the effects can be deadly[3].

One of the leading causes of death and disability worldwide is the cerebrovascular accident stroke[4]. A stroke causes a reduction in cerebral blood flow, preventing the brain or parts of it from receiving energy causing neuronal cell death. The stroke can be due to the rupture (hemorrhagic stroke), or a blockage (ischemic stroke) of a cerebral blood vessel, therefore reducing the cerebral blood flow[5] (Figure 1).

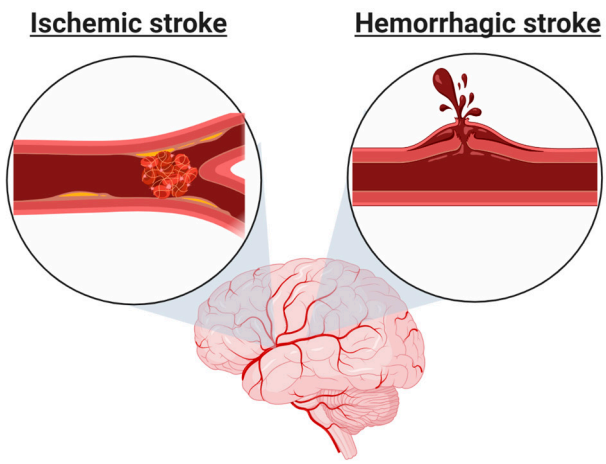


Figure 1.
A stroke can originate in two ways: an ischemic stroke caused by a blood vessel occlusion or a hemorrhagic stroke caused by a blood vessel rupture. In either instance, the stroke will reduce the blood flow within the brain and can lead to irreversible brain damage. Created with BioRender.com.

Over 80 % of all strokes are ischemic in origin[6], and while treatment options exist[7], their use within the clinic is severely limited[8]. The only available drug treatment for acute ischemic stroke is thrombolysis using the recombinant tissue-Plasminogen

Activator (rt-PA). Rt-PA can worsen stroke outcomes despite its beneficial effects due to an increased risk of hemorrhagic transformation following treatment[9, 10]. The detrimental effects of the treatment have limited its use within 4.5 hours from stroke onset[9], drastically reducing eligible patients in the clinic[8, 9].

This thesis aims to enhance rt-PA therapy's safety and improve stroke outcomes. The first part of this thesis concentrates on establishing a rat model that mimics the clinical situation focusing on thrombolysis treatment, allowing us to evaluate new treatment options in a translational stroke model. The following parts aim to assess the ability to reduce the risk of hemorrhagic transformation following rt-PA treatment by targeting matrix metalloproteinase 9 (MMP-9) activity. This specific protease correlates with the increased risk of hemorrhagic transformation of the stroke and thrombolysis treatment[11]. The generated data from this thesis add to the current knowledge and introduces a novel way to improve thrombolysis therapy after stroke.

Ischemic stroke

Ischemic stroke affects over nine million people each year[12]. The occurrence of ischemic stroke is due to the blockage of a cerebral blood vessel, reducing blood flow and preventing the brain from receiving oxygen and glucose[5, 13]. The vessel occlusion can originate from several causes: thromboembolisms, small vessel disease, vasculitis, and arterial dissections[5, 13]. Thromboembolisms stand for most ischemic strokes and are formed primarily by atherosclerosis or cardiac events, predominantly arterial fibrillation[14]. When an occlusion occurs, it will give rise to a hypoperfused area defined as the penumbra[15]. The penumbra quickly becomes hypoxic due to the brain's constant consumption of oxygen, initiating a detrimental reaction of molecular and cellular events known as the ischemic cascade[16]. If blood flow is not restored, the neurons will begin to die within minutes generating the ischemic core[15](Figure 2).

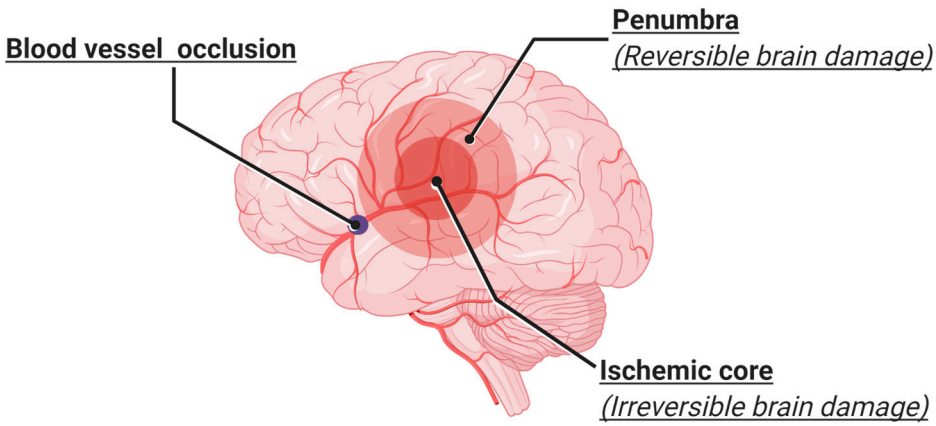


Figure 2.
The occlusion of a cerebral blood vessel will give rise to an ischemic core and an ischemic penumbra. Following vessel occlusion, neurons will start to die within minutes, forming the ischemic core, a region with irreversible brain damage. Surrounding the core is the ischemic penumbra, an area with reduced perfusion still within the threshold of maintaining functionality. Without reperfusion, the cells within the penumbra will cross the threshold of viability and die, transforming the penumbra into the ischemic core. Created with BioRender.com.

The longer the hypoperfusion remains, the further the ischemic core will spread until the penumbra is completely transformed into the core[17]. After the ischemic core has progressed to its maximum volume, there is very little medicine can do to improve stroke outcomes. While some spontaneous recovery occurs and rehabilitation has shown progression in reestablishing neurological function in patients to a degree, many who suffer from ischemic stroke and survive will gain permanent disabilities extending to loss of speech, movement, and cognitive function[9, 17-20].

Pathophysiology

The oxygen and glucose depletion following the occlusion significantly impairs the neuronal cells' energy production, initiating the ischemic cascade. The reduction of adenosine triphosphate (ATP) due to the lack of oxidative phosphorylation prevents the cells from maintaining their ion gradients, causing cytotoxic edema and a detrimental accumulation of calcium[21, 22]. When cytoplasmic calcium levels increase, it prompts adverse cellular responses such as excitotoxicity, membrane degradation, mitochondria damage, and apoptosis[16, 23, 24]. If the generation of ATP is not restored, stopping the ischemic cascade, the adverse cellular responses will lead to irreversible nerve damage and cell death. Furthermore, the affected cells will also start to produce free radicals and pro-inflammatory mediators, which will provoke an inflammatory

response in the brain, further sustained by the debris released by dead neurons[25]. Beyond its effect on the neuronal cells, the lack of oxygen and energy combined with increased inflammation will also influence vascular changes, disrupting the blood-brain barrier (BBB) and further progressing cerebral injury[26-28].

Blood-Brain Barrier

The BBB in the cerebral vasculature makes up a dynamic physical barrier responsible for maintaining the brain's homeostasis by separating the central nervous system from the systemic circulation. The BBB tightly regulates paracellular permeability, nutrient uptake, and ion gradients and prevents unwanted particles from infiltrating the brain. The regulation is achieved by specific endothelial cells, tight junction complexes, and the surrounding basement membrane (Figure 3)[29, 30].

Structure and function

Endothelial cell

The endothelial cells that make up the BBB are unique to their more peripheral counterparts. They contain a higher mitochondria count, less pinocytic activity, and a lack of fenestrations, all of which help with the BBB's strict regulation of paracellular and transcellular transport[31, 32]. In addition, they express lower levels of leucocyte adhesion molecules preventing inflammatory cell migration to the brain[33, 34]. Nevertheless, most prominent is their production of tight junction complexes, which connect the endothelial cells and are the primary mediators of maintaining the limited permeability of the BBB[35].

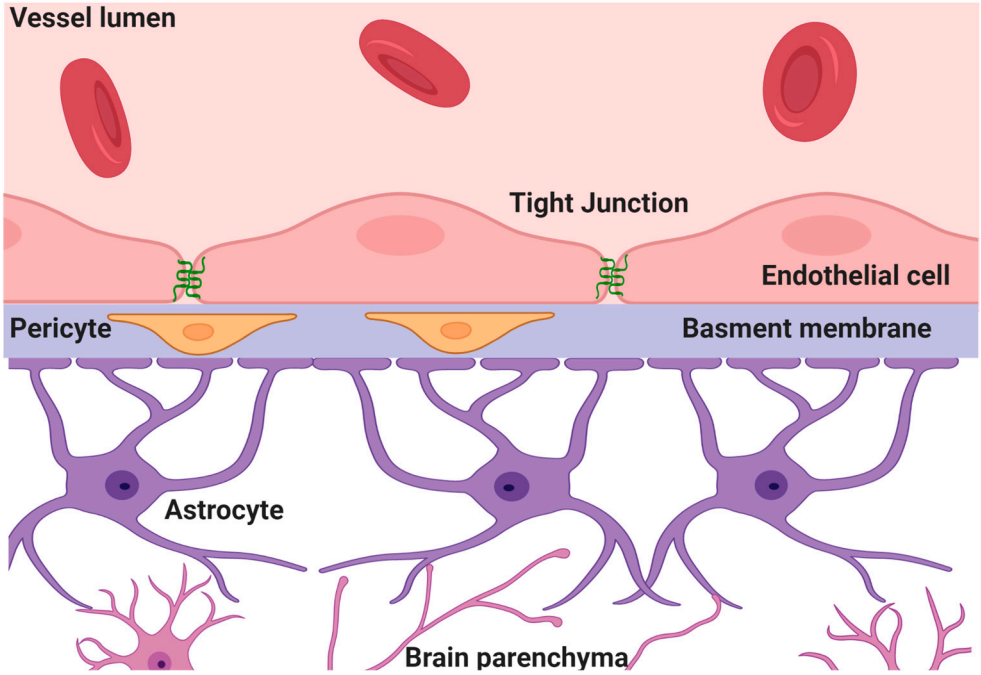


Figure 3.
Illustration of the structure of the BBB. The capillary wall is made of BBB-specific endothelial cells connected by tight junction complexes to maintain the BBB integrity. The basement membrane surrounding the endothelial cells adds structural support to the BBB. The basement membrane also ensheathes BBB supporting pericytes and connects the endothelial cells to the astrocytic endfeet. Created with BioRender.com.

Tight Junctions

Tight junctions are intercellular adhesion complexes and consist of the transmembrane proteins claudin, occludin, and junctional adhesion molecules[27, 36]. The formation of tight junction complexes between the endothelial cells controls the integrity of the BBB[37]. The cytoplasmic regions of occludin and claudin-5 connect to the cell's actin cytoskeleton through scaffolding proteins, while their extracellular regions bind to their twin protein from the adjacent cells forming the tight junction complex[27, 36-39].

Basement membrane

The basement membrane of the endothelial cells further supports the BBB integrity mainly by structural support and intercellular communication[40]. The basement membrane is composed of collagen type IV, laminin, fibronectin, and proteoglycans produced by the endothelial cells and BBB supporting pericytes and astrocytic endfeet[41-46]. The basement membrane forms the connection between pericytes, astrocytic endfeet, and the endothelial cells due to the cells' expression of integrins that grants them to bind the basement membrane components[47]. The interaction allows for

communication between the cerebral vasculature and the neuronal system, facilitating regulation of the cerebral blood flow to meet the brain's demands[44].

BBB disruption following stroke

A crucial part of the pathophysiology of ischemic stroke is the breakdown of the BBB. The ischemic injury and inflammatory processes will reduce the tight junction complexes and break down the basement membrane, increasing the BBBs permeability, which is associated with a poor clinical prognosis[28, 48, 49]. The loss of BBB integrity leads to vasogenic edema, adding to the already ongoing cytotoxic edema, which can lead to deadly outcomes such as herniation of the brain[50]. Furthermore, BBB breakdown can also cause hemorrhagic transformation of the stroke, which also causes worsened outcomes and even death[28, 51, 52].

Increased BBB permeability has been detected as early as six hours following stroke onset and is associated with increased plasma levels of claudin-5 and occludin[49, 53]. Moreover, post-mortem brains following ischemic stroke with hemorrhagic transformation show an evident degradation of collagen IV within the ipsilateral hemisphere, which correlates with increased MMP-9 levels[54].

Matrix Metalloproteinase 9

Matrix metalloproteinases (MMPs) are important proteinases that help reorganize the extracellular matrix and consist of collagenases, stromelysins, matrilysins, membrane-bound MMPs, and gelatinases[55]. Several MMPs have been studied in stroke pathology. One is gelatinase B, also known as MMP-9, which plays a vital role in the breakdown of the BBB.

Activation and function

MMP-9 is a zinc-dependent endopeptidase transcribed as a 92 kDa zymogen, an inactive pro-form. Different signaling pathways can promote its expression by activating its transcription factors, such as nuclear factor kappa B (NFκB)[56-58]. Following transcription, MMP-9s latency depends on the zinc-binding cysteine found in MMP-9s N-terminal in the pro-domain, a domain cleaved upon activation, exposing its catalytic site. Other MMPs, NO, and plasmin, achieve the pro-domain cleavage, and the active MMP-9 (82 kDa) is then further regulated by either degradation or by

its inhibitor tissue inhibitor metalloproteinase-1 (TIMP-1)[57-59]. Active MMP-9 is essential in biological processes such as wound healing, angiogenesis, tissue remodeling, and cellular migration due to its ability to break down extracellular matrix components such as collagenase IV, laminin, and fibronectin (Figure 4)[60, 61]. Though during specific pathologies, MMP-9s' involvement can be severely detrimental.

MMP-9's role in ischemic stroke pathology

During ischemic stroke, MMP-9 levels increase significantly both within the circulation and in the infarct area, with higher levels of MMP-9 expression within the microvasculature compared to the parenchyma[62]. Identical results have also been replicated in animal models of stroke[63]. The increase in MMP-9 activity is associated with an increase in BBB permeability, understandable since MMP-9 can break down the basement membrane components of the cerebral blood vessels. Furthermore, data show that beyond the basement membrane, MMP-9 also breakdowns claudin-5 and occludin, degrading the tight junctions responsible for keeping the BBB intact[64].

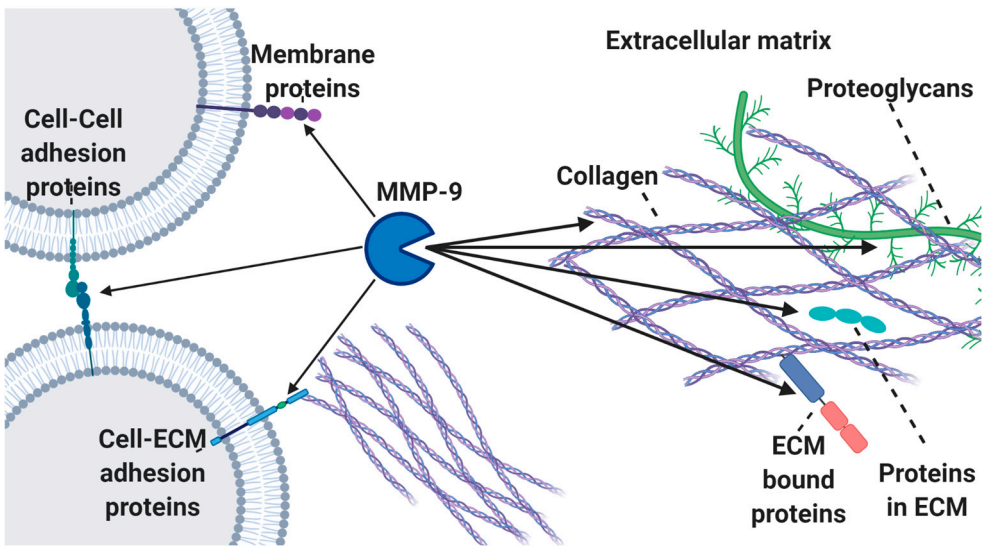


Figure 4.
MMP-9 is required in several biological processes due to its proteolytic activity. MMP-9 can degrade several extracellular matrix (ECM) components, including cell adhesion molecules, collagen IV, proteoglycans, and other proteins within the ECM. Created with BioRender.com.

As mentioned previously, the increase in MMP-9 activity is associated with an increased risk of vascular edema and the hemorrhagic transformation of the stroke[11]. Moreover, MMP-9 knockout studies show improved BBB integrity and decreased infarct volume following ischemic stroke[65].

Lectin-like oxidized low-density lipoprotein receptor-1

The scavenger receptor: lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is primarily expressed on endothelial cells[66]. LOX-1 signaling activates several intracellular pathways involved in inflammation, oxidative stress, apoptosis, and angiogenesis and is, therefore, a critical player in endothelial dysregulation and the formation of atherosclerosis[66, 67].

Regulation and function

LOX-1 transcription is regulated by the transcription factor nuclear factor kappa B NF-κB, which will bind to the LOX-1 gene as a positive feedback loop following LOX-1 activation[68]. When oxidized lectin-like lipoprotein (oxLDL) or other pro-inflammatory proteins bind to LOX-1, it initiates a signaling myriad inhibiting NO synthesis and increases the production of ROS, leukocyte adhesion molecules, pro-apoptotic proteins, and MMPs[69-71]. Altogether, causing endothelial dysregulation. Following activation of the LOX-1 receptor, its function can be regulated by various processes, including internalization of the receptor and cleavage of its extracellular domain[70, 72]. The cleavage of the receptor is performed by MMPs and produces soluble LOX-1 (sLOX-1), which is released into the circulation[73].

LOX-1's role in ischemic stroke pathology

The discoveries of LOX-1s striking influence on endothelial function have earned it a permanent position within the pathology of cardiovascular diseases, including ischemic stroke. Patients show elevated sLOX-1 levels within the circulation following ischemic stroke, indicating an upregulation of LOX-1 expression[74]. This suspicion has been confirmed in preclinical studies where LOX-1 levels increase following experimental stroke[75]. In *in vitro* and *in vivo* studies in myocardial infarctions, LOX-1 inhibition is beneficial in preventing cellular death and infarct lesion progression[68, 76]. In stroke, a knockout study in stroke-prone spontaneously hypertensive rats showed a protective effect in preventing spontaneous brain damage[77]. Furthermore, an increase in LOX-1

expression showed worsened stroke outcome following transient middle cerebral artery occlusion (tMCAO) in mice[78].

One explanation behind the protective effects of LOX-1 inhibition following stroke is the receptors' ability to increase the expression of leukocyte adhesion molecules[71]. Usually, leukocyte infiltration is prevented by the low production of adhesion molecules in the endothelial cells in the BBB[33, 34]. This changes after an ischemic event. Within hours after stroke onset, neutrophils are present within the parenchyma, followed by T-cell and monocyte migration[79]. The inflammatory response following an ischemic stroke can further cause cerebral injury and worsen stroke aftermath[79, 80]. Furthermore, one of the signaling pathways activated by LOX-1 is the extracellular signal-regulated kinase 1/2 (ERK1/2)[81]. A pathway that can lead to increased MMP-9 expression, possibly explaining the increased MMP-9 levels following ischemic stroke.

Extracellular signal-regulated kinase 1/2

The mitogen-activated protein kinases (MAPK) signaling pathways are known for their regulatory role in cellular responses such as proliferation, differentiation, motility, and cell survival[82]. MAPKs are serine/threonine-protein kinases, and in mammals, 14 different MAPKs have been identified. Two are ERK1/2[83].

Activation and function

The ERK1/2 kinases are structurally similar (42 vs. 44kDa) and cause similar responses upon activation. The ERK1/2 signaling cascade is generally prompted by a mitogenic extracellular stimulation of a tyrosine kinase receptor. The signaling myriad is conducted by activating sequential kinases, concluding with the mitogen-activated kinase kinase 1/2's (MEK1/2) phosphorylation of ERK1/2, causing its translocation to the nucleus[82]. Following stimuli, it takes 10 to 20 min for ERK1/2 to translocate to the nucleus activating transcription factors, including NF-κB, causing an upregulation of proliferation, pro-inflammatory proteins, and MMP-9[56, 58, 84, 85].

ERK1/2's role in ischemic pathology

Several stimuli can activate the ERK1/2 signaling pathway during ischemic stroke, one being through the LOX-1 receptor. Human post-mortem brains have shown increased activated ERK1/2 within the ipsilateral penumbra following ischemic stroke, which is also demonstrated in animal models[86, 87]. Targeting ERK1/2 signaling after experimental ischemic stroke has shown beneficial results within the acute phase of the stroke[88-90]. Furthermore, inhibition of the ERK1/2 activation has been seen to prevent the experimental stroke-induced increase of MMP-9 expression within the cerebral vessels[91] (Figure 5).

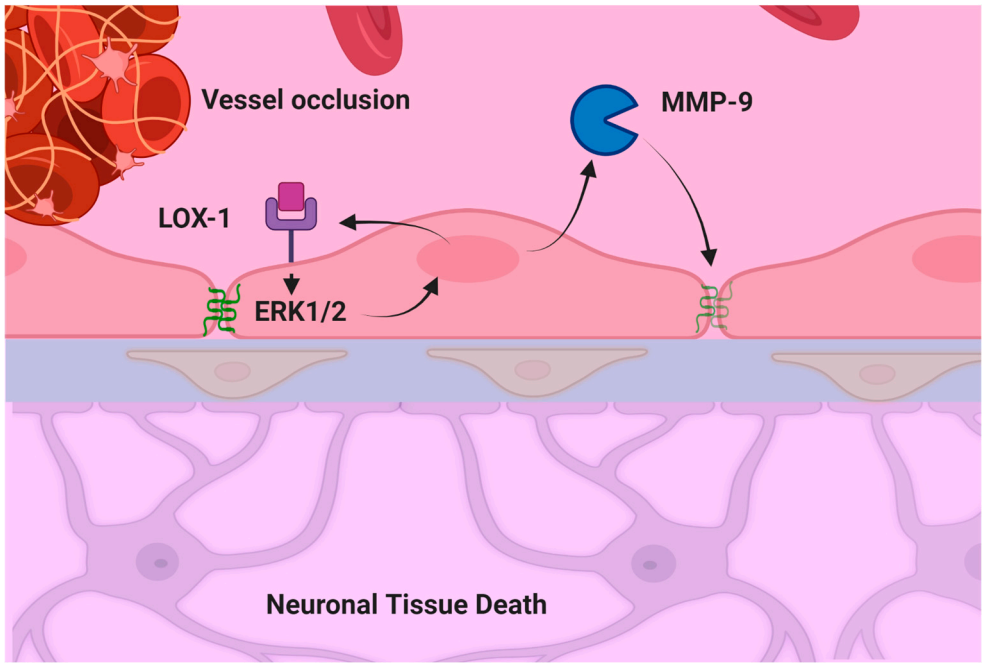


Figure 5.
Following vessel occlusion, blood flow restriction causes neuronal tissue death within the brain parenchyma. The ischemia can also trigger LOX-1 activation leading to the translocation of ERK1/2 into the cell nucleus and upregulating the transcription of its target proteins, including LOX-1 and MMP-9. Following transcription, MMP-9 is released into the vessel lumen, where it can break down tight junction proteins and the basement membrane, disrupting the BBB. Created with BioRender.com.

Acute Ischemic Stroke Treatment

Acute stroke care has improved consequentially in recent years. The formation of stroke care units reduces death and disability and improves independence[92]. Reduction of door-to-needle time has also improved stroke outcome[93], and improved imaging techniques can now identify patients where treatment can be beneficial up to 24 hours after stroke induction[94]. Nonetheless, only two treatment options are available in acute stroke care since recanalization therapy remains the only clinically proven option that improves stroke outcomes. Recanalization can be achieved by thrombolysis with rt-PA and or thrombectomy, the most recent addition for ischemic stroke treatment[9, 10, 95].

Thrombolysis is a drug treatment used to break down the clot, and for ischemic stroke, rt-PA is the only FDA-approved option. Treatment with rt-PA was the first treatment option for acute ischemic stroke, and since its introduction in 1995, it has remained the standard of care[9, 10, 96]. The treatment utilizes the human coagulation system where rt-PA activates plasmin which then can break down the fibrin within the blood clots, causing clot lysis and, therefore, recanalization, improving stroke outcome[97]. Thrombectomy is a surgical procedure where the occlusion is removed mechanically and was first tested in 2004 using the Mechanical Embolus Removal in Cerebral Ischemia (MERCI) retriever[98]. Since then, improved retrievers have had a compelling impact on large vessel occlusions with major improvement of stroke outcomes in patients[95, 99, 100].

Despite the treatments' beneficial effects, their use within the clinic is highly restricted. A study investigating access and incidence of recanalization therapy within 44 European countries shows that 7.3 % of ischemic stroke patients receive rt-PA, and only 1.9 % undergo endovascular treatment. The highest rates within specific countries were 20.6 % for rt-PA and 5 % for endovascular thrombectomy[8]. While the study itself proclaimed the discrepancy in care availability between countries, the treatment's limitations play a crucial role in the low rates. Thrombectomy is only applicable in large vessel occlusions, and due to its logistical requirements, its availability for most ischemic stroke patients is limited[101]. Thrombolysis treatment is the most available option and therefore remains the standard treatment of care; nonetheless, its severe side effects limit its use within the clinic.

The main limitation of rt-PA treatment is its aptitude to increase the risk of hemorrhagic transformation. Due to the risk-benefit dilemma of rt-PA therapy, treatment has a restricted time window of 3-4.5 hours after stroke onset[9, 10]. The restricted time window excludes a substantial amount of stroke patients, particularly in patients with an unknown time of origin which is the case for 8-25 % of all ischemic

stroke patients[102, 103]. Therefore, it is of exceeding importance to increase the safety of the rt-PA, especially now with new evidence showing that recanalization therapy can be effective up to 24 hours after stroke onset[94].

rt-PA induced risk of hemorrhagic transformation

Treatment with rt-PA increases the risk of hemorrhagic by a ten-fold compared to placebo[9]. The risk is due to the disruption of the BBB, allowing blood to enter the brain, and clinically, this correlates with increased levels of the MMP-9[11, 104]. Rt-PA gains its fibrinolytic abilities due to its plasmin activation. Beyond its fibrinolytic ability, plasmin can also activate several MMPs, including MMP-9[105]. In addition, rt-PA also triggers neutrophils to release their MMP-9-containing granules and stimulates endothelial cells to increase their expression of MMP-9, which further explains the increased risk of hemorrhagic transformation following rt-PA administration[106, 107]. Inhibiting MMP-9 after rt-PA therapy can be the solution to improve the safety of rt-PA treatment by preventing the increased risk of hemorrhagic transformation (Figure 6).

MMP-9 inhibition

The protective effect of MMP-9 inhibition following ischemic stroke has been determined previously in preclinical studies[63, 108, 109], but MMP-9 inhibition has not been tested within the clinic due to complications. Clinically, studies using broad-spectrum MMP inhibitors cause dose-limiting toxicities such as musculoskeletal syndrome and gastrointestinal disorders[110-112]. The detrimental effects in the clinical trials have since been discovered to be due to the inhibition of other specific proteases[113], thereby excluding MMP-9 as a trigger for these symptoms[114]. A better understanding of MMP-9 regulation and activation has again opened for its use as a target within several pathologies, including stroke treatment.

In vivo model of ischemic stroke and rt-PA therapy

Preclinical testing of new treatment options for ischemic stroke has had poor translatability into the clinic, questioning the relevancy of the animal models used within stroke research. Only embolic stroke models have successfully predicted a clinically working therapeutic (rt-PA)[17, 115, 116]. While embolic models are essential in

evaluating new thrombolytic strategies or new treatments combined with thrombolytics, their variation of infarct lesion volume/position, fluctuation of measurable neurological dysfunctions, and high mortality levels still limit their ability to produce legitimate results[117-122]. In 2007, a new thromboembolic stroke model was introduced within stroke research, a model with reproducible and translational results[123].

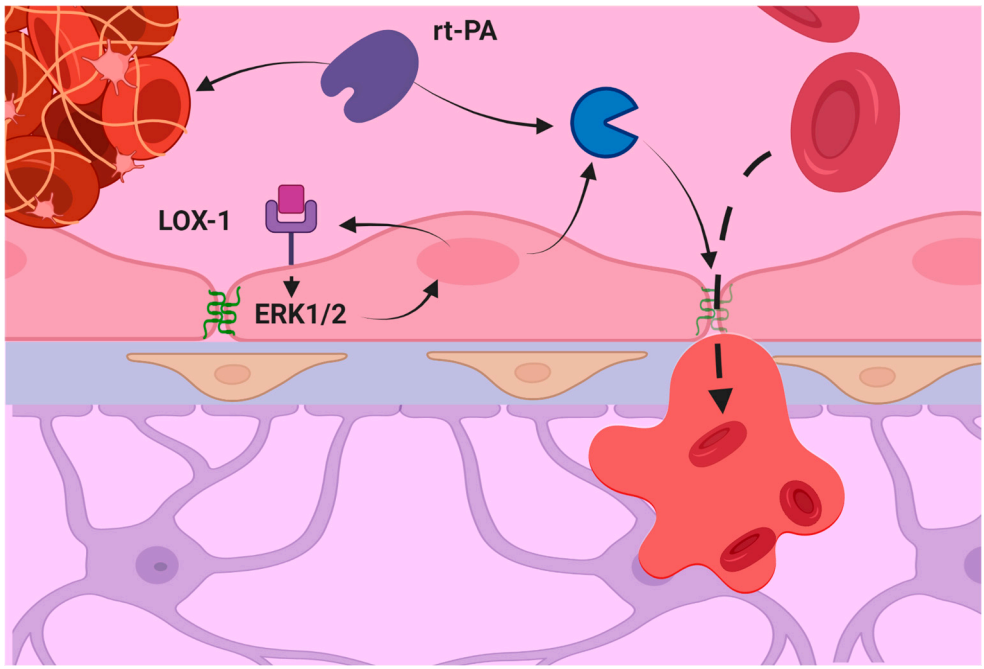


Figure 6.
Treatment with rt-PA has the beneficial effects of causing degradation of the blood clot and preventing the cerebral blood flow. However, rt-PA can also trigger MMP-9 activation, further breaking down the BBB, thereby increasing the risk of hemorrhagic transformation following rt-PA therapy. Created with BioRender.com.

Aims

This thesis aims to enhance rt-PA therapy's safety and improve stroke outcomes. By focusing on preventing the increased levels of MMP-9 associated with the rt-PA-induced risk of hemorrhagic transformation, we intend to improve rt-PA therapy and enable its use within and beyond its current therapeutic window. The objectives of the studies included in this thesis were:

- Establish a novel thromboembolic stroke model in rats that mimic the clinical situation with the focal point on rt-PA therapy
- Investigate if reducing the expression of MMP-9 using an MEK1/2 inhibitor could prevent the rt-PA induced increased risk of hemorrhagic transformation
- Estimate the temporal profile of changes in protein levels, which are important for BBB integrity.
- Assess LOX-1 and MMP-9 inhibition and their ability to affect the expression of proteins that can influence BBB permeability in an in vitro model of ischemia and reperfusion.
- Evaluate if the safety of the rt-PA treatment and improvement of stroke outcome can be achieved by inhibiting the activation of MMP-9 and or by preventing MMP-9 expression using a LOX-1 inhibitor

Results and Discussion

Paper 1. Validation of a stroke model in rat compatible with rt-PA-induced thrombolysis: new hope for successful translation to the clinic

This study aimed to establish and validate the thromboembolic stroke model in rats. The study design is illustrated in Figure 7. We showed that injecting thrombin within the middle cerebral artery (MCA) caused a stable clot formation and induced a reproducible infarct lesion within the cerebral cortex of the rat. The lesion caused a measurable reduction of neurological function, which was prevented with early treatment with rt-PA. The treatment recanalized the MCA and reduced infarct lesion volume when treatment was administrated at one hour. Treatment with rt-PA at four hours did not improve stroke outcomes. The late treatment with rt-PA increased the risk of hemorrhagic transformation and negatively affected stroke outcomes. This study concludes this model's resemblance to the clinical situation and strengthens its use in searching for new treatment options.

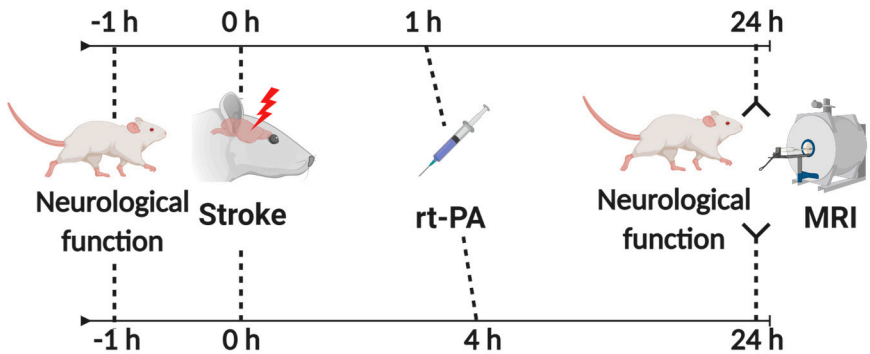


Figure 7.
Illustration of the study design for paper I. Stroke induction was followed by intravenous treatment with either rt-PA or saline at one or four hours. Treatment with rt-PA (3mg/ kg; Actilyse) was injected with a 10% bolus and 90% perfusion for 40 minutes. Neurological function was evaluated through a 28-point neuroscore in all animals prior to surgery and 24 h after stroke induction. Subsequently, magnetic resonance imaging (MRI) scans were used to evaluate infarct lesions and hemorrhagic transformation. Created with BioRender.com.

Discussion

The complex pathophysiology of ischemic stroke has made animal models indispensable in the investigation of specific mechanisms and the search for new therapeutic strategies. Still, years of data have shown a big discrepancy between preclinical and clinical results[124]. All clinical trials on drugs with proven efficacy in animal models have failed, except for thrombolysis with rt-PA[17, 124]. In 2007, *Orset et al*. introduced a new thromboembolic stroke model with high clinical relevance with the aim of reducing the existing translational gap[123]. To continue this work, we have now established the model in rats. Compared to previous embolic models, the thromboembolic stroke model uses direct thrombin injection into one of the MCA's bifurcations, causing an *in-situ* clot formation.

The occlusion of a distal MCA branch causes a rapid drop in the cerebral blood flow within the MCA region. The reduction of blood flow gives rise to an infarct lesion within the rats' cerebral cortex, similar to what is seen in mice (Figure 8)[123]. Due to the clot's placement within a bifurcation, the clot remains in place, giving the model a higher reproducibility than previous models where the injected clots have various distributions within the circulation[118, 119]. The embolic models mimic the clinical stroke where the clot placement varies, but their inconsistency in stroke outcome and high mortality makes their use when evaluating new potential treatments challenging. The infarcts are reproducible within the thromboembolic model, showing a meager mortality rate in mice and rats[123]. In addition, it also mimics the clinical situation when it comes to rt-PA therapy[123, 125, 126].

Treatment with rt-PA show low efficacy (20 - 40 %) in the clinic, and the earlier treatment commences, the higher chance of improved outcome[127-129]. Here, the thrombin-induced infarct lesion within the cerebral cortex of the rats caused a reproducible reduction of neurological function following stroke, which was prevented with early rt-PA treatment (one hour). Early treatment with rt-PA caused recanalization and reperfusion within the MCA region, preventing the spreading of the infarct lesion (Figure 9).

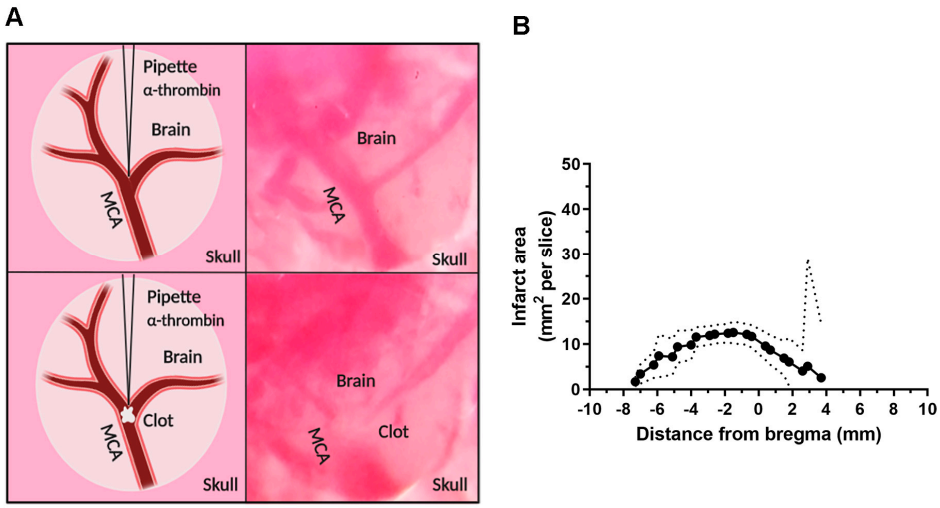


Figure 8.
Injection of thrombin into the MCA causes reproducible infarct lesions. (**A**) Illustration (left column) and visualization (right column) of the in vivo setting after the craniectomy. In the thromboembolic model, the pipette filled with α-thrombin is inserted into the lumen of the MCA bifurcation following thrombin injection (lower row). A formed clot can be visualized within the MCA bifurcation. (**B**) Infarct lesion was reproducible in both size and location, 87.29 (62.97, 111.6) mm^3, starting at 2.91 (2.15, 3.68) mm and ending at – 6.20 (– 7.51, – 4.89) mm in the cerebral cortex (bregma=0 mm, n=7). Data are presented as the area of infarct lesion for each section. Data are expressed as mean and error bars as 95% IC. Created with BioRender.com.

Late administration of rt-PA (four hours) indicated a negative trend in the cerebral blood flow within the infarct area following treatment and did not affect infarct lesion volume or neurological function. This indicates that the infarct core may have reached its maximum volume within four hours from stroke onset in this model, but it needs further confirmation (Figure 10 A-C).

Beyond mimicking the timely manner of clinical efficacy of rt-PA, the model also resembles the detrimental effects of the treatment. Treatment with rt-PA increases the risk of hemorrhagic transformation, limiting its use by restricting its time window for administration within 4.5 hours from stroke onset in the clinic[9, 10]. In the mouse model, the risk of hemorrhagic transformation is 30 % following rt-PA treatment 3 hours after stroke induction[125]. In rats, treatment with rt-PA four hours after stroke onset showed major hemorrhagic events in 25 % of the animals. In contrast, we saw only minor indications of hemorrhagic transformation within saline-treated animals and early treatment with rt-PA (Figure 10 D-F).

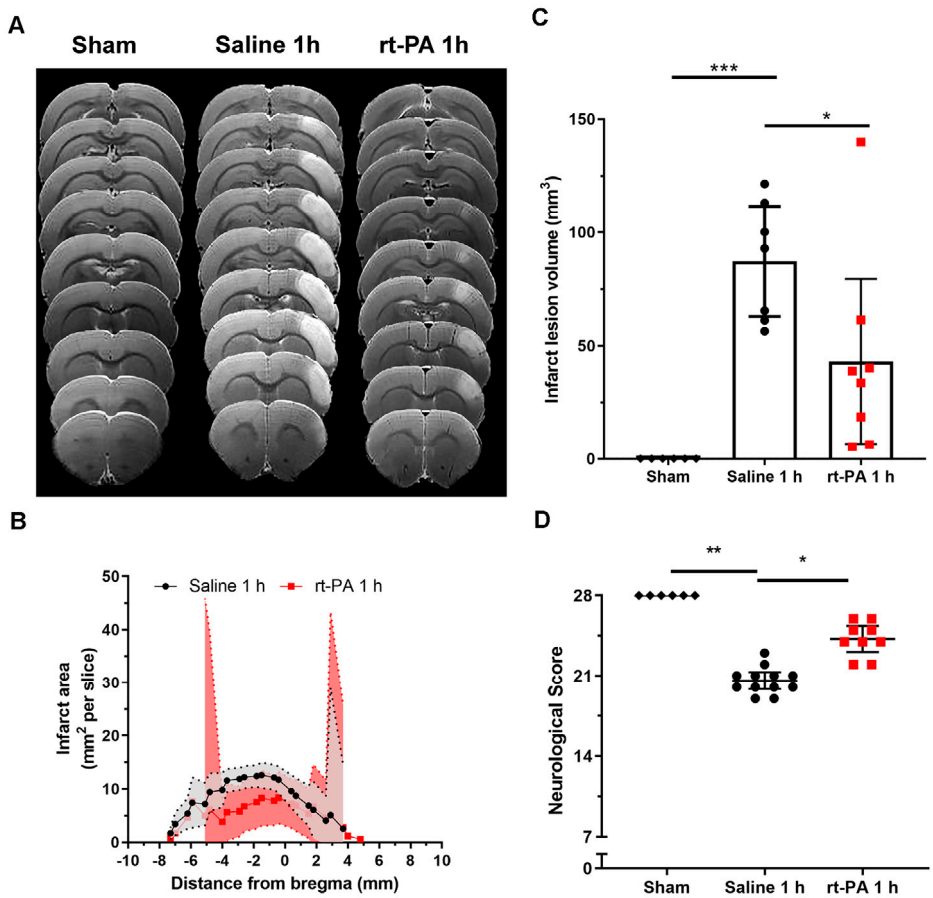


Figure 9.

Early treatment with rt-PA improves outcomes 24 h after stroke induction. (**A**) Representative T_2-weighted image visualizing the infarct lesion within the cerebral cortex of sham-operated, saline, and early rt-PA treated rats. (**B**) In the rt-PA treated animals, the infarct was located between 2.33 (1.15, 3.50) mm and – 4.00 (– 5.55, – 2.45) mm, and in saline-treated animals, between 2.91 (2.15, 3.68) mm and – 6.20 (– 7.51, – 4.89) mm (bregma=0 mm), both within the cerebral cortex. (**C**) Animals treated with rt-PA one hour after stroke onset had significantly smaller infarct lesions than saline-treated animals. (**D**) Neurological function was evaluated 24 h after stroke onset using a 28-point neuroscore. Stroke-induced animals treated with saline one hour after stroke onset showed a significant reduction in neurological function compared to sham-operated animals, which showed no reduction in neurological function]. Treatment with rt-PA resulted in improved neurological function compared to saline. Data are expressed as mean and error bars as 95% IC. Comparison between the treatment groups was made using the uncorrelated Dunn's multiple comparisons test.

In conclusion, we established a thromboembolic stroke model in rats that closely resemble the human ischemic stroke and mimics the effects of rt-PA therapy in terms of infarct formation, neurological function, and hemorrhagic incidence.

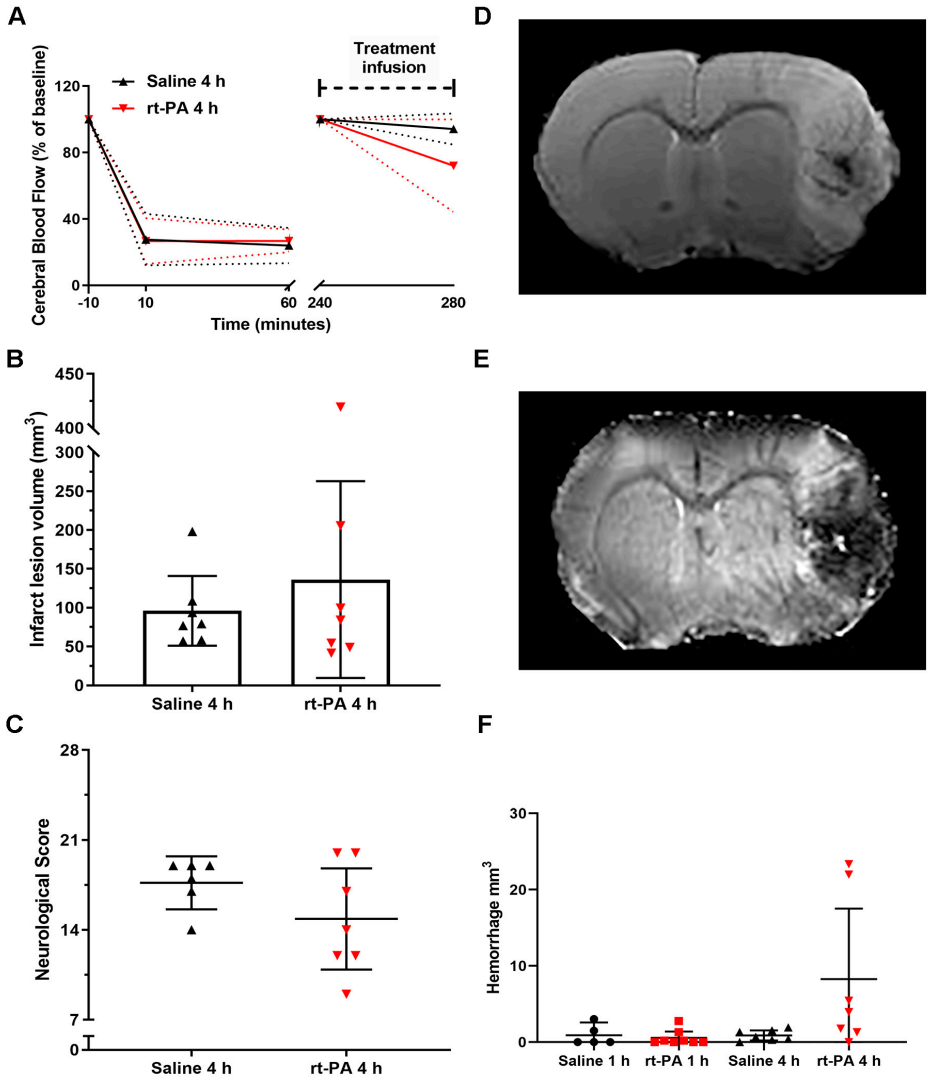


Figure 10.
Late rt-PA treatment shows no beneficial effects on stroke outcomes but increases the risk of hemorrhagic transformation. (**A**) CBF was measured in the MCA region throughout the surgery using a laser Doppler, with a gap between 60–240 min, where the animal was awakened and then re-sedated. Baseline levels measured at – 10 min and then at 240 min after re-sedation was set as 100%. A rapid drop of CBF can be seen after thrombin injection into the MCA, which remained stable for 60 min. Treatment (saline-black or rt-PA-red) was administrated four hours after thrombin injection, and the CBF remained stable for both treatments. (**B**) No difference was observed between either infarct size (**C**) or neurological function between animals treated with rt-PA or saline four hours after stroke onset. (**D**) Representative T_2-weighted (**E**) and T_2*-weighted images of rats treated with rt-PA four hours after stroke onset suffered from a hemorrhagic transformation of the stroke. (**F**) While early rt-PA treatment at one hour after stroke onset did not induce hemorrhagic transformation, delayed treatment at four hours resulted in a major hemorrhagic transformation in 25% of the animals. Data are expressed as mean and error bars as 95% IC. Comparison between the treatment groups was made using the uncorrelated Dunn's multiple comparisons test.

Paper II. Combination treatment with U0126 and rt-PA prevents adverse effects of the delayed rt-PA treatment after acute ischemic stroke

In this study, we aimed to investigate if inhibition of MEK 1/2 to decrease the expression of MMP-9 would prevent the deleterious effect of delayed rt-PA treatment. The study design is illustrated in Figure 11. Using the thromboembolic stroke model in mice, we saw that delayed rt-PA treatment increased the incidence of hemorrhagic events, which was prevented when ERK1/2 activation was inhibited. The inhibition of ERK1/2 activation and delayed rt-PA administration decreased the expression of pERK1/2 within the ischemic region compared to both vehicle and rt-PA treated animals. Similar results were seen in the expression of MMP-9 within the same region. Delayed rt-PA treatment indicated an increase in MMP-9 expression, while the combination treatment caused a significant reduction of MMP-9 expression. This study shows that targeting the expression of MMP-9 has a protective effect against the rt-PA-induced risk of hemorrhagic transformation following experimental ischemic stroke in mice.

Discussion

Ischemic stroke causes endothelial dysfunction within the cerebral vasculature by activating several detrimental signaling pathways, including the ERK1/2 pathway. When ERK1/2 is activated (phosphorylated) by MEK1/2, the kinase is translocated to the cell nuclease activating various transcription factors such as NF-κB, causing an increased expression of MMP-9[56, 85]. Previous studies show that using a MEK1/2 inhibitor, U1026, prevented the endothelial increase of MMP-9 expression by inhibiting ERK1/2 activation[91]. We, therefore, evaluated if U0126 could prevent the rt-PA-induced risk of hemorrhagic transformation in the thromboembolic stroke model in mice by reducing the increased expression of MMP-9.

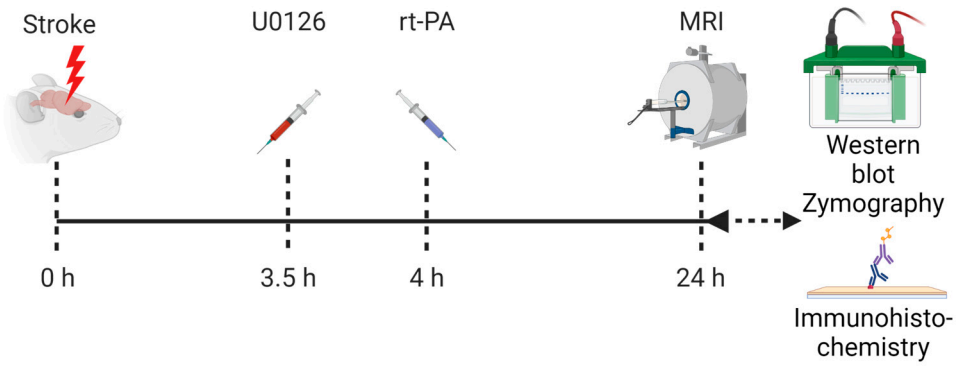


Figure 11.
Illustration of the study design for paper II. Thromboembolic stroke was induced in C57BL/6J mice which were divided into four groups; (i) vehicle-treated, (ii) treated with rt-PA, (iii) treated with rt-PA in combination with U0126, and (iv) treated with U0126. To induce thrombolysis, rt-PA (10 mg/ kg; Actilyse) was administered four hours after thrombin injection. It was injected with a 10% bolus and 90% perfusion for 40 minutes. The MEK1/2 inhibitor U0126 (30 mg/kg;) or vehicle (dimethyl sulfoxide) was injected intraperitoneal at 30 minutes prior to rt-PA administration. After 24 hours, all mice were euthanized and brains removed and either fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for immunohistochemistry and diaminobenzidine (DAB) staining or snap-frozen in isopentane in order to collect tissue for western blot and zymography. Created with BioRender.com.

Within this study, stroke induction caused a significant increase in pERK1/2, which was not influenced by the delayed treatment of rt-PA. The increase of pERK1/2 was previously seen within the smooth muscle cells of cerebral blood vessels following tMCAO, which was prevented when the animals were treated with U1026[130]. Here we saw that the increased immunoreactive expression of pERK1/2 was mainly located within the ischemic core, the peri-infarct region, and vessels. Treatment with U0126 prevented the increase of pERK1/2 immunoreactive expression within the vessels and peri-infarct region, and when the treatment was combined with delayed rt-PA treatment, the difference was significant (Figure 12).

Exploring the immunoreactive expression of MMP-9 within the peri-infarct area showed an increase within the ipsilateral side compared to the contralateral side, simulating the MMP-9 expression within human post-mortem brains following ischemic stroke[54, 62]. Animals treated with rt-PA showed the highest MMP-9 immunoreactivity. The inhibition of ERK1/2 activation decreased the MMP-9 immunoreactivity within the ipsilateral side supporting previously published data[91]; additionally, combining U0126 with rt-PA administration prevented the rt-PA induced increase of MMP-9 (Figure 13).

Evaluating the influence of treatment with U0126 on stroke outcome shows a discrepancy in prior studies, where timing and frequency of treatment with U0126 impact the treatment effect.

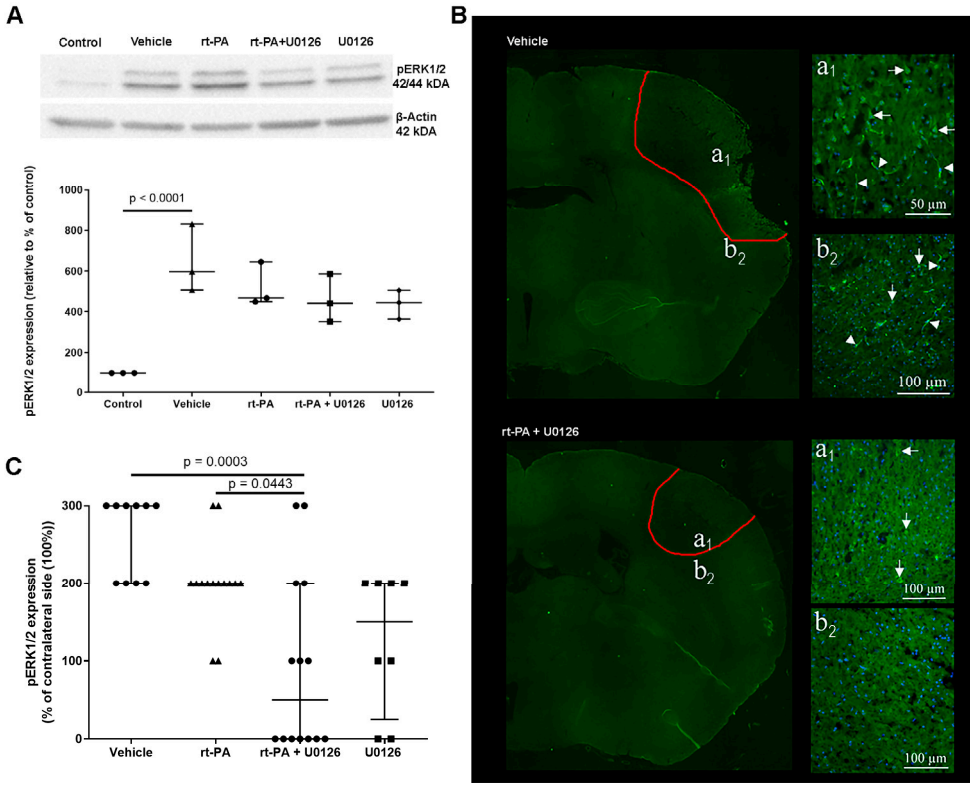


Figure 12.
(**A**) p-ERK1/2 protein expression is shown by representative western blots and quantified by a scatter plot. The protein expression of p-ERK1/2 was normalized to β-actin (loading control). In all comparisons, the average value for the control group was set to 100%. p-ERK1/2 has a molecular weight of 42/44 kDa, and the one of β-actin is 43 kDa. Data are presented as median±IQR, *P<0.05 is considered statistically significant. (**B**) Representative image of pERK1/2 immunoreactivity. Upper panel, saline-treated animal. The area of stroke (outlined in red) is illustrated in a large image to the left. Letters a1 and b2 refer to the illustrations to the right. a1. Te stroke core is shown. Arrows point at pERK1/2 immunoreactive cells and arrowheads at vessels. b2. Immunoreactive cells and vessels were also found close to the stroke area. Lower panel, t-PA+U0126 treated animal. To the left, red-outlined stroke areas are illustrated in a large image. Letters a1 and b2 refer to the illustrations to the right. a1. Some pERK1/2 immunoreactive cells (arrows) were found in the stroke core but not in the area outside the stroke b2. (**C**) Scatter plot showing semi-quantification of protein expression for pERK1/2. Data are presented as median±IQR and normalized to the non-occluded side. Comparison between the treatment groups was made using Dunn's multiple comparisons test.

Delayed and only one dosage of U0126 show little effect on infarct lesion volume and neurological function, while earlier treatment and multiple dosages had a higher impact on stroke outcome[89, 130-132]. Here we used a single dosage given at 3.5 hours following stroke onset, and quantification of infarct lesion volume showed no differences between vehicle, rt-PA, U0126, or U0126 + rt-PA treated animals (Figure 14 A, B). Still, studies with similar results where infarct lesion did not alter also showed improved stroke outcomes following U0126

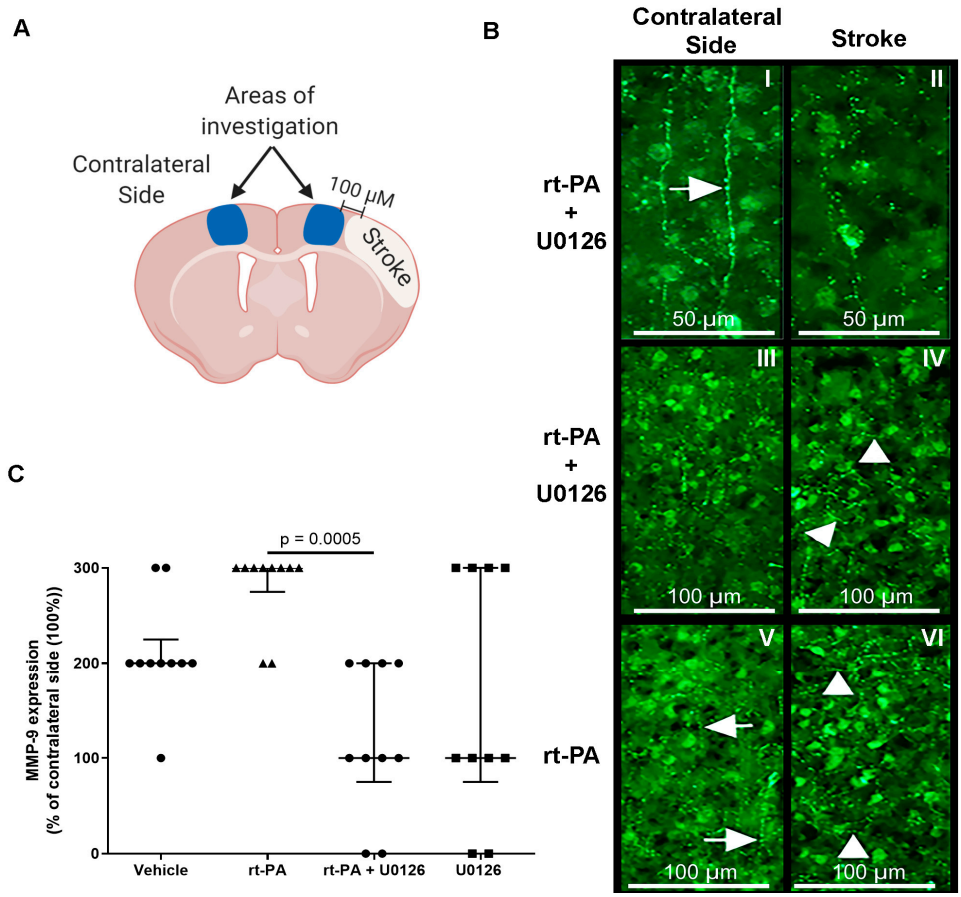


Figure 13.

Representative images of MMP-9 immunoreactivity. (**A**) Illustrationimmunoreactivity images for MMP-9. All images were obtained in cortical layers III–V, 100 µm towards the midline in relation to the stroke area. Created with BioRender.com (**B**) U0126+rt-PA 50 µm: I. The immunoreactivity in the brain's control side was organized in the radial orderliness of the cortical neurons. Arrow points at pearl-like MMP-9 immunoreactivity. II. The corresponding area in the stroke side of the same individual showed less defined structural organization. U0126+rt-PA 100 µm: III. The image demonstrates a lower magnification of the control side of the brain. IV. In the stroke side of the individual in III, it became apparent that, apart from the radial organization, the immunoreactivity was organized more or less perpendicular to the cortical neuronal format (arrowheads), which was not often found in the control side. Rt-PA V. In the control side of the animals treated with rt-PA, the immunoreactivity displayed similar features as for U0126 treated animals shown in I and III, with often radial distribution of the immunoreactivity (arrows). VI. In the stroke area of the animal shown in V, a disorganization of the MMP-9 immunoreactivity was clearly apparent (arrowheads). (**C**) Scatter plot showing semi-quantification of protein expression for MMP-9. Data are presented as median±IQRand normalized to control. Comparison between the treatment groups was made using Dunn's multiple comparisons test.

inhibition[89]. Further studies evaluating neurological function need to be performed to confirm this. However, the difference in the hemorrhagic transformation between the groups may be of greater importance concerning stroke outcomes.

In this study, delayed rt-PA treatment increased the risk of hemorrhagic transformation following stroke and the incidence of larger hemorrhagic events. Which clinically could lead to a worsened stroke outcome[52]. Hemorrhagic transformations are classified as symptomatic or asymptomatic due to their influence on stroke outcomes, whereas symptomatic hemorrhagic transformations have a more substantial influence on worsened stroke outcomes and at a higher rate associated with rt-PA treatment than placebo[133]. Here we show that treatment with U0126 prior to delayed rt-PA treatment prevents the rt-PA-induced risk of hemorrhagic transformation. A result that may be explained by the combination treatments' ability to prevent the rt-PA induced expression of MMP-9 (Figure 14 B, C).

In conclusion, inhibition of ERK1/2 activation did improve the safety of rt-PA treatment, possibly by preventing an increase of MMP-9 expression, but it did not improve stroke outcomes in terms of infarct volume.

Paper III. LOX-1 and MMP-9 inhibition attenuate the detrimental effects of delayed rt-PA therapy and improve outcomes after acute ischemic stroke

The overall aim of this study was to evaluate if we could enhance the safety of the rt-PA treatment and improve stroke outcomes by inhibiting the activity of MMP-9. This study was divided into three objectives.

Firstly, we estimated the temporal profile of protein changes that are important for BBB integrity. The study design is illustrated in Figure 15. Following thromboembolic stroke induction in rats, MMP-9 and Claudin-5 showed momentary changes within the ipsilateral hemisphere. Both MMP-9 activity and Claudin-5 protein levels were significantly increased in the cerebral blood vessels six hours after stroke onset. This suggests a region-specific change in protein levels following ischemic stroke.

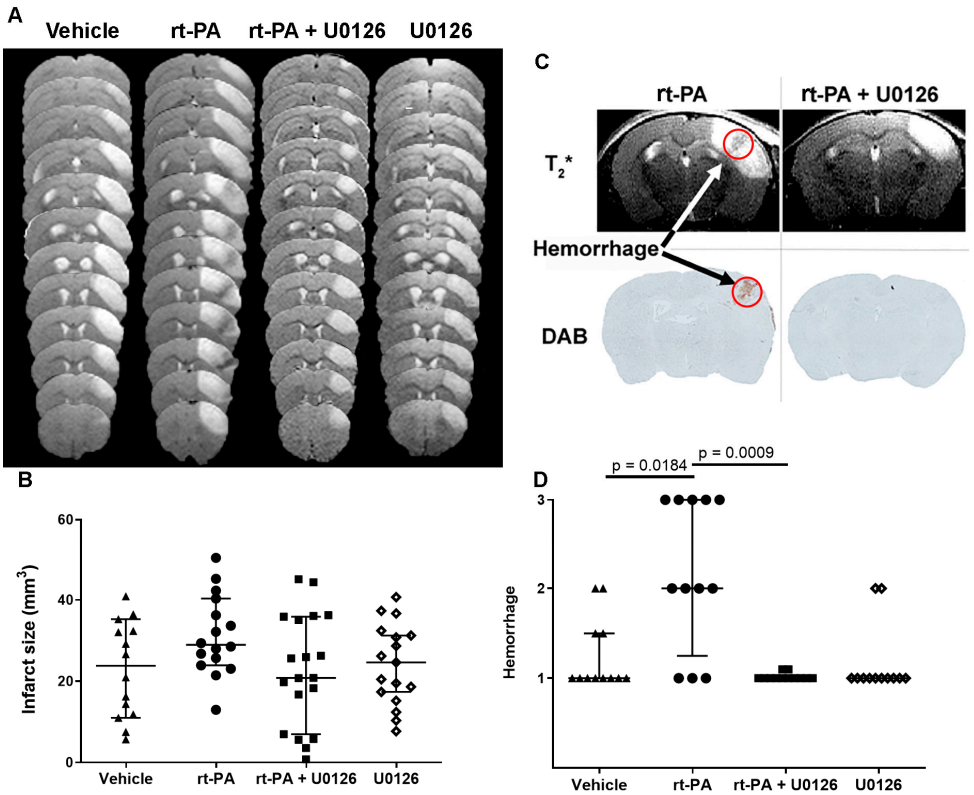


Figure 14.

(**A**) Representative T_2-weighted image visualizing the infarct lesion within the cerebral cortex of the different treatment groups (**B**) and quantification of the infarct lesion volumes. (**C**) Representative T_2*-weighted images and diaminobenzidine staining of the same slice show hemorrhages marked by arrows. (**D**). Quantifcation of hemorrhages were evaluated using DAB staining. Data are presented as median±IQR. . Comparison between the treatment groups was made using Dunn's multiple comparisons test.

Next, we assessed the ability of the LOX-1 inhibitor: BI-0115 and the MMP-9 inhibitor: JNJ0966 to affect the expression of proteins that can influence BBB permeability in an *in vitro* model of ischemia and reperfusion (hypoxia + glucose deprivation-reperfusion, HGD/R) in human brain microvascular endothelial cells (HBMECs). The study design is illustrated in Figure 16. The HGD/R model showed an apparent increase in messenger mRNA expression

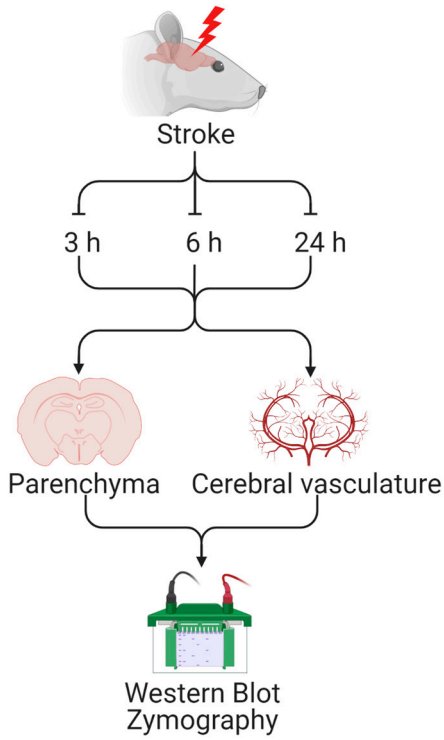


Figure 15.
Illustration of the study design for paper IIIa. Stroke was induced in male Wistar rats using the thromboembolic stroke model, and animals were euthanized using cardiac perfusion at 3, 6, and 24 hours. Following euthanization, vessels and parenchyma were separated to investigate the protein levels of claudin-5 and MMP-9 activity in the different tissue fractions using western blot and zymography. Created with BioRender.com.

of MMP-9 compared to normoxia in HBMECs. The treatment with rt-PA negatively impacted MMP-9 mRNA expression following HGD/R, further sustained by BI-0115 and JNJ0966 treatment. The rt-PA ± BI-0115 and JNJ0966 demonstrate an ability to reduce the hypoxia-induced increase of MMP-9 mRNA, which can help prevent the breakdown of the BBB following ischemic stroke.

Finally, we evaluate if the safety of the rt-PA treatment and improvement of stroke outcome can be achieved by inhibiting the activation of MMP-9 and or by preventing MMP-9 expression using a LOX-1 inhibitor. The study design is illustrated in Figure 17. Following thromboembolic stroke induction in rats, rt-PA administration significantly increased the MMP-9 activity within the ipsilateral cerebral vessels. The increase in MMP-9 activity was sustained when JNJ0966 and BI-0115 were used separately with rt-PA treatment, but the combination of the two inhibitors prevented MMP-9 activation.

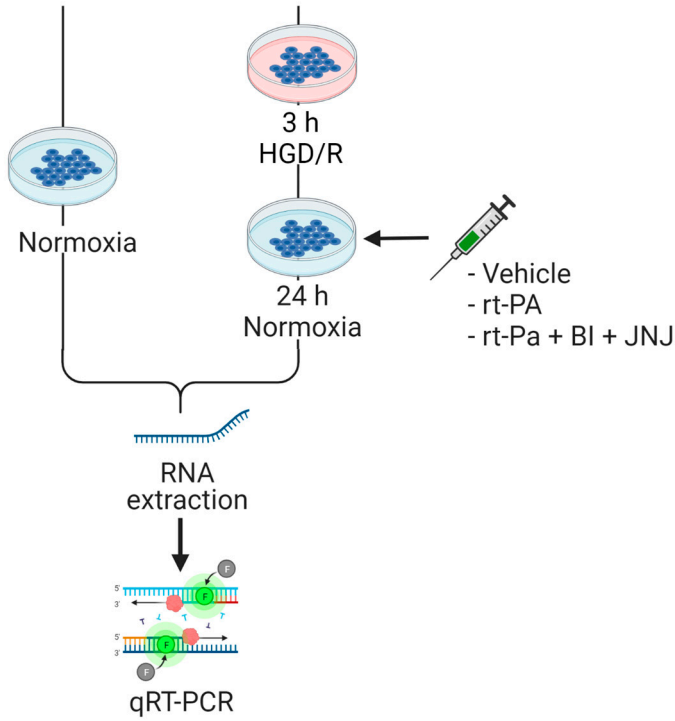


Figure 16.
Illustration of the study design for paper IIIb. The HBMECs were cultured under standard conditions as a control. HBMECs were put under HGD conditions for 3 hours followed by 24 hours of "reperfusion" to stimulate the ischemic condition. Treatment with rt-PA, and the To evaluate the mRNA expression of TIMP-1 and MMP-9, qRT-PA was used. Created with BioRender.com.

The rt-PA induction of MMP-9 activity correlated with the increased risk of hemorrhagic transformation, which was more prevalent and more extensive in these animals. Treatment with BI-0115 and or JNJ0966 all showed beneficial effects against hemorrhagic transformation following rt-PA treatment, and in addition, BI-0115 significantly reduced the percentage of edema measured in rt-PA treated animals. Moreover, treatment with BI-0115 had the most prominent influence on improving neurological function 24 hours after stroke induction. In conclusion, targeting the expression and or the activation of MMP-9 prior to rt-PA administration increases the safety of the treatment, and that inhibition of the LOX-1 receptor may significantly impact stroke outcome.

Discussion

Temporal protein changes within the ipsilateral hemisphere following experimental ischemic stroke

Disruption of the BBB integrity is associated with vascular edema and an increased risk of hemorrhagic transformation, leading to a worsened stroke outcome [51, 134]. Here we wanted to look closer into the longitudinal changes in levels and activation of proteins that directly influence BBB permeability in the acute phase following thromboembolic stroke induction in rats.

In this study, claudin-5 and MMP-9 showed compelling temporal alternations within the ipsilateral hemisphere. At six hours after stroke onset, claudin-5 levels were significantly elevated within the cerebral blood vessel (Figure 18 A). Claudin-5s importance within BBB integrity has previously been validated in which reduced expression increases BBB permeability, and increased expression improves its integrity[135-137]. Following hypoxia and experimental stroke, claudin-5 is rapidly reorganized, decreasing the BBBs resistance for perforation[138]. In the clinic, increased plasma levels of claudin-5 are associated with increased BBB permeability and hemorrhagic events following ischemic stroke[53]. Aiding in reducing the integrity of the BBB is MMP-9.

As with claudin-5, MMP-9 activity increases significantly at six hours following stroke onset within the cerebral vasculature, which also was seen within the cerebral parenchyma (Figure 18 B). With its ability to break down multiple BBB components[64], MMP-9s increase at this time point could have a tremendous impact on the BBBs function within the following time. In fact, in humans, the biggest changes in BBB permeability are seen between six and 48 hours following ischemic stroke, further indicating the importance of this time point[49].

In conclusion, six hours following thromboembolic stroke induction, MMP-9 activity and claudin-5 protein levels increase significantly in rats.

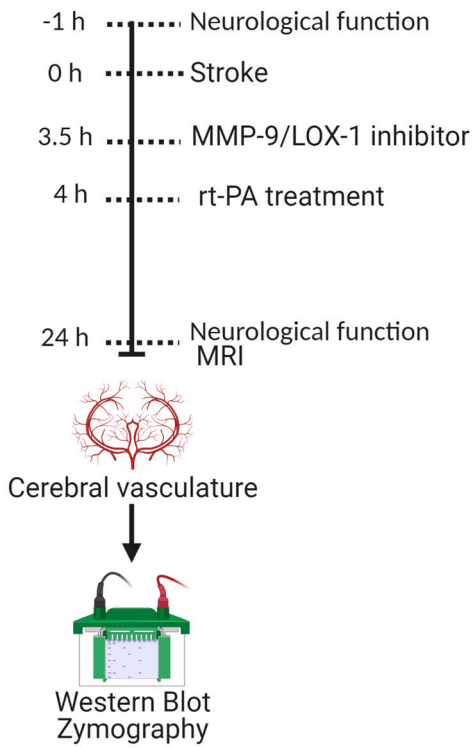


Figure 17.
Illustration of the study design for paper IIIc. Thromboembolic strokes were induced in male Wistar rats, which were then divided into six groups; (i) saline-treated, (ii) treated with rt-PA, (iii) treated with rt-PA in combination with a LOX-1 inhibitor: BI-0115 (iv) treated with rt-PA in combination with an MMP-9 inhibitor: JNJ0966, (v) treated with rt-PA in combination with BI-0115 and JNJ0966 and (vI) sham-operated animals. To induce thrombolysis, rt-PA (3 mg/ kg; Actilyse) was administered four hours after thrombin injection. It was injected with a 10% bolus and 90% perfusion for 40 minutes. The inhibitors (10 mg/kg) were injected intraperitoneal at 30 minutes prior to rt-PA administration. The treatments' effect on stroke outcome was evaluated 24 hours after stroke induction and included neurological function assessment through the 28-point neurological score and the cylinder test. Subsequently, the stroke's infarct volume, brain perfusion, and hemorrhagic transformation were determined using MRI. The cerebral vasculature was then isolated from the collected brains to evaluate MMP-9 activity using zymography and western blotting. Created with BioRender.com.

Treatment with rt-PA ± LOX-1 and MMP-9 inhibitions influence MMP-9 regulation following HGD/R

MMP-9 expression can directly influence the integrity of the BBB following ischemia. From paper II, we could see the protective effects of inhibiting the expression of MMP-9 combined with rt-PA therapy. Moving further, we wanted to investigate both the inhibition of expression and the activation of MMP-9. Since the MEK1/2 inhibitor could prevent the rt-PA induced expression of MMP-9 but not influence the infarct lesion volume, we wanted

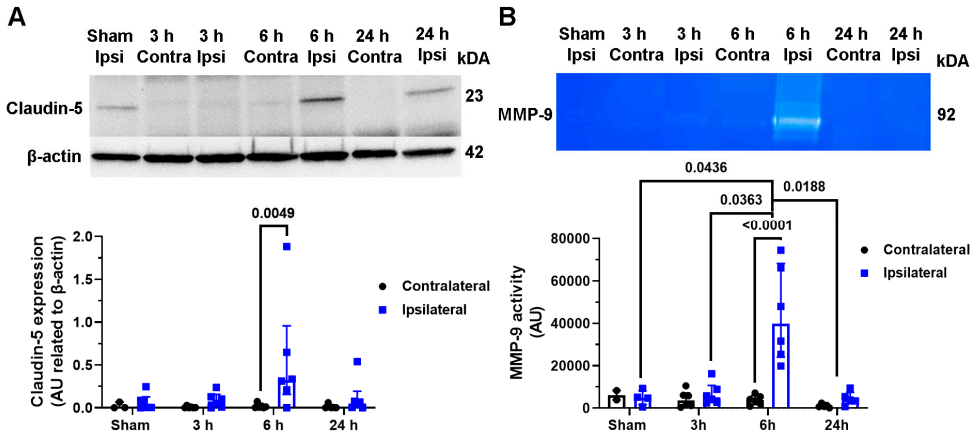


Figure 18.

(**A**) Representative image and quantification of Claudin-5 expression were evaluated by western blot where β-actin was used as a loading control. (**B**) MMP-9 activity was evaluated using zymography shown with a representative zymography gel and quantification of MMP-9 activity. The activity was measured with AU values using ImageJ. Data are shown as median ± IQR. Contra= contralateral, Ipsi= Ipsilateral Comparison between the time points were made using Dunn's multiple comparisons test, where the ipsilateral side was tested separately from the contralateral side. Comparison between the ipsilateral and contralateral hemispheres at each time point was made using Šídák's multiple comparisons test.

to see if an upstream target would have a different effect. Furthermore, increased gene expression of the LOX-1 receptor has been seen in spontaneous hypertensive rats (SHR) and following tMCAO in both SHR and wild-type rats[75, 139].Therefore, we assessed the LOX-1 inhibitor: BI-0115, the MMP-9 inhibitor: JNJ0966, and rt-PA's ability to affect the expression of proteins that can influence BBB permeability in HGD/R, an *in vitro* model of ischemia and reperfusion.

In previous studies, treatment with rt-PA has shown a temporal and dose-dependent increase of MMP-9 activity in normoxic cultured HBMECs, where mRNA levels go back to baseline after 24 hours[140]. Here, we see similar changes. Treatment with rt-PA had no influence on MMP-9 mRNA expression following normoxic conditions of HBMECs at 24h. Contradictory, rt-PA treatment negatively affected the hypoxia-induced increase of MMP-9 mRNA expression, a response which was attenuated with the administration of BI-0115 and JNJ0966. In comparison, mRNA expression of TIMP-1, an endogenous MMP-9 inhibitor, was significantly reduced following hypoxia. The same results were seen when the cells were treated with rt-PA + BI-0115 and JNJ0966 in both normoxic and hypoxic conditions (Figure 19). This suggests that any effect rt-PA ± BI-0115 and JNJ0966 may have on preventing MMP-9 activity will not be through TIMP-1 expression.

In conclusion, MMP-9 mRNA increases significantly during the hypoxic condition in HBMECs, while mRNA for TIMP-1 decreases. The hypoxia-induced increase of MMP-9 mRNA is attenuated by rt-PA ± BI-0115 and JNJ0966.

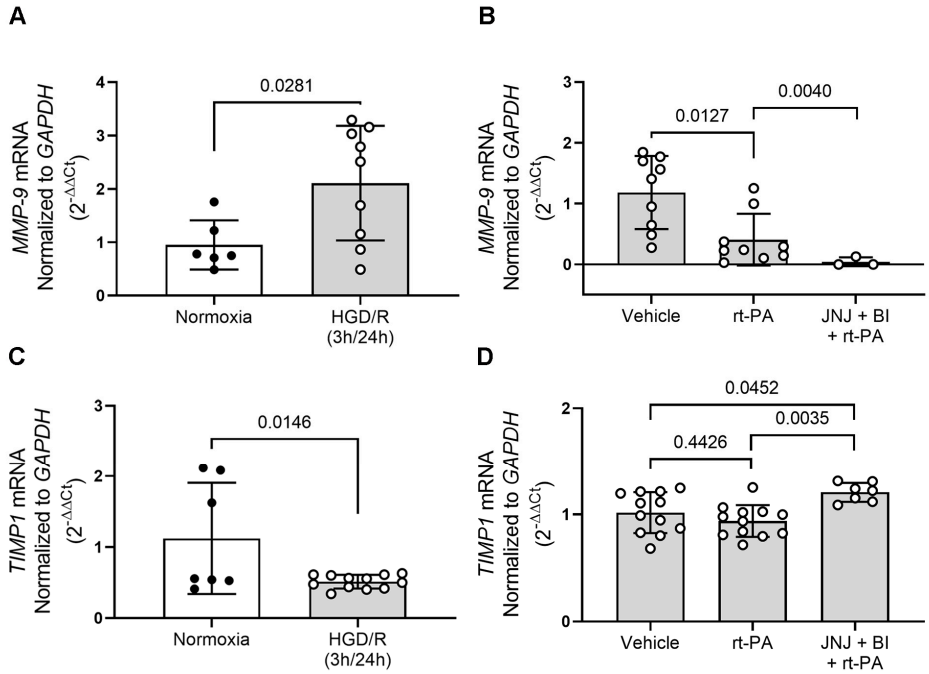


Figure 19.
Bar graph depicts qRT-PCR mediated quantitation of MMP-9 and TIMP-1 mRNA present within HBMECs exposed to either (**A, C**) normoxia or HGD/R (3h/24h) and (**B, D**) HGD/R (3h/24h) treated with either vehicle, rt-PA (12.5µg/mL), or rt-PA + JNJ0966 (5µM) + BI-0115 (10µM) normalized to the housekeeping gene (GAPDH) and expressed as 2^(-ΔΔCt). n = 2-6 independent samples ran in duplicate (data are mean ± SEM). Two-way ANOVA with Tukey's multiple comparisons post hoc test.

LOX-1 and or MMP-9 inhibition effect on rt-PA safety and stroke outcome following a thromboembolic-induced stroke in rats

The LOX-1s role within ischemic stroke has only begun to be elucidated, but studies show clear indications of its detrimental effect on stroke outcome[77, 78]. Furthermore, the *in vitro* study data show that the combination of LOX-1 and MMP-9 inhibition with rt-PA treatment following hypoxia attenuated the hypoxic induced increase of MMP-9 mRNA. In this final part, we, therefore, evaluated if the safety of the rt-PA treatment and improvement of stroke outcome can be achieved by inhibiting the activation of MMP-9 and or by preventing MMP-9 expression using a LOX-1 inhibitor *in vivo*.

As observed both clinically and experimentally before, rt-PA significantly increases MMP-9 activity following ischemic stroke in the thromboembolic stroke model in rats compared to saline-treated animals[141-143]. The increase is regions specific to the ipsilateral hemisphere in the cerebral vasculature. Treatment with either the LOX-1 or the MMP-9 inhibitor showed signs of reducing the rt-PA induced increase of MMP-9 activity. However, the combination of the inhibitors had the most effect. Targeting both the expression and activation of MMP-9 when administrating rt-PA reduced the increase of MMP-9 seen in the cerebral vasculature to similar levels to what was seen in saline-treated animals (Figure 20). The combination treatment's attenuation of the rt-PA induced MMP-9 expression was also seen when evaluating hemorrhagic events.

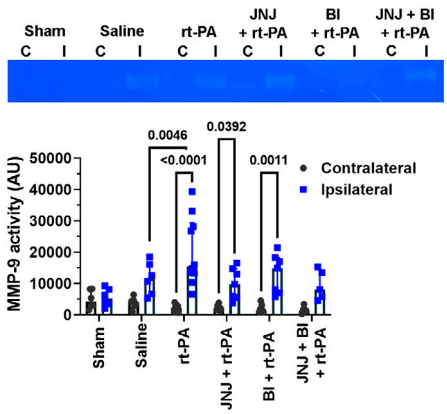


Figure 20.
Following a thromboembolic stroke, animals were either given the MMP-9 inhibitor (JNJ0966 = JNJ) and/or the LOX-1 inhibitor (BI-0115 = BI) before rt-PA administration. At 24 hours after stroke onset, MMP-9 activity was quantified by zymography. Data are shown as median ± IQR. C = contralateral, I = Ipsilateral. Comparison between the time points was made using Dunn's multiple comparisons test, where the ipsilateral side was tested separately from the contralateral side. Comparison between the ipsilateral and contralateral hemispheres at each time point was made using Šídák's multiple comparisons test.

As in the clinic, increased levels of MMP-9 following rt-PA therapy were associated with a significant increase in hemorrhagic events following thromboembolic stroke[11]. The administration of LOX-1 and or MMP-9 inhibitor prior to rt-PA therapy showed protective effects. Hemorrhagic events occurred in all combination treatment groups, but there are signs of reduced incidence and volume in these events. As mentioned in paper II, clinically, hemorrhages can be classified as symptomatic or asymptomatic. Symptomatic hemorrhages cause a higher degree of worsened outcome and are associated with a more considerable blood volume within the infarct area[144-146]. In this study, the inhibition of LOX-1 ± MMP-9 inhibition showed a more considerable influence in preventing more extensive hemorrhages. In addition, the combination

treatment of LOX-1 and rt-PA prevented the rt-PA-induced edema within the ipsilateral hemisphere.

While no significant difference was seen in infarct lesion volume within any treatment group, inhibition of LOX-1 prior to rt-PA treatment showed potential in restraining the spreading of the infarct lesion volume. The effect of LOX-1 inhibition on infarct volume has previously seen similar results following tMCAO in mice, although without the combination with rt-PA administration[78]. Furthermore, when evaluating perfusion within the ipsilateral hemisphere compared to the control side, while saline-treated animals seem to regain perfusion, animals treated with rt-PA still display decreased perfusion within the injured hemisphere. Data may hint at a no-reflow phenomenon occurring following rt-PA treatment. No- reflow is a phenomenon where recanalization does not lead to reperfusion[147, 148]. This has been seen within myocardial infarctions and stroke patients and is suggested to be due to restriction within the microvasculature that remains following recanalization[149, 150]. In this study, treatment with JNJ0966 sustained the rt-PA induced reduction of perfusion, BI-0115 moderate attenuated the reduction, while the combination of the inhibitors had a compelling beneficial effect Figure (21).

An increase in edema, infarct volume, and hemorrhage are all indicators of a worsened stroke outcome[151-153], which in the present study was demonstrated within the rt-PA treated animals. Clinically, the beneficial effects of rt-PA treatment decrease if treatment initiation is delayed[129, 154, 155]. Here we saw that while treatment with JNJ0966 did indicate some beneficial effects, it was BI-0115 that had the most influence in preventing the development of neurological deficiency following stroke and rt-PA treatment (Figure 22). Altogether, inhibition of the LOX-1 and MMP-9 activity improved rt-PA therapy, especially LOX-1, which, beyond improving the safety of rt-PA treatment, also improved the animal's neurological function.

In conclusion, if translational to the clinic, enhancing the safety of the treatment and improving neurological function by combining LOX-1 inhibition with rt-PA therapy would significantly impact outcomes of ischemic stroke patients.

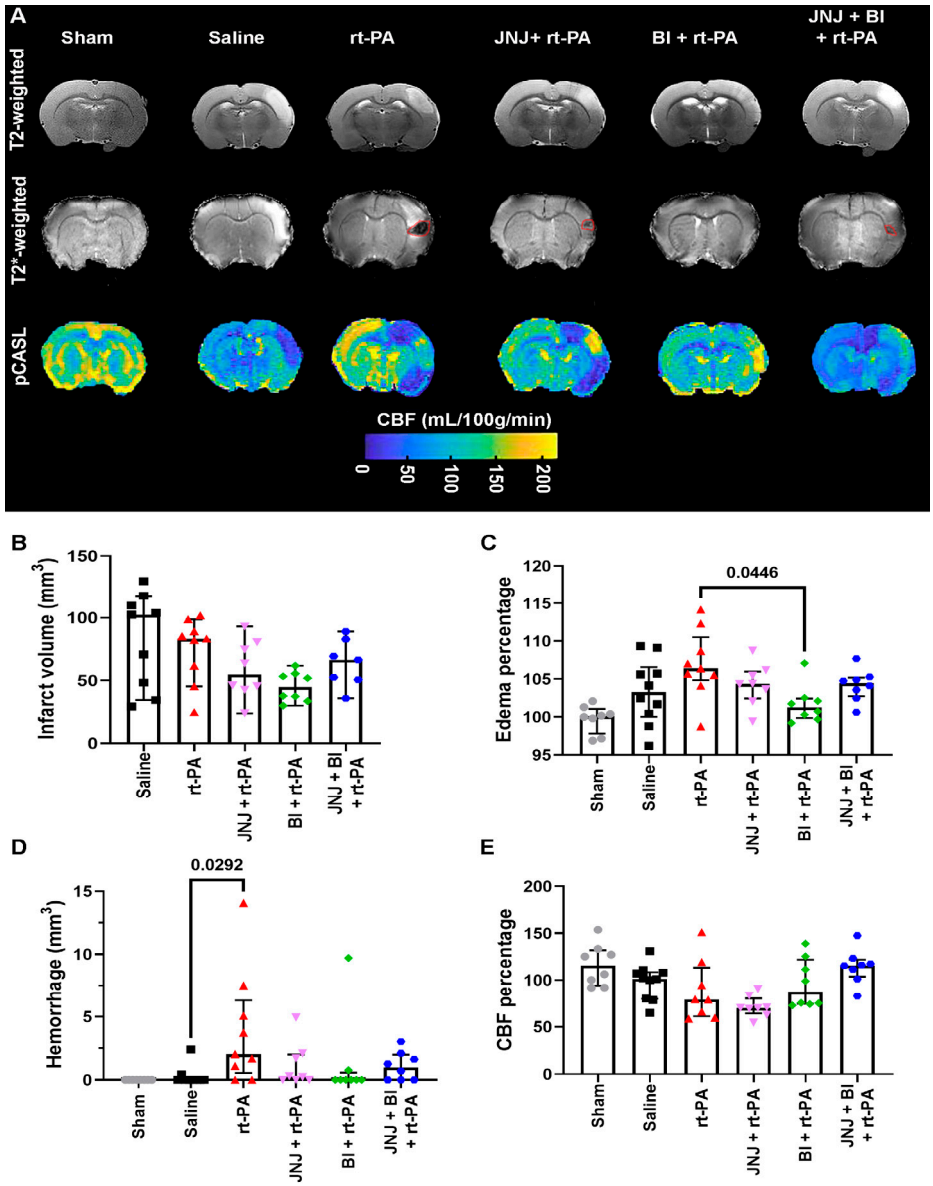


Figure 21.
MMP-9 (JNJ0966 = JNJ) and or LOX-1 (BI-0115 = BI) inhibition conjugated with rt-PA therapy's effect on stroke outcome was evaluated 24 hours after thromboembolic stroke using MRI, compared to saline and rt-PA treated animals. (**A**) Representative T_2-weighted, T_2*-weighted, and pCASL MRI images visualizing the infarct lesions, hemorrhages (circled in red), and perfusion measured in ml/100g/min in all treatment groups. (**B**) Quantification of the infarct lesion measured in mm3 in T2-weighted MRI scans. (**C**) The percentage of edema in the ipsilateral hemisphere compared to its respective contralateral hemisphere was measured from T2-weighted images. (**D**) Hemorrhagic volumes quantified from T2*-weighted MRI images. (**E**) Quantification of the perfusion within the ipsilateral compared to the contralateral hemisphere used as control (100%), measured from 5 consecutive sections within the stroke region. Data are shown as median ± IQR. Comparison between the treatment groups was made using Dunn's multiple comparisons test.

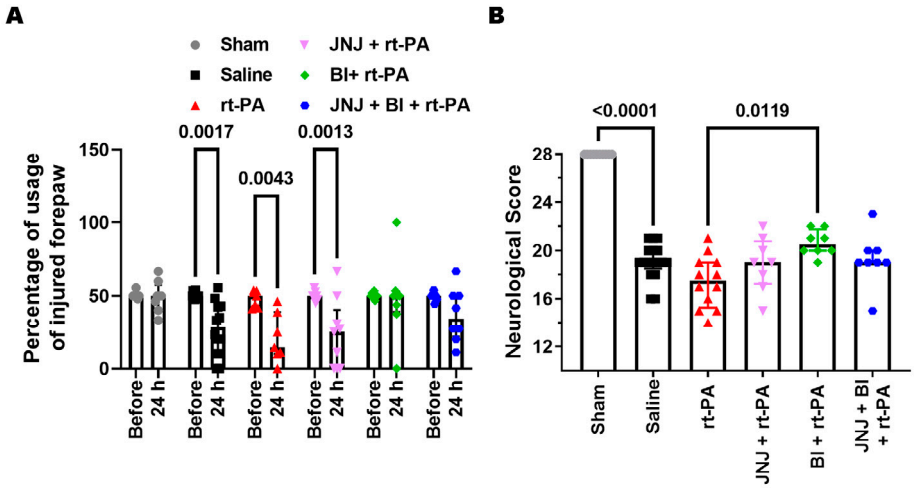


Figure 22.

Stroke was induced using the thromboembolic stroke model, and animals were treated with the MMP-9 inhibitor (JNJ0966 = JNJ) and/or the LOX-1 inhibitor (BI-0115 = BI) combined with rt-PA therapy, or either saline or rt-PA. Neurological function was evaluated using both the cylinder test and the 28-point neuroscore. (**A**) The cylinder test measures the use of the injured (contralateral) forepaw 24 hours after stroke onset compared to baseline levels. Data are shown as median ± IQR. Comparison between the different time points was made using Šídák's multiple comparisons test. (**B**) The 28-point neuroscore is a composite test where the total score of 28-points indicates a healthy rat. Data are shown as median ± IQR. Comparison between treatment groups was made using Dunn's multiple comparisons test.

Concluding Remarks and Future Perspective

This thesis aimed to enhance rt-PA therapy's safety and improve stroke outcomes. By focusing on preventing the increased levels of MMP-9 associated with the rt-PA-induced risk of hemorrhagic transformation, we intended to improve rt-PA therapy and enabling its use within and beyond its current therapeutic window.

Preclinical testing of new treatment options for ischemic stroke has had poor translatability into the clinic, causing questioning if the animal models used within stroke research are relevant. Only embolic stroke models have successfully predicted a clinically working therapeutic, rt-PA. Therefore, in paper I, we aimed to establish the thromboembolic stroke model in rats and solidify its relevance within ischemic stroke research. The thromboembolic model has better reproducibility and lower mortality rates than previous embolic stroke models. Furthermore, the model's ability to mirror the clinical results of treatment with rt-PA, both beneficial and detrimental effects, strengthens its translation capability. While we have evaluated the model within 24 hours of stroke onset, further studies need to investigate its clinical relevance beyond this time frame.

The next part of this thesis focused on the main aim: Improving rt-PA treatment. In this thesis, we evaluated the inhibition of MMP-9 expression by targeting LOX-1 and ERK1/2 and the inhibition of MMP-9 protease activation. The treatments had various effects on the rt-PA-induced activation of MMP-9, but all had a protective impact on preventing hemorrhagic occurrences. The inhibitors also showed a considerable variation in other factors that could influence stroke outcome. Still, the inhibition of LOX-1 combined with rt-PA therapy significantly improved neurological function.

This thesis shows a new way of improving rt-PA therapy that could greatly impact stroke outcomes in patients in the clinic. However, further validation of this new treatment strategy needs to be conducted. Our primary data is based on stroke outcome evaluation 24 hours following stroke induction, with one dosage of the inhibitors followed by rt-PA administration. Further studies focusing on dosage, treatment timing, and long-term evaluation of stroke outcomes must be performed to optimize and better understand the inhibition of LOX-1 with or without rt-PA therapy.

General methodology

Detailed methodologies are described in each paper under the materials and method section.

Stroke induction and treatment

Thromboembolic stroke model (Paper I - III)

In this thesis, we aimed to study thrombolysis treatment and possible new treatment strategies in combination with thrombolysis. Therefore, stroke was induced using the thromboembolic stroke model in rats (paper I, III) and mice (paper II). The model mimics the clinical situation regarding stroke pathophysiology and the outcome of thrombolysis treatment in terms of both its beneficial and detrimental effects[123, 125]. As seen previously, only embolic stroke models have successfully predicted a clinically working therapeutic, rt-PA. However, compared with other embolic stroke models with variation in clot placement, infarct lesion, and neurological deficits, the thromboembolic model has a higher reproducibility of results and a lower mortality rate[123].

Thrombolysis treatment (Paper I - III)

Thrombolysis is achieved by treatment with Alteplase intravenously in the clinic and is administrated as a 10 % bolus followed by 90 % perfusion for 40 minutes; the same treatment and administration were used within this thesis. To establish the thromboembolic stroke model in rats for paper I, thrombolysis was administrated at one- or four- hours following stroke induction. For paper II and III, treatment was administered four hours after stroke induction to mimic the clinical situation with reduced efficacy of the treatment and an increased risk of hemorrhagic transformation.

Inhibitor treatment (Paper II and III)

The inhibitors used within this thesis were:

- U0126: A MEK1/2 inhibitor
- BI-0115: A LOX-1 inhibitor
- JNJ0966: A MMP-9 inhibitor

Administration of the inhibitors was through intraperitoneal injections 30 minutes before thrombolysis treatment. The used administration route allows for quick absorption and translocation of the inhibitors into the systemic circulation. Previous studies determined the time of treatment and evaluation of MMP-9 activation following stroke induction.

Stroke outcome evaluation

Magnetic resonance imaging (Paper I - III)

Evaluation of stroke outcome in terms of infarct volume, hemorrhagic incidence, and perfusion was measured using T_2-weighted, T_2*-weighted, and pCASL MRI scans. The use of MRI imaging reduces the number of animals used within this thesis. Allowing us to determine infarct volume and hemorrhagic transformation on the same animals without using any tissue for staining protocols. In addition, the use of the pCASL scan for determining perfusion within the brain prevents any need for contrast agent administration in animals.

Neurological Function (Paper I and III)

Evaluation of stroke outcome in terms of neurological function was measured using the 28-point neurological score (paper I and III) and the cylinder test (paper III)[156, 157]. The 28-point neurological test evaluates sensorimotor function by assessing the animal's performance on 11 different tasks with various scores (Table 1). The cumulative score of 28 points indicates a healthy animal. The cylinder test evaluates asymmetries in locomotor function by measuring the use of each forepaw when rearing while placed within a transparent cylinder.

Table 1
28-point neuroscore

Test	Maximum score
Circling	4
Motility	3
General Condition	3
Righting reflex when placed on back	1
Paw placement of each paw on the table	4
Ability to pull self up on a horizontal bar	3
Contralateral rotation when held by the tail base	2
Grip strength	2
Visual forepaw reaching	2
Climbing on an inclinded platform	3
Contralateral reflex	1

Ex vivo evaluation

Vessel and parenchyma separation (Paper III)

To allow a more thorough evaluation of tissue-specific changes following thromboembolic stroke, rt-PA, and the use of the different inhibitors within paper III, the cerebral vessels were separated from the parenchyma. Using an improved separation method allowed for a collection of cerebral arteries down to microvessels[158]. This method allowed us to investigate protein changes within the infarcted region in both parenchymal and vessel fractions without the need of pooling tissue of several animals, reducing the number of animals used within this study.

Western Blot (Paper II-III)

We used western blotting to investigate specific proteins and their protein levels. Western blotting is an antibody-based gel electrophoresis method used to determine protein levels. Gel electrophoresis allows for protein separation based on size using an electric current. Transferring the proteins to a membrane allows determining specific protein levels by using protein-specific primary and horseradish peroxidase-conjugated secondary antibodies.

Zymography (Paper II-III)

We used zymography to evaluate MMP-9 activity. Zymography is a gel electrophoresis method allowing for the measurement of proteolytic activity. By incorporating the gel with gelatine (an MMP-9 substrate) and letting our samples incubate in the gel in conditions appropriate for proteolytic activity. Determination of the proteolytic activity of MMP-9 is then performed following coomassie staining of the gel by measuring band intensity in terms of loss of staining.

Immunohistochemistry (Paper II)

We used immunohistochemistry to evaluate protein expression and localization of protein expression. Immunohistochemistry is an antibody-based method using protein-specific primary and fluorescent-conjugated secondary antibodies to determine specific protein distribution in tissue sections.

In vitro evaluation

In vitro model of ischemia and reperfusion (Paper III)

We used HBMECs to evaluate rt-PA ± the LOX-1 and MMP-9 inhibitors *in vitro*. To mimic the clinical situation of ischemia and reperfusion, the cells were under hypoxia and glucose deprivation for three hours, followed by drug administration and "reperfusion" to normal conditions and a glucose-containing medium for 24 hours.

qRT-PCR (Paper III)

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) evaluated the rt-PA ± the LOX-1 and MMP-9 inhibitors' effect on specific mRNAs expression *in vitro*. qRT-PCR is a PCR-based method utilizing a fluorescent reporter dye binding to double-stranded DNA (dsDNA), enabling quantification of the mRNA expression in specific samples.

Acknowledgment

I want to thank all who have made this work possible!

My supervisor, **Saema,** thank you for helping me through these years. I stumbled upon your lab in 2014, almost 8 years ago, and your guidance has made me the scientist I am today. Thank you for all our discussions, whether it be about science, career planning, or life in general. Your thoughts have always been inspirational.

Karsten, my co-supervisor, thank you for your excellent help and support within the laboratory and your invaluable knowledge of stroke research.

To all my colleagues **Anaële, Henrik, André, Frank, Maryam, Philip,** and **Lena,** you have all been a part of my Ph.D. journey and sparkled it with fabulous lunches, discussions, and views of life. Thank you, and I wish you all the best!

A big thank you, **Michael,** for all those hours spent with the MRI. Without your help, this thesis would not have been possible.

While I did retire from my football "career" during my Ph.D., my years in Södra Sandby IF will always be remembered. It was my way of escaping science, and it was you, **Lina, Louise, Cathrine, Kajsa,** and **Bella** that made it amazing. I will never forget our journeys in **Harry**, the singing in the dressing room, and you amazing people!

The **Stockerup** family, thank you all for your support and help for all these years.

My friends from home, **Frida, Sofie, Melina, and Carl**. As cliche as it is, even if we spend months without talking, it never feels as if time has passed when we meet. Thank you for keeping up with my radio silence throughout these years, and for all the good times we have had and will have together.

My friends from Lund, **Emil, Vasiliki, Claes, Ffion, Daniel, Sabine, Andreas, Mirko, Ylva, Clara and Andrés**. I moved down here ten years ago without knowing what I was in for. Thank you for all the laughs, talks, songs, dances, football, training, food, and our time at Ariman's. I have had a wonderful time here in Lund because of you.

And for those closest to me, **my Family.** Thank you for always supporting me, for your guidance, and for being my rock. Thank you for being interested in what I do, you

always want to take care of me, and wishing me the best. Spending time with you over dinner, a card game (which I often end up losing), and fishing (“bottennapp”) has always been and will always be a comfort of mine.

References

1. Azevedo FA, Carvalho LR, Grinberg LT, et al. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol*. 2009; 513: 532-41.
2. Gros C. *Complex and Adaptive Dynamical Systems: A Primer*. Springer Berlin Heidelberg, 2008.
3. Edvinsson L and Krause DN. *Cerebral Blood Flow and Metabolism*. Lippincott Williams & Wilkins, 2002.
4. Katan M and Luft A. Global Burden of Stroke. *Semin Neurol*. 2018; 38: 208-11.
5. Campbell BCV and Khatri P. Stroke. *The Lancet*. 2020; 396: 129-42.
6. Tsao CW, Aday AW, Almarzooq ZI, et al. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. *Circulation*. 2022; 145: e153-e639.
7. Herpich F and Rincon F. Management of Acute Ischemic Stroke. *Crit Care Med*. 2020; 48: 1654-63.
8. Aguiar de Sousa D, von Martial R, Abilleira S, et al. Access to and delivery of acute ischaemic stroke treatments: A survey of national scientific societies and stroke experts in 44 European countries. *Eur Stroke J*. 2019; 4: 13-28.
9. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*. 2008; 359: 1317-29.
10. Hacke W, Kaste M, Fieschi C, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study (ECASS). *JAMA*. 1995; 274: 1017-25.
11. Inzitari D, Giusti B, Nencini P, et al. MMP9 variation after thrombolysis is associated with hemorrhagic transformation of lesion and death. *Stroke*. 2013; 44: 2901-3.
12. Lindsay MP, Norrving B, Sacco RL, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2019. *Int J Stroke*. 2019; 14: 806-17.
13. Campbell BCV, De Silva DA, Macleod MR, et al. Ischaemic stroke. *Nat Rev Dis Primers*. 2019; 5: 70.
14. Allen CL and Bayraktutan U. Risk factors for ischaemic stroke. *Int J Stroke*. 2008; 3: 105-16.

15. Astrup J, Siesjo BK and Symon L. Thresholds in cerebral ischemia - the ischemic penumbra. *Stroke*. 1981; 12: 723-5.
16. Dirnagl U, Iadecola C and Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci*. 1999; 22: 391-7.
17. Hossmann KA. The two pathophysiologies of focal brain ischemia: implications for translational stroke research. *J Cereb Blood Flow Metab*. 2012; 32: 1310-6.
18. Lee KB, Lim SH, Kim KH, et al. Six-month functional recovery of stroke patients: a multi-time-point study. *Int J Rehabil Res*. 2015; 38: 173-80.
19. Verheyden G, Nieuwboer A, De Wit L, et al. Time course of trunk, arm, leg, and functional recovery after ischemic stroke. *Neurorehabilitation and neural repair*. 2008; 22: 173-9.
20. Delavaran H, Aked J, Sjunnesson H, et al. Spontaneous Recovery of Upper Extremity Motor Impairment After Ischemic Stroke: Implications for Stem Cell-Based Therapeutic Approaches. *Transl Stroke Res*. 2017; 8: 351-61.
21. Martin RL, Lloyd HG and Cowan AI. The early events of oxygen and glucose deprivation: setting the scene for neuronal death? *Trends Neurosci*. 1994; 17: 251-7.
22. Erecinska M and Silver IA. Tissue oxygen tension and brain sensitivity to hypoxia. *Respir Physiol*. 2001; 128: 263-76.
23. Xing C, Arai K, Lo EH and Hommel M. Pathophysiologic cascades in ischemic stroke. *Int J Stroke*. 2012; 7: 378-85.
24. Moskowitz MA, Lo EH and Iadecola C. The science of stroke: mechanisms in search of treatments. *Neuron*. 2010; 67: 181-98.
25. Lo EH, Dalkara T and Moskowitz MA. Mechanisms, challenges and opportunities in stroke. *Nat Rev Neurosci*. 2003; 4: 399-415.
26. Wang Q, Tang XN and Yenari MA. The inflammatory response in stroke. *J Neuroimmunol*. 2007; 184: 53-68.
27. Sandoval KE and Witt KA. Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis*. 2008; 32: 200-19.
28. Bernardo-Castro S, Sousa JA, Bras A, et al. Pathophysiology of Blood-Brain Barrier Permeability Throughout the Different Stages of Ischemic Stroke and Its Implication on Hemorrhagic Transformation and Recovery. *Front Neurol*. 2020; 11: 594672.
29. Baeten KM and Akassoglou K. Extracellular matrix and matrix receptors in blood-brain barrier formation and stroke. *Dev Neurobiol*. 2011; 71: 1018-39.
30. Obermeier B, Verma A and Ransohoff RM. Chapter 3 - The blood–brain barrier. In: Pittock SJ and Vincent A, (eds.). *Handbook of Clinical Neurology*. Elsevier, 2016, p. 39-59.
31. Fenstermacher J, Gross P, Sposito N, Acuff V, Pettersen S and Gruber K. Structural and functional variations in capillary systems within the brain. *Ann N Y Acad Sci*. 1988; 529: 21-30.

32. Sedlakova R, Shivers RR and Del Maestro RF. Ultrastructure of the blood-brain barrier in the rabbit. *J Submicrosc Cytol Pathol*. 1999; 31: 149-61.

33. Piccio L, Rossi B, Scarpini E, et al. Molecular mechanisms involved in lymphocyte recruitment in inflamed brain microvessels: critical roles for P-selectin glycoprotein ligand-1 and heterotrimeric G(i)-linked receptors. *J Immunol*. 2002; 168: 1940-9.

34. Hauser SL, Bhan AK, Gilles FH, et al. Immunohistochemical staining of human brain with monoclonal antibodies that identify lymphocytes, monocytes, and the Ia antigen. *Journal of Neuroimmunology*. 1983; 5: 197-205.

35. Luissint AC, Artus C, Glacial F, Ganeshamoorthy K and Couraud PO. Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. *Fluids Barriers CNS*. 2012; 9: 23.

36. Lochhead JJ, Yang J, Ronaldson PT and Davis TP. Structure, Function, and Regulation of the Blood-Brain Barrier Tight Junction in Central Nervous System Disorders. *Front Physiol*. 2020; 11: 914.

37. Greene C, Hanley N and Campbell M. Claudin-5: gatekeeper of neurological function. *Fluids Barriers CNS*. 2019; 16: 3.

38. Farquhar MG and Palade GE. Junctional complexes in various epithelia. *The Journal of cell biology*. 1963; 17: 375-412.

39. Fanning AS, Jameson BJ, Jesaitis LA and Anderson JM. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J Biol Chem*. 1998; 273: 29745-53.

40. Thomsen MS, Routhe LJ and Moos T. The vascular basement membrane in the healthy and pathological brain. *J Cereb Blood Flow Metab*. 2017; 37: 3300-17.

41. Hellstrom M, Gerhardt H, Kalen M, et al. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol*. 2001; 153: 543-53.

42. von Tell D, Armulik A and Betsholtz C. Pericytes and vascular stability. *Exp Cell Res*. 2006; 312: 623-9.

43. Abbott NJ, Rönnbäck L and Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. *Nature Reviews Neuroscience*. 2006; 7: 41-53.

44. Filosa JA and Iddings JA. Astrocyte regulation of cerebral vascular tone. *Am J Physiol Heart Circ Physiol*. 2013; 305: H609-19.

45. Tilling T, Engelbertz C, Decker S, Korte D, Hüwel S and Galla HJ. Expression and adhesive properties of basement membrane proteins in cerebral capillary endothelial cell cultures. *Cell and tissue research*. 2002; 310: 19-29.

46. Xu L, Nirwane A and Yao Y. Basement membrane and blood-brain barrier. *Stroke Vasc Neurol*. 2019; 4: 78-82.

47. Osada T, Gu YH, Kanazawa M, et al. Interendothelial claudin-5 expression depends on cerebral endothelial cell-matrix adhesion by beta(1)-integrins. *J Cereb Blood Flow Metab*. 2011; 31: 1972-85.

48. Nadareishvili Z, Simpkins AN, Hitomi E, Reyes D and Leigh R. Post-Stroke Blood-Brain Barrier Disruption and Poor Functional Outcome in Patients Receiving Thrombolytic Therapy. *Cerebrovascular diseases (Basel, Switzerland)*. 2019; 47: 135-42.
49. Merali Z, Huang K, Mikulis D, Silver F and Kassner A. Evolution of blood-brain-barrier permeability after acute ischemic stroke. *PLoS One*. 2017; 12: e0171558.
50. Firlik AD, Yonas H, Kaufmann AM, et al. Relationship between cerebral blood flow and the development of swelling and life-threatening herniation in acute ischemic stroke. *Journal of Neurosurgery*. 1998; 89: 243-9.
51. Ozkul-Wermester O, Guegan-Massardier E, Triquenot A, Borden A, Perot G and Gerardin E. Increased blood-brain barrier permeability on perfusion computed tomography predicts hemorrhagic transformation in acute ischemic stroke. *Eur Neurol*. 2014; 72: 45-53.
52. Annan M, Gaudron M, Cottier JP, et al. Functional Outcome of Hemorrhagic Transformation after Thrombolysis for Ischemic Stroke: A Prospective Study. *Cerebrovasc Dis Extra*. 2015; 5: 103-6.
53. Kazmierski R, Michalak S, Wencel-Warot A and Nowinski WL. Serum tight-junction proteins predict hemorrhagic transformation in ischemic stroke patients. *Neurology*. 2012; 79: 1677-85.
54. Rosell A, Cuadrado E, Ortega-Aznar A, Hernandez-Guillamon M, Lo EH and Montaner J. MMP-9-positive neutrophil infiltration is associated to blood-brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. *Stroke*. 2008; 39: 1121-6.
55. Klein T and Bischoff R. Physiology and pathophysiology of matrix metalloproteases. *Amino Acids*. 2011; 41: 271-90.
56. Moon S-K, Cha B-Y and Kim C-H. ERK1/2 mediates TNF-α-induced matrix metalloproteinase-9 expression in human vascular smooth muscle cells via the regulation of NF-κB and AP-1: Involvement of the ras dependent pathway. *Journal of Cellular Physiology*. 2004; 198: 417-27.
57. Van den Steen PE, Dubois B, Nelissen I, Rudd PM, Dwek RA and Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit Rev Biochem Mol Biol*. 2002; 37: 375-536.
58. Vandooren J, Van den Steen PE and Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. *Crit Rev Biochem Mol Biol*. 2013; 48: 222-72.
59. Davis GE, Pintar Allen KA, Salazar R and Maxwell SA. Matrix metalloproteinase-1 and -9 activation by plasmin regulates a novel endothelial cell-mediated mechanism of collagen gel contraction and capillary tube regression in three-dimensional collagen matrices. *J Cell Sci*. 2001; 114: 917-30.

60. Chelladurai P, Seeger W and Pullamsetti SS. Matrix metalloproteinases and their inhibitors in pulmonary hypertension. *European Respiratory Journal*. 2012: erj02099-2011.

61. Farina AR and Mackay AR. Gelatinase B/MMP-9 in Tumour Pathogenesis and Progression. *Cancers*. 2014; 6: 240 - 96.

62. Cuadrado E, Rosell A, Penalba A, et al. Vascular MMP-9/TIMP-2 and neuronal MMP-10 up-regulation in human brain after stroke: a combined laser microdissection and protein array study. *J Proteome Res*. 2009; 8: 3191-7.

63. Romanic AM, White RF, Arleth AJ, Ohlstein EH and Barone FC. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats: inhibition of matrix metalloproteinase-9 reduces infarct size. *Stroke*. 1998; 29: 1020-30.

64. Chen F, Ohashi N, Li W, Eckman C and Nguyen JH. Disruptions of occludin and claudin-5 in brain endothelial cells in vitro and in brains of mice with acute liver failure. *Hepatology*. 2009; 50: 1914-23.

65. Wang G, Guo Q, Hossain M, et al. Bone marrow-derived cells are the major source of MMP-9 contributing to blood-brain barrier dysfunction and infarct formation after ischemic stroke in mice. *Brain Res*. 2009; 1294: 183-92.

66. Mehta JL, Chen J, Hermonat PL, Romeo F and Novelli G. Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): a critical player in the development of atherosclerosis and related disorders. *Cardiovasc Res*. 2006; 69: 36-45.

67. Yoshimoto R, Fujita Y, Kakino A, Iwamoto S, Takaya T and Sawamura T. The discovery of LOX-1, its ligands and clinical significance. *Cardiovasc Drugs Ther*. 2011; 25: 379-91.

68. Li D and Mehta JL. Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells: evidence from use of antisense LOX-1 mRNA and chemical inhibitors. *Arterioscler Thromb Vasc Biol*. 2000; 20: 1116-22.

69. Sugimoto K, Ishibashi T, Sawamura T, et al. LOX-1-MT1-MMP axis is crucial for RhoA and Rac1 activation induced by oxidized low-density lipoprotein in endothelial cells. *Cardiovasc Res*. 2009; 84: 127-36.

70. Mentrup T, Cabrera-Cabrera F and Schröder B. Proteolytic Regulation of the Lectin-Like Oxidized Lipoprotein Receptor LOX-1. *Frontiers in cardiovascular medicine*. 2020; 7: 594441.

71. Minami M, Kume N, Kataoka H, et al. Transforming growth factor-beta(1) increases the expression of lectin-like oxidized low-density lipoprotein receptor-1. *Biochem Biophys Res Commun*. 2000; 272: 357-61.

72. Murphy JE, Vohra RS, Dunn S, et al. Oxidised LDL internalisation by the LOX-1 scavenger receptor is dependent on a novel cytoplasmic motif and is regulated by dynamin-2. *Journal of Cell Science*. 2008; 121: 2136-47.

73. Gioia M, Vindigni G, Testa B, et al. Membrane Cholesterol Modulates LOX-1 Shedding in Endothelial Cells. *PLoS One*. 2015; 10: e0141270.

74. Skarpengland T, Skjelland M, Kong XY, et al. Increased Levels of Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 in Ischemic Stroke and Transient Ischemic Attack. *J Am Heart Assoc*. 2018; 7.

75. Grell AS, Mostajeran M, Frederiksen SD, Edvinsson L and Ansar S. Cerebrovascular Gene Expression in Spontaneously Hypertensive Rats After Transient Middle Cerebral Artery Occlusion. *Neuroscience*. 2017; 367: 219-32.

76. Li D, Williams V, Liu L, et al. LOX-1 inhibition in myocardial ischemia-reperfusion injury: modulation of MMP-1 and inflammation. *Am J Physiol Heart Circ Physiol*. 2002; 283: H1795-801.

77. Liang YQ, Kakino A, Matsuzaka Y, et al. LOX-1 (Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1) Deletion Has Protective Effects on Stroke in the Genetic Background of Stroke-Prone Spontaneously Hypertensive Rat. *Stroke*. 2020; 51: 1835-43.

78. Akhmedov A, Bonetti NR, Reiner MF, et al. Deleterious role of endothelial lectin-like oxidized low-density lipoprotein receptor-1 in ischaemia/reperfusion cerebral injury. *J Cereb Blood Flow Metab*. 2019; 39: 2233-45.

79. Gelderblom M, Leypoldt F, Steinbach K, et al. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke*. 2009; 40: 1849-57.

80. Jin R, Liu L, Zhang S, Nanda A and Li G. Role of inflammation and its mediators in acute ischemic stroke. *J Cardiovasc Transl Res*. 2013; 6: 834-51.

81. Tanigawa H, Miura S-i, Zhang B, et al. Low-density lipoprotein oxidized to various degrees activates ERK1/2 through Lox-1. *Atherosclerosis*. 2006; 188: 245-50.

82. Sun J and Nan G. The Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway as a Discovery Target in Stroke. *Journal of Molecular Neuroscience*. 2016; 59: 90-8.

83. Cargnello M and Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol Mol Biol Rev*. 2011; 75: 50-83.

84. Chen RH, Sarnecki C and Blenis J. Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Mol Cell Biol*. 1992; 12: 915-27.

85. Cho A, Graves J and Reidy MA. Mitogen-activated protein kinases mediate matrix metalloproteinase-9 expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2000; 20: 2527-32.

86. Slevin M, Krupinski J, Slowik A, Rubio F, Szczudlik A and Gaffney J. Activation of MAP kinase (ERK-1/ERK-2), tyrosine kinase and VEGF in the human brain following acute ischaemic stroke. *Neuroreport*. 2000; 11: 2759-64.

87. Irving EA, Barone FC, Reith AD, Hadingham SJ and Parsons AA. Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischaemia in the rat. *Brain Res Mol Brain Res*. 2000; 77: 65-75.

88. Namura S, Iihara K, Takami S, et al. Intravenous administration of MEK inhibitor U0126 affords brain protection against forebrain ischemia and focal cerebral ischemia. *Proc Natl Acad Sci U S A*. 2001; 98: 11569-74.

89. Ahnstedt H, Mostajeran M, Blixt FW, et al. U0126 attenuates cerebral vasoconstriction and improves long-term neurologic outcome after stroke in female rats. *J Cereb Blood Flow Metab*. 2015; 35: 454-60.

90. Wang X, Wang H, Xu L, et al. Significant neuroprotection against ischemic brain injury by inhibition of the MEK1 protein kinase in mice: exploration of potential mechanism associated with apoptosis. *J Pharmacol Exp Ther*. 2003; 304: 172-8.

91. Maddahi A, Chen Q and Edvinsson L. Enhanced cerebrovascular expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 via the MEK/ERK pathway during cerebral ischemia in the rat. *BMC Neurosci*. 2009; 10: 56.

92. Stroke Unit Trialists C. Organised inpatient (stroke unit) care for stroke. *Cochrane Database Syst Rev*. 2013: CD000197.

93. Ajmi SC, Advani R, Fjetland L, et al. Reducing door-to-needle times in stroke thrombolysis to 13 min through protocol revision and simulation training: a quality improvement project in a Norwegian stroke centre. *BMJ quality & safety*. 2019; 28: 939-48.

94. Nogueira RG, Jadhav AP, Haussen DC, et al. Thrombectomy 6 to 24 Hours after Stroke with a Mismatch between Deficit and Infarct. *N Engl J Med*. 2018; 378: 11-21.

95. Tong X, Wang Y, Fiehler J, et al. Thrombectomy Versus Combined Thrombolysis and Thrombectomy in Patients With Acute Stroke: A Matched-Control Study. *Stroke*. 2021; 52: 1589-600.

96. Powers WJ, Rabinstein AA, Ackerson T, et al. Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*. 2019; 50: e344-e418.

97. Jilani TN and Siddiqui AH. Tissue plasminogen activator. *StatPearls [Internet]*. StatPearls Publishing, 2021.

98. Gobin YP, Starkman S, Duckwiler GR, et al. MERCI 1: a phase 1 study of Mechanical Embolus Removal in Cerebral Ischemia. *Stroke*. 2004; 35: 2848-54.

99. Turk AS, 3rd, Siddiqui A, Fifi JT, et al. Aspiration thrombectomy versus stent retriever thrombectomy as first-line approach for large vessel occlusion (COMPASS): a multicentre, randomised, open label, blinded outcome, non-inferiority trial. *Lancet*. 2019; 393: 998-1008.

100. Bernsen MLE, Bruggeman AAE, Brouwer J, et al. Aspiration Versus Stent Retriever Thrombectomy for Posterior Circulation Stroke. *Stroke*. 2022; 53: 749-57.

101. Mokin M, Ansari SA, McTaggart RA, et al. Indications for thrombectomy in acute ischemic stroke from emergent large vessel occlusion (ELVO): report of the SNIS Standards and Guidelines Committee. *J Neurointerv Surg*. 2019; 11: 215-20.

102. Barber PA, Zhang J, Demchuk AM, Hill MD and Buchan AM. Why are stroke patients excluded from TPA therapy? An analysis of patient eligibility. *Neurology*. 2001; 56: 1015-20.

103. Mackey J, Kleindorfer D, Sucharew H, et al. Population-based study of wake-up strokes. *Neurology*. 2011; 76: 1662-7.

104. Montaner J, Molina CA, Monasterio J, et al. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. *Circulation*. 2003; 107: 598-603.

105. Ramos-DeSimone N, Hahn-Dantona E, Sipley J, Nagase H, French DL and Quigley JP. Activation of matrix metalloproteinase-9 (MMP-9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. *J Biol Chem*. 1999; 274: 13066-76.

106. Cuadrado E, Ortega L, Hernandez-Guillamon M, et al. Tissue plasminogen activator (t-PA) promotes neutrophil degranulation and MMP-9 release. *J Leukoc Biol*. 2008; 84: 207-14.

107. Jin X, Sun Y, Xu J and Liu W. Caveolin-1 mediates tissue plasminogen activator-induced MMP-9 up-regulation in cultured brain microvascular endothelial cells. *J Neurochem*. 2015; 132: 724-30.

108. Cui J, Chen S, Zhang C, et al. Inhibition of MMP-9 by a selective gelatinase inhibitor protects neurovasculature from embolic focal cerebral ischemia. *Mol Neurodegener*. 2012; 7: 21.

109. Lapchak PA, Chapman DF and Zivin JA. Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. *Stroke*. 2000; 31: 3034-40.

110. Tierney GM, Griffin NR, Stuart RC, et al. A pilot study of the safety and effects of the matrix metalloproteinase inhibitor marimastat in gastric cancer. *Eur J Cancer*. 1999; 35: 563-8.

111. Bramhall SR, Schulz J, Nemunaitis J, Brown PD, Baillet M and Buckels JA. A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. *Br J Cancer*. 2002; 87: 161-7.

112. Rosemurgy A, Harris J, Langleben A, Casper E, Goode S and Rasmussen H. Marimastat in patients with advanced pancreatic cancer: a dose-finding study. *Am J Clin Oncol*. 1999; 22: 247-52.

113. Winer A, Adams S and Mignatti P. Matrix Metalloproteinase Inhibitors in Cancer Therapy: Turning Past Failures Into Future Successes. *Molecular Cancer Therapeutics*. 2018; 17: 1147-55.

114. Maquoi E, Sounni NE, Devy L, et al. Anti-invasive, antitumoral, and antiangiogenic efficacy of a pyrimidine-2,4,6-trione derivative, an orally active and selective matrix metalloproteinases inhibitor. *Clin Cancer Res*. 2004; 10: 4038-47.

115. Kilic E, Hermann DM and Hossmann KA. A reproducible model of thromboembolic stroke in mice. *Neuroreport*. 1998; 9: 2967-70.

116. Zhang Z, Zhang RL, Jiang Q, Raman SB, Cantwell L and Chopp M. A new rat model of thrombotic focal cerebral ischemia. *J Cereb Blood Flow Metab*. 1997; 17: 123-35.

117. Rasmussen RS, Overgaard K, Pakola S and Boysen G. Effects of microplasmin on recovery in a rat embolic stroke model. *Neurol Res*. 2008; 30: 75-81.

118. Dirnagl U. *Rodent Models of Stroke*. 2016.

119. Macrae IM. Preclinical stroke research--advantages and disadvantages of the most common rodent models of focal ischaemia. *Br J Pharmacol*. 2011; 164: 1062-78.

120. Kaneko D, Nakamura N and Ogawa T. Cerebral infarction in rats using homologous blood emboli: development of a new experimental model. *Stroke*. 1985; 16: 76-84.

121. Kudo M, Aoyama A, Ichimori S and Fukunaga N. An animal model of cerebral infarction. Homologous blood clot emboli in rats. *Stroke*. 1982; 13: 505-8.

122. Ren M, Lin Z-J, Qian H, et al. Embolic middle cerebral artery occlusion model using thrombin and fibrinogen composed clots in rat. *Journal of neuroscience methods*. 2012; 211: 296-304.

123. Orset C, Macrez R, Young AR, et al. Mouse model of in situ thromboembolic stroke and reperfusion. *Stroke*. 2007; 38: 2771-8.

124. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH and Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol*. 2006; 59: 467-77.

125. Garcia-Culebras A, Palma-Tortosa S, Moraga A, et al. Toll-Like Receptor 4 Mediates Hemorrhagic Transformation After Delayed Tissue Plasminogen Activator Administration in In Situ Thromboembolic Stroke. *Stroke*. 2017; 48: 1695-9.

126. Orset C, Haelewyn B, Allan SM, et al. Efficacy of Alteplase in a Mouse Model of Acute Ischemic Stroke: A Retrospective Pooled Analysis. *Stroke*. 2016; 47: 1312-8.

127. Bhatia R, Hill MD, Shobha N, et al. Low rates of acute recanalization with intravenous recombinant tissue plasminogen activator in ischemic stroke: real-world experience and a call for action. *Stroke*. 2010; 41: 2254-8.

128. Lee KY, Han SW, Kim SH, et al. Early recanalization after intravenous administration of recombinant tissue plasminogen activator as assessed by pre- and post-thrombolytic angiography in acute ischemic stroke patients. *Stroke*. 2007; 38: 192-3.

129. Man S, Xian Y, Holmes DN, et al. Association Between Thrombolytic Door-to-Needle Time and 1-Year Mortality and Readmission in Patients With Acute Ischemic Stroke. *JAMA*. 2020; 323: 2170-84.

130. Maddahi A and Edvinsson L. Cerebral ischemia induces microvascular pro-inflammatory cytokine expression via the MEK/ERK pathway. *J Neuroinflammation*. 2010; 7: 14.

131. Mostajeran M, Edvinsson L, Warfvinge K, Singh R and Ansar S. Inhibition of mitogen-activated protein kinase 1/2 in the acute phase of stroke improves long-term neurological outcome and promotes recovery processes in rats. *Acta Physiol (Oxf)*. 2017; 219: 814-24.

132. Mostajeran M, Wetterling F, Blixt FW, Edvinsson L and Ansar S. Acute mitogen-activated protein kinase 1/2 inhibition improves functional recovery and vascular changes after ischaemic stroke in rat-monitored by 9.4 T magnetic resonance imaging. *Acta Physiol (Oxf)*. 2018; 223: e12985.

133. Yaghi S, Willey JZ, Cucchiara B, et al. Treatment and Outcome of Hemorrhagic Transformation After Intravenous Alteplase in Acute Ischemic Stroke: A Scientific Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*. 2017; 48: e343-e61.

134. Kim T, Koo J, Kim SH, Song IU, Chung SW and Lee KS. Blood-brain barrier permeability assessed by perfusion computed tomography predicts hemorrhagic transformation in acute reperfusion therapy. *Neurol Sci*. 2018; 39: 1579-84.

135. Berndt P, Winkler L, Cording J, et al. Tight junction proteins at the blood-brain barrier: far more than claudin-5. *Cell Mol Life Sci*. 2019; 76: 1987-2002.

136. Nitta T, Hata M, Gotoh S, et al. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *The Journal of cell biology*. 2003; 161: 653-60.

137. Gericke B, Romermann K, Noack A, et al. A face-to-face comparison of claudin-5 transduced human brain endothelial (hCMEC/D3) cells with porcine brain endothelial cells as blood-brain barrier models for drug transport studies. *Fluids Barriers CNS*. 2020; 17: 53.

138. Liu J, Jin X, Liu KJ and Liu W. Matrix metalloproteinase-2-mediated occludin degradation and caveolin-1-mediated claudin-5 redistribution contribute to blood-brain barrier damage in early ischemic stroke stage. *J Neurosci*. 2012; 32: 3044-57.

139. Grell AS, Frederiksen SD, Edvinsson L and Ansar S. Cerebrovascular gene expression in spontaneously hypertensive rats. *PLoS One*. 2017; 12: e0184233.

140. Wang X, Lee SR, Arai K, et al. Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. *Nat Med*. 2003; 9: 1313-7.

141. Golab P, Boguszewska-Czubara A, Kielbus M and Kurzepa J. The rtPA increases MMP-9 activity in serum during ischaemic stroke. *Neurol Neurochir Pol*. 2014; 48: 309-14.

142. Saleem S, Wang D, Zhao T, Sullivan RD and Reed GL. Matrix Metalloproteinase-9 Expression is Enhanced by Ischemia and Tissue Plasminogen Activator and Induces Hemorrhage, Disability and Mortality in Experimental Stroke. *Neuroscience*. 2021; 460: 120-9.

143. Ning M, Furie KL, Koroshetz WJ, et al. Association between tPA therapy and raised early matrix metalloproteinase-9 in acute stroke. *Neurology*. 2006; 66: 1550-5.

144. Trouillas P and von Kummer R. Classification and pathogenesis of cerebral hemorrhages after thrombolysis in ischemic stroke. *Stroke*. 2006; 37: 556-61.

145. Fiorelli M, Bastianello S, von Kummer R, et al. Hemorrhagic transformation within 36 hours of a cerebral infarct: relationships with early clinical deterioration and 3-month outcome in the European Cooperative Acute Stroke Study I (ECASS I) cohort. *Stroke*. 1999; 30: 2280-4.

146. Berger C, Fiorelli M, Steiner T, et al. Hemorrhagic transformation of ischemic brain tissue: asymptomatic or symptomatic? *Stroke*. 2001; 32: 1330-5.

147. Kloner RA, King KS and Harrington MG. No-reflow phenomenon in the heart and brain. *Am J Physiol Heart Circ Physiol*. 2018; 315: H550-H62.

148. Soares BP, Tong E, Hom J, et al. Reperfusion Is a More Accurate Predictor of Follow-Up Infarct Volume Than Recanalization. *Stroke*. 2010; 41: e34-e40.

149. Yemisci M, Gursoy-Ozdemir Y, Vural A, Can A, Topalkara K and Dalkara T. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nat Med*. 2009; 15: 1031-7.

150. El Amki M, Gluck C, Binder N, et al. Neutrophils Obstructing Brain Capillaries Are a Major Cause of No-Reflow in Ischemic Stroke. *Cell Rep*. 2020; 33: 108260.

151. Yoo AJ, Chaudhry ZA, Nogueira RG, et al. Infarct volume is a pivotal biomarker after intra-arterial stroke therapy. *Stroke*. 2012; 43: 1323-30.

152. Battey TW, Karki M, Singhal AB, et al. Brain edema predicts outcome after nonlacunar ischemic stroke. *Stroke*. 2014; 45: 3643-8.

153. Paciaroni M, Agnelli G, Corea F, et al. Early hemorrhagic transformation of brain infarction: rate, predictive factors, and influence on clinical outcome: results of a prospective multicenter study. *Stroke*. 2008; 39: 2249-56.

154. Yafasova A, Fosbol EL, Johnsen SP, et al. Time to Thrombolysis and Long-Term Outcomes in Patients With Acute Ischemic Stroke: A Nationwide Study. *Stroke*. 2021; 52: 1724-32.

155. Hacke W, Donnan G, Fieschi C, et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. *Lancet*. 2004; 363: 768-74.

156. Encarnacion A, Horie N, Keren-Gill H, Bliss TM, Steinberg GK and Shamloo M. Long-term behavioral assessment of function in an experimental model for ischemic stroke. *J Neurosci Methods*. 2011; 196: 247-57.

157. Choi-Lundberg DL, Lin Q, Schallert T, et al. Behavioral and cellular protection of rat dopaminergic neurons by an adenoviral vector encoding glial cell line-derived neurotrophic factor. *Exp Neurol*. 1998; 154: 261-75.

158. Matthes F, Matuskova H, Arkelius K, Ansar S, Lundgaard I and Meissner A. An Improved Method for Physical Separation of Cerebral Vasculature and Parenchyma Enables Detection of Blood-Brain-Barrier Dysfunction. *NeuroSci*. 2021; 2: 59-74.

Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Science, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:101
ISBN 978-91-8021-262-5
ISSN 1652-8220