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NEUROPROTECTIVE EFFECT OF THEOPHYLLINE IN ACUTE ISCHEMIC STROKE

BY
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Neuroprotective effect of theophylline in acute ischemic stroke

PhD Dissertation by

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Preface

The introduction of thrombolysis in 2004 and thrombectomy in 2015 were important breakthroughs in the history of acute stroke treatment in Denmark. There are ongoing efforts to improve acute stroke treatment further, and numerous clinical trials have been performed in order to identify a neuroprotective agent although to date none have been successful. The concept of using theophylline as a neuroprotective agent in patients with stroke began in the 1940´s; theophylline was used for this purpose widely in the 1960´s-1970´s, but its use was stopped in the early 1980´s due to a lack of evidence about its clinical effectiveness.

The rationale for this thesis was based on the idea of combining new effective treatments and new imaging techniques with a historical putative neuroprotective agent (theophylline) to attempt to improve acute stroke treatment. This led to the Theophylline in Acute Ischemic Stroke (TEA-Stroke) trial conducted at Aalborg University Hospital in cooperation with: Aarhus University Hospital; the Centre of Functionally Integrative Neuroscience, Aarhus University; University Medical Center Hamburg-Eppendorf, Germany; Stanford University, Palo Alto, CA, USA; and the University of Calgary, Canada.

The work was carried out during my employment at the Department of Neurology, Aalborg University Hospital in the period 2014-2020.

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I am very grateful to Niels Hjort, who handled the challenge of being a principal investigator in Aarhus with great dedication and offered tremendous help as my sparring partner in all stages of this project. In this context, I would also like to thank my supervisors Grethe Andersen, Flemming Winther Bach, and Henrik Vorum for their continuing support. I owe special thanks to the study nurses Schiela Jensen, Kristina Eiskjær Sørensen, Rikke Bay Thomsen, Marlene Dyran, Tinna Antonsen, Nina Hjort Jensen, and the GCP-coordinator nurse Kirsten Østergaard Nielsen for their diligence and enthusiasm. Jan Plougmann Povlsen and Helle Wulf Eskildsen are thanked for their accurate and careful work as central imaging reading boards, and Götz Thomalla, Caspar Brekenfeld, and Bernhard Vens-Cappell from the University Medical Center Hamburg-Eppendorf in Germany are thanked for their advice and support as members of the data safety monitoring board.

My studies required the understanding, postprocessing, and analysis of large quantities of imaging data. Gregory Albers and Søren Christensen at Stanford University in Palo Alto, CA, USA, introduced me to the world of multiparametric imaging reading during my overseas studies at Stanford University. Irene Klærke Mikkelsen, Mikkel Bo Hansen, Kim Mouridsen, and Leif Østergaard from the Centre of Functionally Integrative Neuroscience at Aarhus University helped me to postprocess and analyze the imaging data. Nils Forkert and Anthony Winder from the University of Calgary in Canada introduced me to AnToNIa and artificial intelligence, and I am very grateful for the great time that I had during my overseas studies at the University of Calgary.

I am also very grateful to Martin Nygård Johansen for his great willingness to provide endless precious statistical advice and helpful comments.

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Last but certainly not least, I would like to thank my lovely family. Being on-call for acute recruitment into the study 24 hours a day, 7 days a week for several years has been a challenge. I give a huge hug to my sons Jan and Malte for having patience with me and tolerating my distractions. Finally, my dear wife, words are simply not enough. Thank you, dear Ivy.

List of Papers

This PhD thesis is based on the following papers:

I. Modrau B, Hjort N, Østergaard L, Mouridsen K, Andersen G, Bach FW. Theophylline as an add-on to thrombolytic therapy in acute ischaemic stroke (TEA-Stroke): A randomized, double-blinded, placebo-controlled, two-centre phase II study. Eur Stroke J. 2016; 1: 248–254.

II. Modrau B, Andersen G, Mikkelsen IK, Nielsen A, Hansen MB, Johansen MB, Eskildsen HW, Plougmann JP, Yavarian Y, Mouridsen K, Østergaard L, Bach FW, Hjort N. Theophylline as an add-on to thrombolytic therapy in acute ischemic stroke: A randomized placebo-controlled trial. Stroke 2020; 51: 1983–1990

III. Modrau B, Winder A, Hjort N, Johansen MN, Andersen G, Fiehler J, Vorum H, Forkert N. Prediction of brain tissue infarction in patients with acute ischemic stroke treated with theophylline as an add-on to thrombolytic therapy: A randomized clinical trial sub-group analysis (Front Neurol. 2020; submitted)

IV. Modrau B, Winder A, Hjort N, Andersen G, Johansen MN, Vorum H, Fiehler J, Forkert N. Perfusion changes in the infarct core and penumbra in patients with ischemic stroke treated with theophylline as an add-on to thrombolytic therapy: A randomized clinical trial subgroup analysis (Clin Neuroradiol. 2020; submitted)

The Roman numerals I–IV will be used as references to these papers in this PhD thesis

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Thesis at Glance

Paper I

Aim: A clinical trial protocol that evaluates the neuroprotective effect of theophylline in patients with acute ischemic stroke.

Hypothesis: Previous clinical trials, which evaluate the neuroprotective effect of theophylline in stroke, had important limitations.

Methods: Literature research

Results: A phase II, proof-of-concept, randomized, placebo-controlled clinical trial protocol

Conclusions: The literature is limited, dated, and previous clinical trials have important limitations. A new protocol was designed to offset these shortcomings.

Paper II

Aim: A randomized controlled trial to evaluate the safety and efficiency of theophylline, as an add-on to thrombolytic therapy, in patients with acute ischemic stroke.

Primary outcome: No clinically significant safety concerns were identified. Neither co-primary end points, early clinical improvement, and infarct volume growth at the 24-hour follow-up were significantly different after post hoc correction for multiplicity.

Conclusion: The trial was terminated early due to slow recruitment, and the small study size precludes a conclusion as to whether theophylline has a neuroprotective effect. However, early clinical improvement as a single endpoint would have been significantly better in patients treated with theophylline.

Paper III

Aim: A sub-study to evaluate the neuroprotective effect of theophylline on the imaging endpoint final infarct volume

Design: A machine-learning model using voxel-by-voxel information from multi-parametric magnetic resonance imaging data and clinical parameters was used to predict the infarct volume for each individual patient virtually treated with theophylline and placebo.

Results: The predicted follow-up lesion volumes of brain tissue infarction for each patient were not significantly different for virtually treatment with theophylline or placebo.

Conclusion: The neuroprotective effect of theophylline, namely, reduced brain infarction, as shown in preclinical stroke models, was not present in acute ischemic stroke patients.

Paper IV

Aim: A sub-study to evaluate the effect of theophylline on cerebral perfusion in the infarct core and penumbra

Design: Perfusion-weighted magnetic resonance imaging data at baseline and 3-hour follow-up were used to assess the cerebral blood flow, cerebral blood volume, and mean transit time in the infarct core, penumbra, and unaffected brain tissue.

Results: Theophylline in acute ischemic stroke did not change the perfusion in the salvageable penumbra, but only affected the cerebral blood volume in the infarct core.

Conclusion: In contrast to the penumbra, the infarct core is very unlikely to be salvageable, which might explain why theophylline failed to reduce the final infarct volume and failed to substantially improve the clinical outcome.

Abbreviations

CBF	cerebral blood flow
CT	computed tomography
DWI	diffusion-weighted imaging
FLAIR	fluid-attenuated inversion recovery
mcg	microgram
mg	milligram
ml	milliliter
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
PWI	perfusion-weighted imaging
TEA-Stroke	Theophylline in Acute Ischemic Stroke
TIMI score	Thrombolysis in Myocardial Infarction score

English Summary

As the second commonest cause of death and the third commonest cause of disability worldwide, stroke is a major public health burden. Of the different types of stroke, most stroke patients are diagnosed with acute ischemic stroke, in which early reperfusion of the salvageable penumbra is crucial for good clinical outcomes. Theophylline is a vasoactive agent that might facilitate neuroprotection of the penumbra, based on evidence showing that it is associated with the redistribution of cerebral perfusion and increased cerebral blood flow in the area of a stroke. Data from the use of theophylline in preclinical stroke models have shown that it can reduce brain infarction volume, brain tissue edema, and mortality, and clinical case series have reported temporary clinical improvements in selected patients with stroke that received theophylline. Although these promising results were not replicated in two previous randomized clinical trials, these trials had important limitations regarding patient selection, delayed intervention, and the lack of use of additional revascularization therapies.

The aim of this dissertation was to address the shortcomings of previous clinical trials and to evaluate the neuroprotective effect of theophylline in patients with acute ischemic stroke. The Theophylline in Acute Ischemic Stroke (TEA-Stroke) trial was a proof-of-concept trial designed to incorporate two sub-studies: one used a machine learning approach to assess the neuroprotective effect of theophylline on infarct volume growth, and the other involved a voxel-by-voxel-based evaluation of the effect of theophylline on cerebral perfusion in the penumbra and the infarct core.

The main finding of this dissertation was that the co-primary endpoints of early clinical improvement and infarct volume growth were not significantly different in the theophylline group compared to the control group. The clinical endpoint alone indicated a potential benefit but after correction for multiplicity the difference was not statistically

significant. No significant safety concerns were identified. The prediction of follow-up lesions for each patient virtually treated with theophylline and placebo confirmed that theophylline did not reduce the growth in infarct volume. Finally, the cerebral perfusion analysis showed that theophylline did not change the perfusion in the salvageable penumbra, but only affected the cerebral blood volume in the infarct core. In contrast to the penumbra, the infarct core is highly unlikely to be salvageable. This might explain why theophylline failed to reduce the final infarct volume and failed to substantially improve clinical outcomes. In conclusion, the data of this thesis are not strong enough to support further research on the use of theophylline as a neuroprotective agent, because the balance between clinically meaningful benefits and potential harm associated with the use of theophylline in acute ischemic stroke remains questionable.

Dansk Resume

Stroke er en hyppig og alvorlig sygdom, som globalt er den næst hyppigste årsag til død og tredje hyppigste årsag til handicap. Langt de fleste strokes er iskæmiskbetingede og en omgående reperfusion af det truede hjernevæv, penumbraen, er afgørende for et godt resultat. Theophylline er et vasoaktivt præparat, som formodes at være neuroprotektiv ved at omfordele den cerebrale perfusion til gavn for det iskæmiske område. Theophylline har vist reduceret hjerneødem, hjerneskade og mortalitet i prækliniske stroke-modeller. Derudover beskriver case-rapports forbigående klinisk forbedring hos nogle stroke-patienter. Disse lovende resultater kun ikke reproduceres i kliniske forsøg, som dog har haft betydelige svagheder: Patientselektion, forsinket intervention og manglende revaskulariserende behandling.

Formålet med afhandlingen var at belyse den neuroprotektive effekt af theophylline hos patienter med akut cerebral iskæmi. I designet af protokollen "Theophylline in acute ischemic stroke" (TEA-Stroke) blev der taget hensyn til de udfordringer fra de tidligere studier og inkluderede to substudier: Et studie anvendte kunstig intelligens til at detektere en eventuel subtil neuroprotektiv effekt af theophylline og det andet studie vurderede effekten af theophylline på den cerebrale perfusion i penumbraen og i infarktkernen.

Hovedresultatet af afhandlingen, er at de to primære endepunkter tidlig klinisk forbedring og infarktvækst ikke viste signifikant forskel mellem theophylline- og kontrolgruppen. Tidlig klinisk forbedring alene ville have været til fordel for theophylline, men betragtes ikke statistisk signifikant efter korrektur for flere målinger ved to primære endepunkter. Studiet viste ingen kliniske relevante bivirkninger. Substudierne bekræftede at theophylline ikke reducerede infarktvæksten og viste at theophylline ikke ændrede perfusionen i penumbraen. Theophylline påvirkede udelukkende den cerebrale blodvolumen i infarktkernen. I modsætning til den potentielle reversible skade i penumbraen er skaden i infarktkernen som regel irreversibel og det kan forklare hvorfor theophylline ikke reducerede

den endelige infarktvolumen og ikke substantielt forbedrede det kliniske resultat.

Der konkluderes, at resultaterne fra denne afhandling ikke underbygger ny forskning som undersøger den neuroprotektive effekt af theophylline, idet det fremstår tvivlsomt, hvorvidt den viste kliniske effekt står mål med de potentielle bivirkninger.

Introduction

The main purpose of this dissertation was to evaluate the neuroprotective effect of theophylline in patients with acute ischemic stroke.

Stroke

As the second commonest cause of death and the third commonest cause of disability worldwide, stroke is a major public health problem.(1) In Denmark, there were approximately 12.000 new stroke cases in 2019, with an associated mortality rate of 8% within the first month.(2) In the majority of stroke patients (85%), the etiology of the stroke is acute ischemia, which is characterized by an insufficient blood supply to the brain secondary to cerebral vessel occlusion. Persistent vessel occlusion leads to enlargement of the ischemic area(3) and worsening of the clinical outcome.(4) For every minute that a typical acute ischemic stroke patient with occlusion of the middle cerebral artery is left untreated, it is estimated that approximately 1.9 million neurons are lost.(5) Accordingly, early reperfusion of the ischemic area is crucial for a good clinical outcome.

The treatment of acute ischemic stroke has advanced markedly over the last few years. Acute revascularization with intravenous recombinant tissue plasminogen activator within a time window of 4.5 hours after symptom onset is the current treatment standard in Denmark.(4,6,7) Thrombolysis has also been shown to be effective in patients with an unknown time of stroke onset, if the patients are selected using specific brain imaging criteria.(8) However, recent studies have shown conflicting results for the use of thrombolysis in an extended time window up to 9 hours from the onset of stroke.(9,10)

Endovascular thrombectomy, which involves mechanical removal of the blood clot within the first 6 hours after the onset of stroke, is an established therapy in cases of large vessel occlusion(11,12) and became a standard treatment in Denmark in 2015. Recent clinical trials

of endovascular thrombectomy in the extended time window of up to 24 hours from the onset of stroke have shown excellent clinical outcomes, with a very low number-needed-to-treat in terms of achieving daily functional independence.(13,14)

The selection of patients with acute ischemic stroke eligible for revascularization therapies depends not only on the time of symptom onset but also on acute brain imaging.

Brain imaging in acute ischemic stroke

Brain imaging using computed tomography (CT) or magnetic resonance imaging (MRI), in combination with CT angiography or magnetic resonance angiography, is essential to exclude stroke mimics and hemorrhage, to define the extent of the acute ischemia, and to determine the cause and mechanism of the stroke particularly in relation to the identification of large vessel occlusion.(15) Perfusion-weighted positron emission tomography imaging facilitates differentiation between the core of acute ischemia that turns into infarction despite recanalization, the oligemic area that rarely turns into infarcted tissue, and the hypoperfused area of brain tissue with preserved but at-risk neuronal integrity known as the ischemic penumbra.(16) The existence of an ischemic penumbra in stroke, comprising an area of brain tissue surrounding the infarct core with slower cell death, was supported by a baboon stroke model described by Astrup et al. in 1977.(17) The brain cells in the ischemic penumbra were found to be functionally inactive (electrically silent), but with preserved structure and the ability to survive for a period of time.

Diffusion-weighted MRI (DWI) is an established technique routinely used in the clinical setting to delineate the core of acute ischemia in patients with ischemic stroke (18) and perfusion-weighted MRI (PWI) is used to detect the area of cerebral hypoperfusion(19). The DWI/PWI-mismatch is believed to represent the penumbra.(20) The infarct volume and the proportion of ischemic penumbra salvaged correlate well with clinical outcomes in stroke patients.(21) Patients with

DWI/PWI-mismatch respond better to early recanalization than patients without mismatch.(22) Even though the presence of an ischemic penumbra appears to be a favorable selection criteria, and some phase II clinical trials have shown that the mismatch concept is suitable for selecting patients most likely to benefit from acute treatment(23,24), the overall results of subsequent clinical trials have been conflicting.(25–29) As a result, multimodal imaging, including imaging of the ischemic penumbra outside of clinical trials, is currently not recommended in order to prevent delayed revascularization within the conventional time windows. However, the penumbra selection criteria are recommended in the extended time window to select patients for endovascular therapy(30) if the remaining eligibility criteria of the corresponding clinical trials are fulfilled.(13,14)

Delayed revascularization in acute ischemic stroke, whether achieved through thrombolytic therapy or endovascular thrombectomy, is associated with poor clinical outcomes.(6,31) In a meta-analysis of pooled individual patient data from randomized clinical trials, the time from symptom onset to randomization was 121–240 minutes in the majority of patients.(31) In another meta-analysis of clinical trials evaluating the use of thrombolytic therapy, the median onset to treatment time was 144 minutes.(32) To contrast, in another meta-analysis, the onset to treatment time was >180 minutes in more than two-thirds of all included patients.(4) Thrombolysis data from the Danish Stroke Registry in 2019 showed a median time of 100 minutes from onset to admission and a median time of 28 minutes from admission to thrombolysis (resulting in an overall median onset to treatment time of 128 minutes).(33)

Neuroprotective therapy in acute ischemic stroke

Even though the time periods of pre- and inter-hospital management of acute stroke patients have shortened substantially over the past 20 years, the current data still show a notable delay from onset to treatment. For this reason, a neuroprotective treatment that can salvage the ischemic penumbra and thus preserve neuronal integrity and prevent subsequent

brain damage is desirable. Without sufficient revascularization and reperfusion of the penumbra, the brain cells in the penumbra will die and the infarct core will expend.(34)

The exact molecular mechanisms behind cell death in stroke are still a matter of debate, but it is widely accepted that excitotoxicity, oxidative stress, free radicals, and apoptosis are involved.(35–37) A large number of different neuroprotective strategies have been assessed in animal models of stroke. To date, these promising preclinical data have not been translated into clinical trials demonstrating improved clinical outcomes in patients with acute ischemic stroke.(38)

Currently, the only recommended therapy for acute ischemic stroke is revascularization with thrombolysis and/or endovascular thrombectomy.(30) Thrombolysis with tissue plasmin activator has been shown to be associated with clinical benefit but has also shown neurotoxic effects in animal models of stroke.(39,40) However, the results from preclinical data have led to the conclusion that a combination of drugs might improve neuroprotection in a way that is not possible with monotherapy.(41) The integration of neurovascular revascularization therapy and neuroprotective cellular therapy may result in further improvements in the safety of thrombolysis and, crucially, might extend the therapeutic time window.(42) The recent ESCAPE-NA1 randomized controlled clinical trial was able to combine vascular and cellular neuroprotective approaches while assessing use of the neuroprotective agent nerinetide in acute ischemic stroke patients treated with thrombolysis and/or endovascular thrombectomy.(43) The trial results were neutral in relation to the primary endpoint, which was defined as a good clinical outcome. Even though the trial was well-designed, the promising neuroprotective effect demonstrated in a preclinical stroke model was not translated into clinical efficacy in humans.(44) However, the authors reported a treatment effect among patients not treated with thrombolysis, probably related to a pharmacological interaction between nerinetide and the thrombolytic

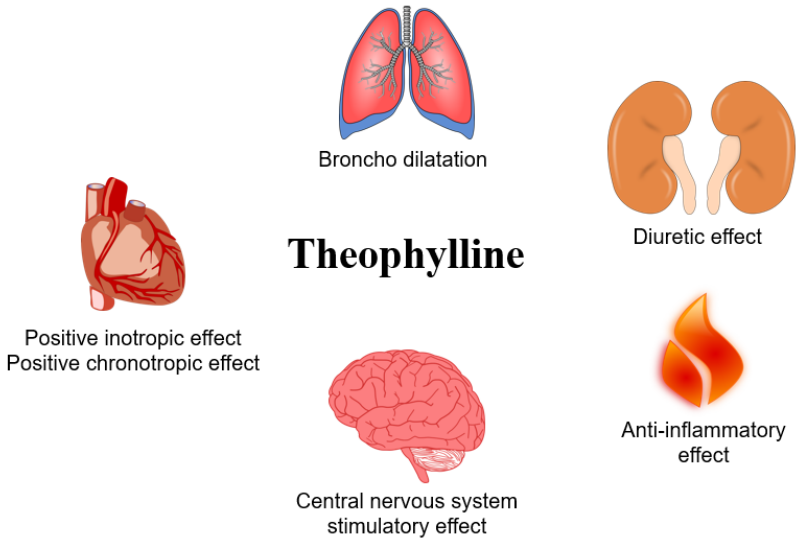
agent alteplase, and they suggested that a neuroprotective effect in human stroke might still be possible.(43)

Theophylline

Methylxanthines are alkaloids found naturally in chocolate, coffee, and tea. The most abundant methylxanthines in chocolate, coffee, and tea are theobromine, caffeine, and theophylline, respectively.(45). Theophylline is thought to act as a nonselective phosphodiesterase inhibitor, as an adenosine receptor antagonist with an effect on smooth muscles, and as an anti-inflammatory agent (because of its roles as an inhibitor of nuclear factor-κB, inhibitor of phosphoinositide 3 kinase-δ, activator of interleukin-10 secretion, activator of apoptosis of inflammatory cells, inhibitor of cell death via inhibition of poly[ADP-ribose]polymerase-1, and activator of histone deacetylase activity).(46) Theophylline is a pharmacologically active substance that is combined with ethylenediamine for better solubility. Previous publications have referred to theophylline as well as the combination of theophylline and ethylenediamine, otherwise known as aminophylline. Henceforth, theophyllamine and aminophylline will be referred to as theophylline.

Theophylline has a number of well-known clinical actions (Figure 1).

Figure 1:



Images obtained from https://pixabay.com

In Denmark, theophylline is licensed for the treatment of asthma and bronchial spasm. Off-label applications rely on the central nervous system stimulatory effect in the treatment of apnea in preterm infants(47) and the diuretic effect to prevent contrast-induced renal failure(48).

Theophylline and stroke

Previous studies have demonstrated an inverse relationship between tea consumption and the risk of stroke and cerebrovascular disease in general.(49–51) However, the neuroprotective effect of theophylline in acute ischemic stroke is controversial.

The first response of brain tissue to ischemia is vasodilatation in the involved vascular territory in an attempt to increase the regional cerebral blood volume and maintain cerebral blood flow.(52) Naturally, adenosine acts as an endogenous mediator between cerebral metabolism and blood flow via pial vasodilatation(53,54).

Phosphodiesterase inhibition increases the intracellular concentration of cyclic adenosine monophosphate causing vasodilatation through the activation of calcium-activated potassium channels and adenosine triphosphate-sensitive potassium channels.(55) Theophylline acts as a phosphodiesterase inhibitor causing vasodilatation; however, it also acts as a competitive adenosine receptor antagonist(56) thereby, also causing cerebral vasoconstriction.(57,58) This controversy is the reason that the vasoactive effects of theophylline on ischemic brain tissue are still unexplained. Furthermore, the cerebral vasoconstrictive effect of theophylline, associated with reduced global and regional cerebral blood flow, has been shown in previous studies.(59–61) Skinhøj and Paulson evaluated stroke patients using the Xenon-133 arterial injection technique and showed decreased regional cerebral blood flow in areas of normal brain tissue and, in some patients, increased regional cerebral blood flow in the areas of brain tissue affected by stroke.(59) The authors concluded that this "inverse intracerebral steal phenomenon" might explain the favorable clinical effect of theophylline. For instance, the authors observed a remarkable and reproducible clinical improvement, with diminished right hemiparesis and aphasia, in one patient with an occluded internal carotid artery and increased cerebral blood flow in the territory of the left middle cerebral artery following the administration of theophylline. The opposite of this, a "reverse intracerebral steal phenomenon", has been described in patients with MRI-verified acute ischemic stroke, in association with the presence of a perfusion/diffusion-mismatch and fluctuating clinical symptoms suspicious for a salvageable penumbra; these patients showed vasodilatation of the intracerebral vessels in areas of normal brain tissue, leading to reduced cerebral blood flow in the area of infarction when exposed to hypercapnia from hypoventilation or treatment with acetazolamide.(62)

Animal stroke models with pre- or per-conditioning using theophylline have shown reduced ischemic brain damage, perifocal edema, and mortality.(63–65) The effect of theophylline in stroke in humans has

been described in several case series and two randomized clinical trials; the case series described a considerable and immediate clinical improvement, albeit only temporarily and not in all patients.(66–72)

Two randomized double-blind, placebo-controlled studies have been performed with theophylline in stroke patients.(73,74) The study by Geismar et al. included 79 patients who received a bolus of theophylline or placebo and showed a significant increase in the frequency of early improvement in the group treated with theophylline (38% versus 15%). However, there was no significant difference at the 3-week follow-up point(73). Britton et al. randomized 46 patients to receive either a bolus followed by continuous infusion of theophylline or placebo over 3 days.(74) The authors described a trend toward better functional outcome in the theophylline group at 3 weeks, but without a significant difference when deterioration and mortality were accounted for. Both of these studies had important limitations, as follows:

1. The diagnosis of stroke was made without brain imaging and was based on clinical evaluation.

2. The mortality of 22–23% within 3 weeks was high in both studies and indicated a selection bias towards patients with severe stroke and predominantly irreversible brain damage.

3. The time of the study intervention was, on average, delayed by 20 hours (one-third of patients were treated beyond 24 hours; Geismar et al.) and 40 hours (range 18–114 hours; Britton et al.) following the onset of stroke. Thus, most patients in these two trials were treated at a time point where established irreversible brain infarction was presumed to have occurred and, presumably, there was little, or no salvageable penumbra left.

4. Theophylline has no known thrombolytic effect. Clinical improvement and reduction in infarct volume growth due to improved perfusion of the penumbra will presumably be only temporary, as no

thrombolytic agent or revascularization therapy was available at the time the studies were performed.

Despite the substantial limitations of these studies, which can be summarized as inappropriate patient selection, delayed intervention, and a lack of revascularization therapy, no further clinical trials of theophylline in acute ischemic stroke have been performed. Consequently, a Cochrane review, primarily based on the limited data from the aforementioned clinical trials, concluded that there is not enough evidence to assess whether theophylline or its analogs are safe and improve outcomes in patients with acute ischemic stroke.(75)

Based on these data, it remains unknown whether theophylline has a neuroprotective effect in acute ischemic stroke. Reperfusion of the salvageable penumbra despite occlusion of the culprit vessel may explain the neuroprotective effect of theophylline as an adjunct to thrombolytic therapy. This may offer the potential to reduce the final infarct volume with improved clinical outcome but may also offer an extended time window for revascularization therapy. No previous study has evaluated theophylline as an add-on to thrombolytic therapy and there are no ongoing trials on this topic registered with the U.S. National Institutes of Health (www.ClinicalTrials.gov) or EudraCT (www.clinicaltrialsregister.eu).

Aims

The overall aim of this PhD thesis was to evaluate the potential neuroprotective effect of theophylline in patients with acute ischemic stroke. This was achieved through four studies, as follows:

Study I

A clinical trial protocol that evaluated the neuroprotective effect of theophylline in patients with acute ischemic stroke.

Study II

A randomized controlled trial to evaluate the safety and efficiency of theophylline as an adjunct to thrombolytic therapy in patients with acute ischemic stroke.

Study III

A sub-study to evaluate the neuroprotective effect of theophylline on the imaging endpoint of final infarct volume.

Study IV

A sub-study to evaluate the effect of theophylline on cerebral perfusion in the infarct core and penumbra.

Material and Methods

This PhD dissertation is based on the **Th**e**E**ophylline in **A**cute Ischemic **Stroke** (TEA-Stroke) trial, an investigator-initiated two-center, proof-of-concept, phase II clinical trial with a randomized, double-blind, placebo-controlled design.

Ethical considerations and dose justification

This study was performed in accordance with the Declaration of Helsinki and was approved by the Danish Health and Medicines Authorities (ref no 2013050908) and the local institutional ethics review board (ref no N-20130034). Written informed consent was obtained from all subjects before participation

Theophylline is not currently licensed for the treatment of acute ischemic stroke. In previous randomized stroke trials, theophylline was administrated as a repetitive bolus or as a bolus followed by continuous infusion. In these trials, none or only mild adverse events such as nausea were registered.(73,74) There is no documented evidence in the literature that theophylline causes bleeding complications, including intracerebral hemorrhage.

The usual loading dose of theophylline is 5 mg/kg body weight in patients with acute bronchial spasm that have not been preloaded with theophylline. It is recommended that the calculation of theophylline dosage is based on ideal body weight, as theophylline distributes poorly within body fat. On average, a dose of 4.6 mg/kg theophylline administrated over 30 minutes will produce a maximum post-distribution serum concentration of 10 mcg/ml, with a range of 6–16 mcg/ml (Food and Drug Administration-approved theophylline information for healthcare professionals [www.drugs.com]). Clinically important pulmonary improvement usually requires serum theophylline concentrations to be greater than 10 mcg/ml. Adverse reactions with peak serum theophylline concentrations less than 20 mcg/ml are generally mild and mainly transient caffeine-like adverse reactions. The

frequency and severity of adverse reactions are increased with peak serum theophylline concentrations greater than 20 mcg/ml. More severe adverse reactions are seen in acute or chronic overdose associated with theophylline concentrations greater than 30 mcg/ml.

A dose-escalation study of theophylline in acute ischemic stroke does not exist. The bolus dose used in most of the case series and in both randomized clinical trials in acute ischemic stroke was less than the bolus dose usually used in patients with acute bronchial spasm. An intravenous bolus of 200 mg and 230 mg theophylline without adjustment for body weight was used by Britton et al.(74) and Geismar et al.(73), respectively. Assuming an average body weight of 70 kg, this corresponds to a theophylline concentration of 2.9 mg/kg and 3.3 mg/kg, respectively. On average, these dosages would be too small to reach theophylline concentrations greater than 10 mcg/ml, which makes the risk of typical adverse reactions less likely. Nevertheless, a relevant effect in the TEA-Stroke trial could be expected, as almost the same dosage (an intravenous bolus of 250 mg theophylline) has been shown to result in cerebral vasoconstriction when measured 5 minutes, 25 minutes, and 45 minutes after administration.(76) The same authors confirmed the elimination of theophylline from serum by first order rates with half-lives of 5.4–9.0 hours. This is consistent with the temporary clinical improvement observed up to six hours after the administration of 220 mg theophylline.(59)

Studies I and II

The trial protocol considered the important limitations of the previous clinical trials and provided, for the first time, a trial design incorporating theophylline and revascularization therapy together. Eligibility for thrombolytic therapy within 4.5 hours of symptom onset and MRI-verified acute ischemic stroke with perfusion/diffusion mismatch suspicious for salvageable penumbra were chosen as the main inclusion criteria. The trial was designed as a double-blind, placebo-controlled, two-center trial. A clinical assessment of stroke severity using the National Institute of Health Stroke Scale (NIHSS) was performed at

baseline, and at 3- (range 2–3) and 24- (range 22–32) hour follow-up.(77,78) The NIHSS measures the severity of a stroke (NIHSS scores 0–4 correspond to mild stroke symptoms, NIHSS scores 5–15 to moderate, NIHSS scores 16–20 to moderate-to-severe, and NIHSS scores ≥ 20 to severe stroke; Appendix 1). The modified Rankin Scale (mRS) was applied to assess the functional outcome of patients at the 3-month follow-up point (0 = no symptoms, 1 = no significant disability, 2 = slight disability, 3 = moderate disability, 4 = moderate severe disability, 5 = severe disability, and 6 = dead; Appendix 2).(77,79–81) Imaging assessment with multiparametric MRI was performed at baseline, 3-hour follow-up, and 24-hour follow-up. An overview of the patient flow is published in the European Stroke Journal (82) and the detailed schedule of assessments is shown in figure 2. The 24-hour follow-up was the timepoint for the primary endpoint assessment and the 3-hour follow-up was added for the sub-study assessing the effect of theophylline on the infarct core and penumbra.

Figure 2: Patient flow and assessment

TEA Stroke trial	Screening for rtPA	Screening for TEA-Stroke	Enrolment Randomization	Study medication	Follow-up			
		Visit 0 Day 0		Visit 1 Day 0	Monitoring Day 0-1	Visit 2 2-3 h	Visit 3 22-32 h	Visit 4 3 months
Inclusion-/exclusion criteria	X	X						
Information material		X						
Informed consent		X						
Demographic data	X							
Medical history	X	X						
History of previous medication	X	X						
Vital sign, body temperature	X			X				
Physical examination	X						X	X
mRs	X							X
NIHSS	X					X	X	X
Laboratory tests	X					X	X	
Pregnancy test*	X							
Routine-ECG	X					X	X	
MRI cerebrum	X					X	X	
Application of study medication				X				
ECG-monitoring				X	X	X		
Vital signs monitoring				X	X	X		
Concomitant medication	X			X	X	X	X	X**
Adverse events	X			X	X	X	X	X***

- * Mandatory for fertile woman below the age of 55 years
- ** only Anticoagulation, platelet inhibition therapy
- *** only SAE, SUSAR
- TEA-Stroke Trial related activity

ECG: electrocardiography; MRI: magnetic resonance imaging; mRs: modified Rankin Scale; NIHSS: National Institute of Health Stroke Scale; rtPA: recombinant tissue plasmin activator

Primary outcome

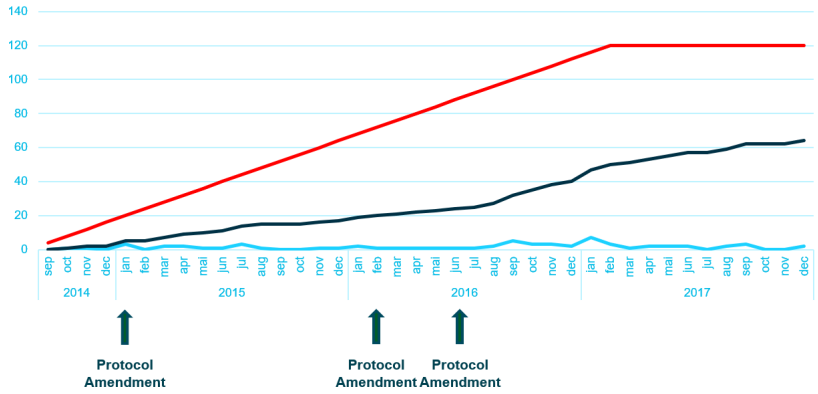
The following co-primary end points at the 24-hour follow-up point were chosen:

- Clinical improvement, defined as the absolute change in the NIHSS score from baseline to follow-up; and
- The proportion of the penumbra salvaged, defined as the proportion of the diffusion/perfusion mismatch on the baseline imaging that did not progress to final infarction on the imaging performed during follow-up.

Early clinical improvement and penumbra salvage were the surrogate markers chosen as co-primary end points as they were associated with good long-term clinical outcomes (25,83–85) and have been successfully applied in a well-designed clinical trial with a small sample volume.(70) The effect size of theophylline as an add-on to thrombolytic therapy is unknown. A sample size of 60 patients in each treatment group was chosen in the TEA-Stroke trial protocol (Study I). This calculation was based on the sample size estimates for proof-of-principle phase II MRI studies in stroke by Donnan et al.(86) However, the interpretation of this reference was incorrect and was thus replaced by a post hoc sample size calculation (Study II). Furthermore, a post hoc Bonferroni correction was performed in order to consider the co-primary end points using multiple comparisons.

The TEA-Stroke trial was performed at the stroke units of Aalborg University Hospital and Aarhus University Hospital. The trial protocol included 120 patients with acute ischemic stroke and a significant penumbral mismatch as determined by MRI. Patients were randomized 1:1 to receive either 200 mg theophylline or placebo as an add-on to standard thrombolytic therapy. Three protocol amendments were made due to the unexpected low recruitment rate (Figure 3).

Figure 3: Recruitment rate



Red = expected recruitment, blue = monthly recruitment, black = total recruitment.

Protocol amendment PA01 (January 28th, 2015)

Most patients who were otherwise eligible for thrombolytic therapy did have a less severe stroke defined by an NIHSS score <6. In cases of severe stroke, patients were often unable to provide informed consent. The inclusion criterion NIHSS score was changed from ≥ 6 to ≥ 4 in order to improve the recruitment rate.

Protocol amendment PA02 (February 24th, 2016)

In the TEA-Stroke protocol, a semi-automatic in-house software was chosen to assess the MRI perfusion/diffusion mismatch. Unfortunately, several potential trial candidates were excluded, as the semi-automatic calculation was not possible in time due to technical reasons. To improve the recruitment rate, the visual assessment of the perfusion/diffusion mismatch based on the MRI-scanner software was allowed if the semi-automatic calculation was not available in a timely manner.

Protocol amendment PA03 (June 20th, 2016)

The study period was extended to February 1st, 2019 to compensate for the delayed start of recruitment and the unexpected low recruitment rate. Furthermore, the technically challenging and time-consuming perfusion/diffusion-mismatch inclusion criterion was omitted by simplifying the scanning protocol to further improve the recruitment rate. For that same reason, visit 2 at 2–3 hours was omitted to improve the feasibility of the study. An interim analysis was scheduled after the inclusion of 60 patients.

The protocol amendments necessitated a change in the co-primary end points, as omitting the perfusion-weighted imaging meant that it was no longer possible to assess the proportion of penumbral salvage as an end point. The study outcomes were finally adapted as follows:

Co-primary end points

- The clinical improvement, defined as the absolute change in the NIHSS score between baseline and follow-up.
- The proportion of infarct volume growth, defined as the proportion of co-registered DWI lesions at the 24-hour follow-up that were not present at baseline ($[DWI^{follow\text{-}up} - DWI^{baseline}]/DWI^{baseline} \times 100\%$).

Safety end points

- Parenchymal hemorrhage(s) at the 24-hour follow-up point
- Symptomatic intracerebral hemorrhage(s) at the 24-hour follow-up point
- Death within 90 days

Secondary clinical endpoints

- Major clinical improvement (≥50% improvement in NIHSS score between baseline and 24-hour follow-up)
- Favorable functional outcome at 90 days (mRS score 0 or 1)
- Improved categorical functional outcome at 90 days

Secondary imaging endpoints

- Recanalization rate
- Penumbral salvage

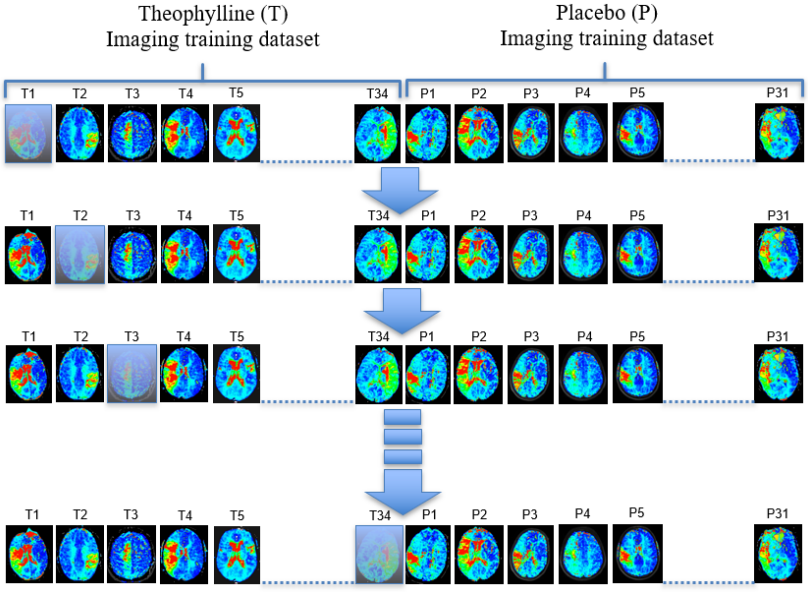
The steering committee decided to stop the trial after 3.5 years of recruitment as the recruitment rate remained slow despite the above amendments.

Study III

This pre-planned TEA-Stroke subgroup analysis was designed to detect a subtle neuroprotective effect of theophylline on infarct volume growth. The main selection criteria for this subgroup analysis were a multiparametric MRI including diffusion-and perfusion MRI at baseline, and a follow-up MRI at 24 hours. The AnToNIa software tool(87) was used to extract voxel-by-voxel information on: the tissue type probability, anatomical location, distance to the infarct core, apparent diffusion coefficient (ADC) value, cerebral blood flow (CBF) value, cerebral blood volume (CBV) value, mean transit time (MTT) value, and time-to-maximum (Tmax) value.(87) These imaging features were combined with the following clinical parameters: age, sex, baseline NIHSS score, and time from stroke onset to time of study administration. A predictive modeling approach was chosen to compare the follow-up lesions of patients virtually treated with theophylline and placebo. Previous studies using this approach showed a better

performance in tissue outcome prediction compared to simple perfusion parameter thresholding(88–91), and this approach has been used successfully for intra-individual comparisons of virtual treatments.(92) Two random forest machine learning models were trained, one with the training sets from patients with theophylline and another with the training sets from patients treated with placebo. Finally, the lesion outcome for both treatment options in each patient was predicted, while the patient used for lesion outcome prediction was excluded from the training set in order to prevent double-dipping (the leave-one-out approach is illustrated in Figure 4). This approach allowed the assessment of the final imaging outcome based on 8 imaging and 4 clinical parameters, and essentially doubled the sample size by simulating both treatment outcomes for each patient.

Figure 4: Leave-one-out approach



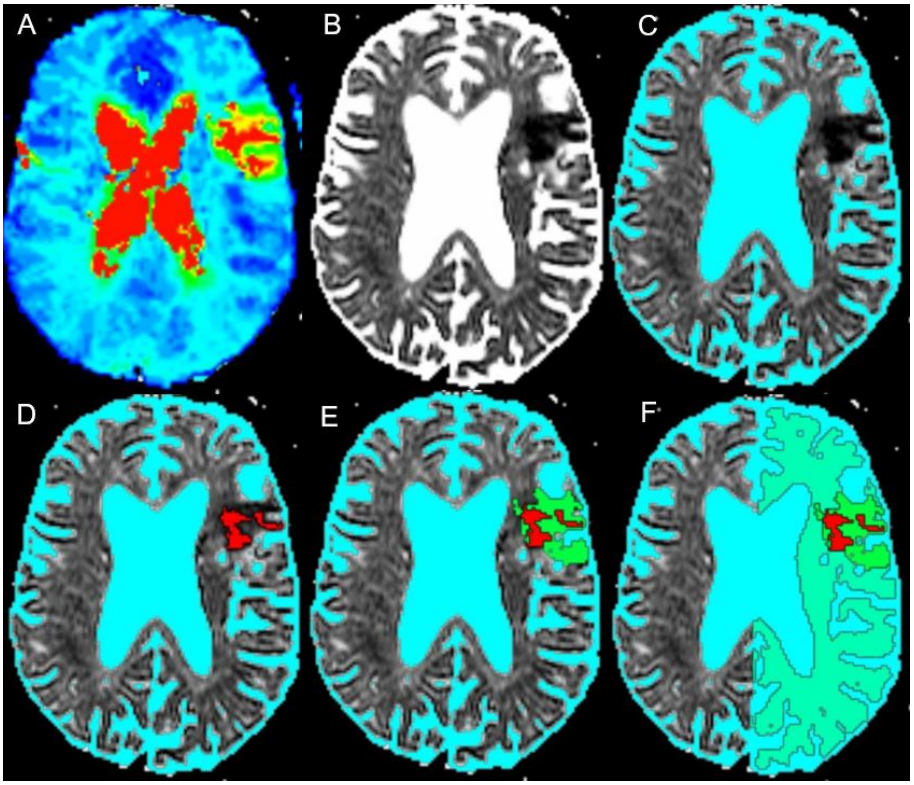
The predicted infarct volume for T1, virtually treated with theophylline, was based the imaging data in the theophylline group without the T1 dataset (leave-one-out), and the predicted infarct volume when virtually

treated with placebo was based on all imaging data from the placebo group. The same was applied for T2, T3, …T34, and *vice versa* for each patient in the placebo group.

Study IV

This pre-planned TEA-Stroke subgroup analysis was designed to evaluate the effect of theophylline on cerebral perfusion. The main selection criteria for this subgroup analysis were multiparametric MRI scans including diffusion and perfusion-weighted imaging at baseline and 3-hour follow-up. Theophylline has a half-life of 3–9 hours, and it was expected that the effect of theophylline on cerebral perfusion was still present at the 3-hour follow-up point. The AnToNIa software tool was used to calculate the CBF, CBV, MTT, and Tmax of the residual function. Relative perfusion maps (rCBF, rCBV, rMTT, and rTmax) were computed using the mean values form the contralateral hemisphere. The mean values for each parameter were calculated from the infarct core region of interest (ROI), the penumbra ROI (defined by perfusion-diffusion mismatch), and the unaffected tissue ROI (infarct core and penumbra ROI subtracted from the affected hemisphere; Figure 5). The same baseline ROI for infarct core, penumbra, and unaffected tissue was used to calculate the mean rCBF, rCBV, and rMTT at the 3-hour follow-up point. To evaluate the dynamics in the penumbra over time, two different penumbra ROIs at the 3-hour follow-up point were applied: the ROI mask reflecting the penumbra at baseline ($penumbra^{ROI\text{-}0h}$) and the ROI mask reflecting the actual penumbra at 3 hours ($penumbra^{ROI\text{-}3h}$). Finally, the absolute values and the change from baseline to 3-hour follow-up were compared between the theophylline group and the control group. Regarding the clinical assessment, the NIHSS score at baseline and the 3-hour follow-up point was assessed to determine for the presence of early clinical improvement, and vital signs were measured to assess the positive inotropic and chronotropic effects of theophylline.

Figure 5: Semi-automatic imaging post-processing using the AnToNIa software tool



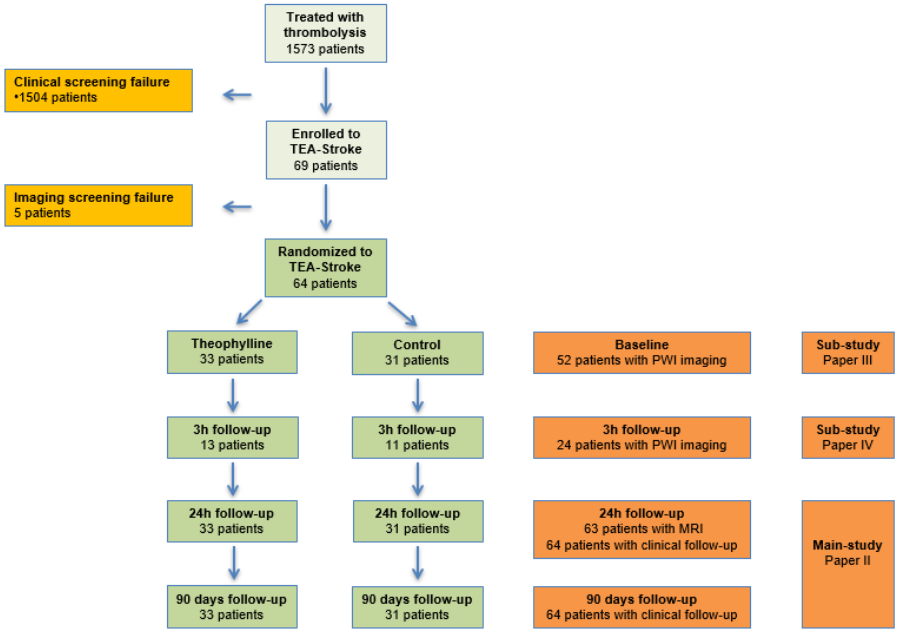
A = T-max map with a hypoperfused cortical area in the left middle cerebral artery territory; B = apparent diffusion coefficient (ADC) map with hypodensity in the hypoperfused area, indicating the infarct core; C = ADC map with cerebrospinal fluid segmentation (blue); D = segmentation of the infarct core region of interest (ROI; red), E = segmentation of the infarct core ROI (red) and penumbra ROI (bright green); F = segmentation of infarct core ROI (red), penumbra ROI (bright green), and unaffected tissue ROI (light green).

Results

Study population

During the study period from September 2014 to December 2017, the recruiting sites of Aalborg University Hospital and Aarhus University Hospital treated 1.573 stroke patients with thrombolysis. Sixty-seven patients were enrolled, and 64 patients were finally randomized to the TEA-Stroke trial (Figure 6). The reasons for not being enrolled in the trial and the reasons for screening failure are shown in the Consolidated Standards of Reporting Trials (CONSORT) flow diagram published in Stroke (93).

Figure 6: CONSORT flow diagram (Study II, III, IV)



MRI indicates magnetic resonance imaging; PWI perfusion weighted imaging

Finally, 33 patients were randomized to the theophylline group and 31 patients were randomized to the control group (Study II). Fifty-two patients fulfilled the criteria for the subgroup analysis to predict the lesion outcome for both treatment options in each patient (Study III), and 24 patients fulfilled the criteria for the subgroup analysis to evaluate the effect of theophylline on cerebral perfusion (Study IV).

The baseline characteristics, imaging characteristics, and procedural data were similar between the theophylline group and the control group in Studies II, III, and IV except for diabetes mellitus, which was commoner in patients in the theophylline group than in the control group (significant difference in Study III only).

Study II

Theophylline was associated with an improved NIHSS score between baseline and the 24-hour follow-up point of 4.7 points (standard deviation [SD] 5.6) compared to an improvement of 1.3 points (SD 7.5) in the control group (p=0.044). Growth in infarct volume was on average 141.6% (SD 126.5) in the theophylline group and 104.1% (SD 62.5) in the control group (p=0.146).

No clinically significant safety concerns were identified, and the safety end points, secondary clinical endpoints, and secondary imaging end points were similar between the two groups (Table 1).

Table 1: Efficacy and safety outcomes (93) © 2020 AHA/ASA Journals

	Theophylline Group (N=33)	Control Group (N=31)	EFFECT Measure	Crude Value* (95% CI)	*P* Value*	Adjusted Value† (95% CI)	*P* Value†
Primary efficacy outcome							
Mean improvement in NIHSS score at 24 h	−4.7 (SD, 5.6)	−1.3 (SD, 7.5)	Mean difference	−3.4 (−6.7 to −0.1)	0.044‡	−3.6 (−7.1 to −0.1)	0.043‡
Mean proportion of infarct growth at 24 h	141.6% (SD, 126.5)	104.1%§ (SD, 62.5)	Mean difference	37.7% (−13.5 to 89.0)	0.146‡	35.2% (−17.4 to 87.7)	0.185‡
Safety outcome							
New ischemic lesion at 24 h, No.	2 (6%)	2 (6%)	Odds ratio	1.0 (0.1 to 11.8)	0.987	NA	NA
Parenchymal hematoma type II and I at 24 h, No.	5 (15%)	6 (19%)	Odds ratio	0.7 (0.2 to 2.9)	0.604	NA	NA
Symptomatic intracerebral hemorrhage at 24 h, No.	0 (0%)	1 (3%)		NA	0.484‖	NA	NA
Any kind of intracerebral hemorrhage at 24 h, No.	10 (30%)	8 (26%)	Odds ratio	1.3 (0.4 to 4.2)	0.697	1.4 (0.4 to 5.2)	0.581
New stroke within 90 days, No.	0 (0%)	1 (3%)		NA	0.484‖	NA	NA
Death within 90 days, No.	0 (0%)	2 (6%)		NA	0.231‖	NA	NA
Secondary clinical efficacy outcome							
Major neurological improvement at 24 h, No.	22 (67%)	14 (45%)	Odds ratio	2.6 (0.9 to 7.5)	0.070	3.0 (1.0 to 9.0)	0.056
Functional independence at 90 days, No.	20 (61%)	18 (58%)	Odds ratio	1.1 (0.4 to 3.1)	0.802	1.3 (0.4 to 4.0)	0.640
Secondary imaging efficacy outcome							
Mean penumbra salvage volume at 24 h¶	27 mL (SD, 37)	18 mL (SD, 24)	Mean difference	9.5 mL (−10.6 to 29.7)	0.343	10.4 mL (−9.1 to 30.0)	0.287
Recanalization at 24 h (TIMI 2–3), No.#	9 (64%)	10 (83%)		0.3 (0.0 to 2.2)	0.235	0.1 (0.0 to 2.5)	0.160

NA indicates not applicable due to small sample size; NIHSS, National Institute of Health Stroke Scale; and TIMI, Thrombolysis in Myocardial Infarction grading of arterial obstruction (score 0, complete occlusion; 1, severe stenosis; 2, mild to moderate stenosis; and 3, normal arterial caliber).

*Adjusted for age (≤60 or >60 y) and stroke severity (NIHSS of ≤15 or >15) at randomization.

†Adjusted for age (≤60 or >60 y) and stroke severity (NIHSS of ≤15 or >15) at randomization and prespecified potential confounding variables.

‡Post hoc Bonferroni correction applied.

§N=30 for median proportion of infarct growth in the control group as MRI follow-up at 24 h was not possible in 1 patient with severe neurological deterioration due to remote ICH.

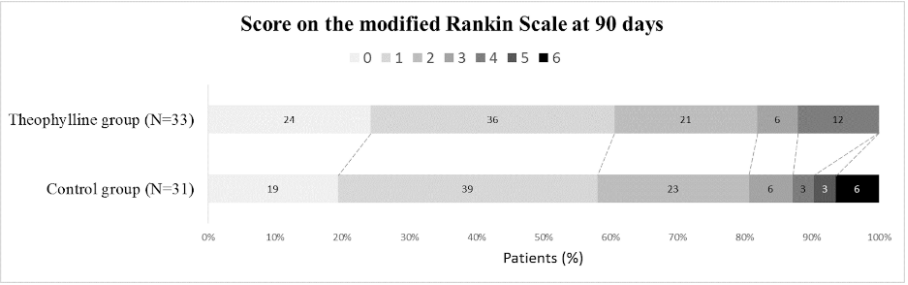
‖Fisher exact test due to low number of patients.

¶Penumbra salvage assessment was available in 22 patients in the theophylline group and in 21 patients in the control group.

#Recanalization was achieved in 9 out of 14 patients in the theophylline group and in 10 out of 12 patients in the control group.

The improvement of categorical functional outcome on the modified Rankin Scale at 90 days is illustrated in Figure 7.

Figure 7: Functional outcome at 90 days: (93) © 2020 AHA/ASA Journals



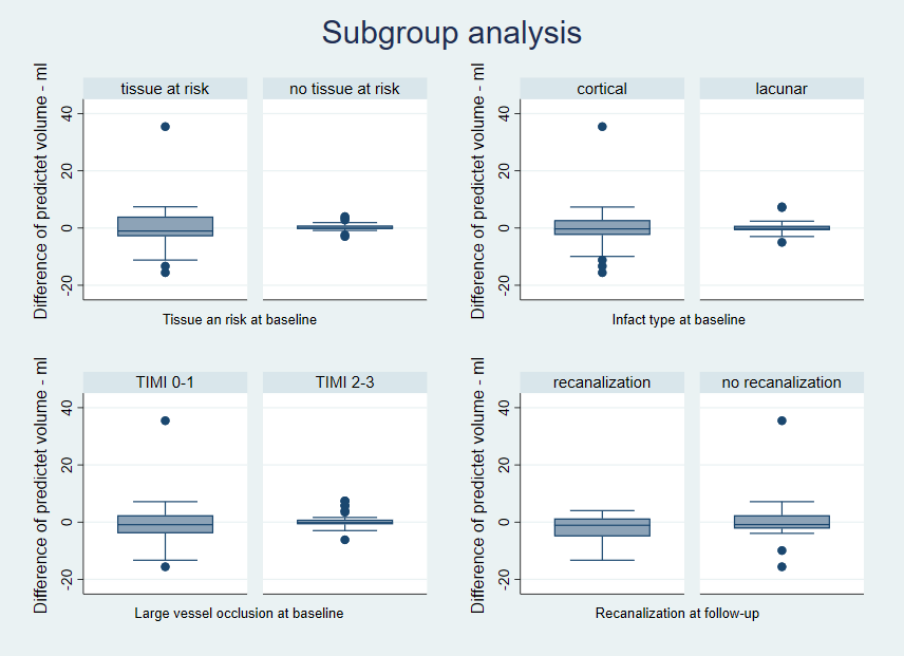
Patients in the theophylline group were treated with theophylline as an add-on to thrombolytic therapy. Patients in the control group were treated with thrombolytic therapy alone. Scores on the modified Rankin scale range from 0 to 6 (0, no symptoms; 1, no clinically significant disability; 2, slight disability; 3, moderate disability; 4, moderately severe disability; 5, severe disability; and 6, death). There were no patients with modified Rankin Scale score of 5 or 6 in the theophylline group. The adjusted common odds ratio for favorable outcome in the theophylline group was 1.44 (95% CI, 0.58–3.59; P=0.432).

Study III

The mean ischemic lesion volume at the 24-hour follow-up point based on the follow-up T2-FLAIR MRI imaging was 13.9 ml (SD 20.3) for the theophylline group and 11.7 ml (SD 19.3) for the control group (p=0.92). When applying the predictive modeling approach, the mean predicted ischemic lesion volume was 11.4 ml (SD 18.7) for patients virtually treated with theophylline and 11.2 ml (SD 17.3) for patients virtually treated with placebo (p=0.86).

The predicted ischemic lesion volumes were similar in patients with and without the presence of a penumbra (p=0.89), in patients with cortical versus lacunar infarctions (p=0.88), patients with and without large vessel occlusion (p=0.57), and in patients with and without revascularization at follow-up (p=0.35; Figure 8).

Figure 8: Subgroup analysis (Study III)

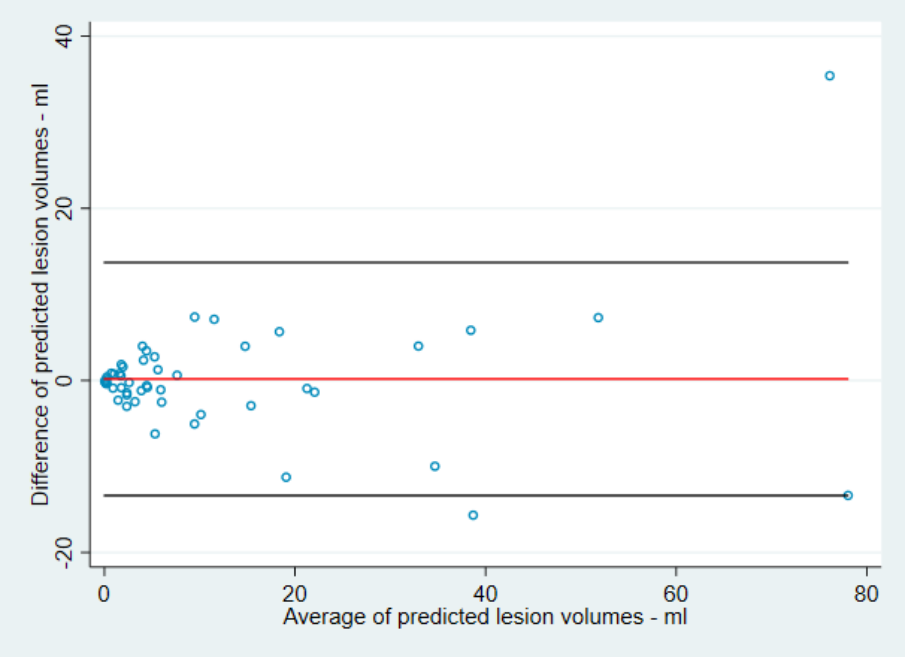


Box-whisker diagrams depicting the predicted volume of follow-up lesions plotted as the difference between the two predictions

(theophylline and placebo) for each patient. Values <0 indicate that theophylline is better than placebo, and values >0 indicate that placebo is better than theophylline, indicating no significant difference for patients with and without tissue-at-risk at baseline for patients with: cortical vs. lacunar infarction, with and without large vessel occlusion at baseline, and with and without recanalization at follow-up.

Likewise, the difference between the predicted ischemic lesion volume for each patient virtually treated with theophylline or placebo was not significantly different between small and large infarct volumes (Figure 9).

Figure 9: Bland-Altman plot of the difference between the two follow-up lesion volumes for theophylline and placebo over the average of the two predicted volumes for each patient (Study III)

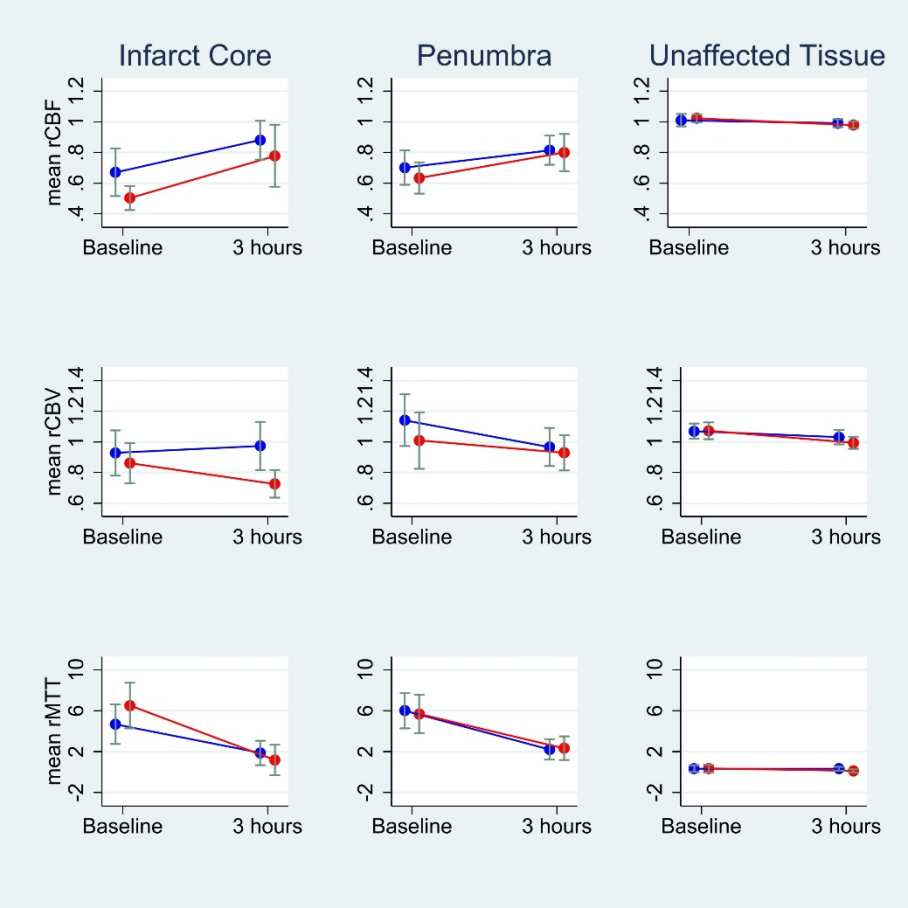


Values <0 indicate that theophylline is better than placebo while values >0 indicate that placebo is better than theophylline. The middle line indicates the mean difference between the predicted values, and the upper and lower lines indicate the higher and lower limits of agreement (twice the standard deviation), respectively.

Study IV

The mean rCBF, rCBV, and rMTT values at baseline, at 3-hour follow-up, and the change from baseline to 3-hour follow-up in relation to the infarct core, the penumbra, and the unaffected tissue were similar between the theophylline group and the control group (Figure 10).

Figure 10: Mean relative cerebral blood flow (rCBF), relative cerebral blood volume (rCBV), and relative mean transit time (rMTT); Study IV



Mean values with confidence interval (red=theophylline group, blue=control group)

The only exception was the mean rCBV in the infarct core. There was no significant difference between the theophylline group and the control group at baseline ($p=0.34$), but rCBV was significantly higher at the 3-hour follow-up point ($p<0.01$) in the theophylline group. The mean

rCBV change from baseline to follow-up was significantly different (p=0.04) in the theophylline group.

Table 2: Perfusion parameters (Study IV)

	INFARCT CORE			PENUMBRA			UNAFFECTED TISSUE		
	Theo-phylline (n = 13)	Control (n = 11)	p-value	Theo-phylline (n = 13)	Control (n = 11)	p-value	Theo-phylline (n = 13)	Control (n = 11)	p-value
Baseline									
rCBF (SD)	0.67 (0.28)	0.50 (0.13)	0.16[a]	0.70 (0.21)	0.63 (0.17)	0.51[a]	1.01 (0.07)	1.02 (0.04)	0.14[a]
rCBV (SD)	0.93 (0.27)	0.86 (0.22)	0.34[a]	1.14 (0.31)	1.01 (0.31)	0.28[a]	1.06 (0.09)	1.07 (0.09)	0.75[a]
rMTT (SD)	4.7 (3.6)	6.5 (3.8)	0.21[a]	6.02 (3.17)	5.68 (3.17)	0.93[a]	0.34 (0.64)	0.35 (0.60)	0.58[a]
3 h follow-up									
rCBF (SD)	0.88 (0.23)	0.78 (0.34)	0.28[a]	0.82 (0.17)	0.80 (0.21)	0.66[a]	0.99 (0.05)	0.98 (0.04)	0.47[a]
rCBV (SD)	0.97 (0.29)	0.73 (0.15)	0.01[a]	0.97 (0.23)	0.93 (0.38)	0.66[a]	1.03 (0.09)	0.99 (0.07)	0.34[a]
rMTT (SD)	1.87 (2.17)	1.18 (2.53)	0.34[a]	2.22 (1.85)	2.34 (1.95)	0.88[a]	0.34 (0.36)	0.12 (0.29)	0.11[a]
Mean Δ 0-3 h									
rCBF (SD)	0.21 (0.33)	0.27 (0.36)	0.65[b]	0.11 (0.21)	0.17 (0.16)	0.51[b]	-0.02 (0.05)	-0.04 (0.04)	0.20[b]
rCBV (SD)	0.05 (0.18)	-0.14 (0.24)	0.04[b]	-0.17 (0.25)	-0.08 (0.20)	0.32[b]	-0.04 (0.07)	-0.08 (0.07)	0.18[b]
rMTT (SD)	-2.82 (4.26)	-5.32 (4.43)	0.17[b]	-3.80 (4.17)	-3.35 (3.16)	0.77[b]	-0.00 (0.64)	-0.22 (0.54)	0.39[b]

Abbreviation: SD = standard deviation.

a Two-sample Wilcoxon rank-sum test, b Two-sample *t*-test with equal variances

Early clinical improvement with an improved NIHSS score ≥4 points between baseline and the 3-hour follow-up point was present in 1 (8%) patient in the theophylline group compared to 2 (18%) patients in the

control group (p=1). The rate of early recanalization was 100% (7/7) in the theophylline group and 50% (2/4) in the control group (p=0.109). The mean heart rate increased significantly by 11 beats per minute (SD 26) in the theophylline group compared to a decrease by 9 beats per minute (SD 14) in the control group ($p<0.05$). The mean systolic and diastolic blood pressures were similar in both groups.

The mean difference in rCBF, rCBV, and rMTT from baseline to the 3-hour follow-up point in the follow-up penumbra defined by the baseline ROI ($penumbra^{ROI\text{-}0h}$) and based on the actual penumbra at follow-up ($penumbra^{ROI\text{-}3h}$) was similar in the theophylline group and the control group (Table 3).

Table 3: Perfusion parameters within the different penumbra ROIs at baseline and follow-up

	Theophylline group (n = 13)			**Control group (n = 11)**			
$Penumbra^{ROI\text{-}0h}$	**0 h**	**3 h**	**Mean Δ**	**0 h**	**3 h**	**Mean Δ**	**p-value***
Mean rCBV	1.14 (0.31)	0.97 (0.23)	-0.17 (0.25)	1.01 (0.31)	0.93 (0.19)	-0.08 (0.21)	0.3236
Mean rMTT	6.02 (3.17)	2.22 (1.85)	-3.80 (4.17)	5.68 (3.17)	2.34 (1.95)	-3.35 (3.16)	0.7701
Mean rCBF	0.70 (0.21)	0.82 (0.17)	0.11 (0.21)	0.63 (0.17)	0.80 (0.21)	0.17 (0.16)	0.5079
$Penumbra^{ROI\text{-}3h}$							
Mean rCBF	0.70 (0.21)	0.81 (0.19)	0.08 (0.20)	0.63 (0.17)	0.57 (0.18)	-0.04 (0.20)	0.1971
Mean rCBV	1.14 (0.31)	1.20 (0.34)	0.05 (0.36)	1.01 (0.31)	0.93 (0.37)	-0.11 (0.44)	0.3524
Mean rMTT	6.02 (3.17)	3.77 (1.05)	-1.18 (2.54)	5.68 (3.17)	4.08 (2.39)	-2.65 (1.99)	0.1732

* Two-sample *t*-test with equal variances.

Abbreviations: rCBF, cerebral blood flow; rCBV, cerebral blood volume; rMTT, mean transit time; ROI, region of interest.

Discussion

The aim of this thesis was to evaluate the neuroprotective effect of theophylline in patients with acute ischemic stroke.

The TEA-Stroke trial protocol provided a study design involving the administration of theophylline as an adjunct to thrombolytic therapy that considered the important limitations of previous clinical trials (Study I). The trial was terminated early because of the low recruitment rate. No safety concerns were identified. The main outcome findings were: The co-primary endpoints clinical improvement and infarct volume growth at the 24-hour follow-up point were not significantly different between the theophylline group and the control group after correction for multiplicity (Study II). Voxel-by-voxel analysis of the multi-parametric MRI data, through the application of a machine learning model to predict the follow-up brain lesion volumes, revealed no significant difference in patients virtually treated with theophylline and placebo (Study III). Theophylline did not change the perfusion in the penumbra at the 3-hour follow-up, but affected the rCBV in the infarct core, with significantly higher values observed in the theophylline group (Study IV).

The clinical and imaging outcomes of the main TEA-Stroke trial and the sub-studies are somewhat contradictory.

Clinical endpoints

Theophylline improved the NIHSS score between baseline and 24-hour follow-up by 4.7 points, compared with an improvement of 1.3 points in the control group ($p=0.044$). This was not statistically significant after correction for multiplicity with a significance level of $\alpha=0.025$, as the clinical improvement was part of the co-primary endpoint (Study II). The trial was primarily designed to include patients with moderate to severe symptoms of acute ischemic stroke and an MRI indicative of a salvageable penumbra, as these patients might benefit most from a putative neuroprotective effect of theophylline. The subgroup analysis of patients with more severe stroke symptoms did support our

hypothesis that these patients benefit most, but the low sample size precluded the formation of a definitive conclusion. The low number of patients subsequently recruited, and the early termination of the trial was a result of patients with more severe stroke symptoms often unable to provide informed consent, a recognized rate-limiting step in acute clinical stroke trials.(94,95)

The secondary endpoints of early clinical improvement from baseline to the 3-hour follow-up point and functional clinical outcome at 90 days (functional independence and categorical functional outcome on the mRS) were not significantly different between the two treatment groups but were not statistically powered to detect meaningful differences. The fact that there was no death or severe disability in the theophylline group compared to three patients in the control group might be due to chance.

The trend towards clinical improvement, but without statistical significance, is in line with previous clinical trials with theophylline in stroke patients.(73,74) Although they share the problem of low sample sizes, the previous trials additionally suffered from inadequate patient selection, lack of revascularization therapy, and delayed intervention as presumed reasons for their failure to show statistically significant results.

Imaging endpoints

The co-primary imaging endpoint did not confirm reduced infarct volume growth at the 24-hour follow-up point in the theophylline group compared to the control group. Infarct volume growth was selected as a co-primary endpoint because infarct volume growth has been previously described as a surrogate marker for good long-term outcome.(25,83) However, two recent randomized clinical trials involving acute interventions in ischemic stroke demonstrated significant clinical improvements despite no significant reduction in infarct volume growth being observed. The MRI-Guided Thrombolysis for Stroke With Unknown Time of Onset (wake-up) trial in patients

with acute ischemic stroke of unknown onset treated with thrombolysis,(96) and the Thrombectomy for Stroke at 6 to 16 Hours with selection by perfusion imaging (DEFUSE III) trial,(13) both showed low number-needed-to-treat values in achieving functional independence of stroke patients at 90 days. Despite highly favorable clinical outcomes, no significant reduction in the growth of infarct volume was observed, indicating that infarct volume growth at 24-hour follow-up might be less suitable as an imaging surrogate endpoint.

However, the infarct volume growth information in these trials, and in the main TEA-Stroke trial (Study II), was limited to follow-up T2-FLAIR MRI segmentation. Thus, it was unclear whether the small sample size and the considerably large variation of stroke volumes in the TEA-Stroke trial might prevent the detection of a subtle neuroprotective effect of theophylline. The sub-study was designed with the voxel-by-voxel analysis of 8 multi-parametric MRI parameters, and 4 clinical parameters, to amplify the outcome information (Study III). Furthermore, the machine learning model approach to predict the follow-up brain lesion volumes essentially doubled the sample size. However, the predicted follow-up lesion volumes were almost identical in patients virtually treated with theophylline and placebo. This was also true for patients with penumbra and/or subsequent revascularization, a highly selected group of patients that would be most likely to benefit from neuroprotection. The lack of a neuroprotective effect of theophylline on infarct volume growth might be explained by the results of the last sub-study designed to evaluate the change in cerebral perfusion induced by theophylline (Study IV). This analysis showed that theophylline did not change cerebral perfusion in the salvageable penumbra and only affected the rCBV in the infarct core. It should be mentioned that even though CBF, CBV, and MTT were used in previous studies to define the penumbra and infarct core,(52,97,98) the precise thresholds to distinguish these two areas from each other, as well as the final outcome prediction, varies between studies.(99–101) The decreased signal in the ADC maps was

used to define the infarct core in this sub-study analysis. Dreier et al. depict spreading depolarization as the principal mechanism that mediates neuronal death in ischemic stroke.(102) This mechanism was described as a near-complete breakdown with cessation of spontaneous electrical activity, collapse of ion gradients, and consecutive cytotoxic edema. The cytotoxic edema is detectable as restricted diffusion with decreased signal in the ADC maps. However, normalization of the ADC signal may occur in acute stroke after tissue reperfusion, especially within 3 hours after stroke onset and brain tissue with initially decreased ADC signal may also include tissue at risk.(103) In the penumbra, a cluster of repetitive prolonged spreading depolarization is typical and initially reversible, whereas in the infarct core, it is often a single terminal event causing neuronal death.(102) This contributes to the uncertainty of the correct differentiation and the dynamic over time of the infarct core and penumbra that might limit the interpretation of the perfusion changes in this sub-study. Another issue is the presence of experimental evidence showing that adenosine released by astrocytes during ischemia might have a protective effect on neurons and the microcirculation(58,104) by activating adenosine triphosphate-dependent membrane pumps required in the recovery from spreading depolarization.(102) Theophylline, however, acts as competitive adenosine receptor antagonist(56) and antagonization of adenosine in the infarct core and penumbra might be counterproductive in the concept of neuroprotection by theophylline.

Regarding the sub-study results of this dissertation, it is uncertain whether the increased rCBV in the infarct core in the theophylline group was caused by the potentially positive chronotropic and inotropic effects of theophylline, but the significant difference in heart rate with higher values in the theophylline group might support this theory. The inverse intracerebral steal phenomenon or ‘Robin Hood effect’ of theophylline described in previous trials(59) might provide another explanation. However, the data from this sub-study do not support the theory of redistribution from healthy brain tissue to the stroke-affected

areas, as the perfusion in the unaffected brain tissue and the penumbra remained unchanged. If this was present at all, it was limited to the infarct core and unlikely to be salvageable. This might explain why theophylline did not show a more substantial clinical improvement (Study II) and did not reduce the infarct volume growth (Study III).

The main strength of the TEA-Stroke trial and sub-studies was that they were based on a randomized, double-blind, placebo-controlled study design. The protocol was designed to select patients with MRI-verified ischemic stroke within the acute phase of 4.5 hours after symptom onset, with the presence of salvageable penumbra, and as an adjunct to thrombolytic therapy to offset the shortcomings of previous clinical trials (Study I). The trial randomization and treatment concealment were effective, and the outcome assessment was blinded and complete (Study II). The quality of all perfusion MRI datasets was sufficient for analysis (studies III and IV). However, the main limitation was the slow recruitment rate and the early termination of the trial despite three amendments. The inability of patients with moderate and severe stroke symptoms to provide informed consent was a major factor contributing to screening failure. Consent by proxy was not permitted by the Danish Health and Medicine Authorities when the study was designed back in 2013. Furthermore, recruitment of new sites in Denmark and Europe was not possible, as the trial protocol and in particular the acute multi-parametric MRI studies were challenging for other sites.

Conclusion

The aim of this thesis was met as it evaluated the neuroprotective effect of theophylline as an adjunct to thrombolytic therapy in patients with acute ischemic stroke. The TEA-Stroke trial was designed and performed to offset the shortcomings of previous clinical trials. No significant safety concerns were identified. The co-primary clinical endpoint alone could have shown early clinical improvement in the theophylline group but was not statistically significant after correction for multiplicity. The co-primary endpoint of infarct volume growth was similar in both treatment groups. A machine learning approach to predict the follow-up lesions for each patient virtually treated with theophylline and placebo was applied. This approach adjusted for the large variation of stroke volumes in a small sample size and confirmed that theophylline did not reduce the infarct volume growth. Furthermore, the analysis of cerebral perfusion showed that theophylline did not change the perfusion in the salvageable penumbra, but only affected the cerebral blood volume in the infarct core. In contrast to the penumbra, the infarct core is very unlikely to be salvageable, which might explain why theophylline failed to reduce the final infarct volume and failed to substantially improve the clinical outcome.

Suggestions for Further Research

The results of the TEA-Stroke trial should be added to the growing body of neuroprotective clinical trials that have failed to translate promising results from pre-clinical stroke models to humans.(105) However, the traditional scientific definition of neuroprotection refers to the minimization of the harmful effect of ischemia at the level of the neuron and, from the pragmatic point of view of patients and physicians, neuroprotection means maintaining neuronal damage under the threshold of symptom manifestation.(38) With this in mind, this proof-

of-concept trial might encourage a further clinical trial designed to search for a clinically meaningful neuroprotective effect of theophylline. However, the clinical effect in the TEA-Stroke trial was small, and the potential neuroprotective effect could not be supported by the imaging data. Furthermore, it must be remembered that theophylline has potentially serious side effects, although this trial had no clinically relevant safety concerns. In summary, the data of this thesis are not strong enough to support further research on theophylline as a neuroprotective agent, as the balance between potential clinically meaningful benefit and harm of theophylline in acute ischemic stroke remains questionable.

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Appendices

Appendix A: National Institute of Health Stroke Scale (NIHSS)

Appendix B: Modified Rankin Scale (mRS)

Appendix C: Study I: Eur Stroke J. 2016; 1: 248–254.

Appendix D: Study II: Stroke 2020; 51: 1983–1990

Appendix E: Study III: Front Neurol. 2020; submitted

Appendix F: Study IV: Clin Neuroradiol. 2020; submitted

Appendix A (1/6): National Institute of Health Stroke Scale (NIHSS)

N I H STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

Time: ___ ___:___ ___ [ ]am [ ]pm

Person Administering Scale _____

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = **Alert;** keenly responsive. 1 = **Not alert**; but arousable by minor stimulation to obey, answer, or respond. 2 = **Not alert**; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.	_____
1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = **Answers** both questions correctly. 1 = **Answers** one question correctly. 2 = **Answers** neither question correctly.	_____
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = **Performs** both tasks correctly. 1 = **Performs** one task correctly. 2 = **Performs** neither task correctly.	_____
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = **Normal.** 1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic maneuver.	_____

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Appendix A (2/6): National Institute of Health Stroke Scale (NIHSS)

N I H
STROKE
SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = **No visual loss.** 1 = **Partial hemianopia.** 2 = **Complete hemianopia.** 3 = **Bilateral hemianopia** (blind including cortical blindness).	_____
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	0 = **Normal** symmetrical movements. 1 = **Minor paralysis** (flattened nasolabial fold, asymmetry on smiling). 2 = **Partial paralysis** (total or near-total paralysis of lower face). 3 = **Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face).	_____
5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** limb holds 90 (or 45) degrees for full 10 seconds. 1 = **Drift;** limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. 2 = **Some effort against gravity;** limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = **No effort against gravity;** limb falls. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **5a. Left Arm** **5b. Right Arm**	_____ _____
6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** leg holds 30-degree position for full 5 seconds. 1 = **Drift;** leg falls by the end of the 5-second period but does not hit bed. 2 = **Some effort against gravity;** leg falls to bed by 5 seconds, but has some effort against gravity. 3 = **No effort against gravity;** leg falls to bed immediately. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **6a. Left Leg** **6b. Right Leg**	_____

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Appendix A (3/6): National Institute of Health Stroke Scale (NIHSS)

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = **Absent.** 1 = **Present in one limb.** 2 = **Present in two limbs.** UN = **Amputation** or joint fusion, explain: _____	_____
8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	0 = **Normal;** no sensory loss. 1 = **Mild-to-moderate sensory loss;** patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = **Severe to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.	_____
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = **No aphasia;** normal. 1 = **Mild-to-moderate aphasia;** some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = **Severe aphasia;** all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. 3 = **Mute, global aphasia;** no usable speech or auditory comprehension.	_____
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = **Normal.** 1 = **Mild-to-moderate dysarthria;** patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = **Severe dysarthria;** patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = **Intubated** or other physical barrier, explain:_____	_____

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Appendix A (4/6): National Institute of Health Stroke Scale (NIHSS)

N I H
STROKE
SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = **No abnormality.** 1 = **Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = **Profound hemi-inattention or extinction to more than one modality;** does not recognize own hand or orients to only one side of space.	_____

_____

_____

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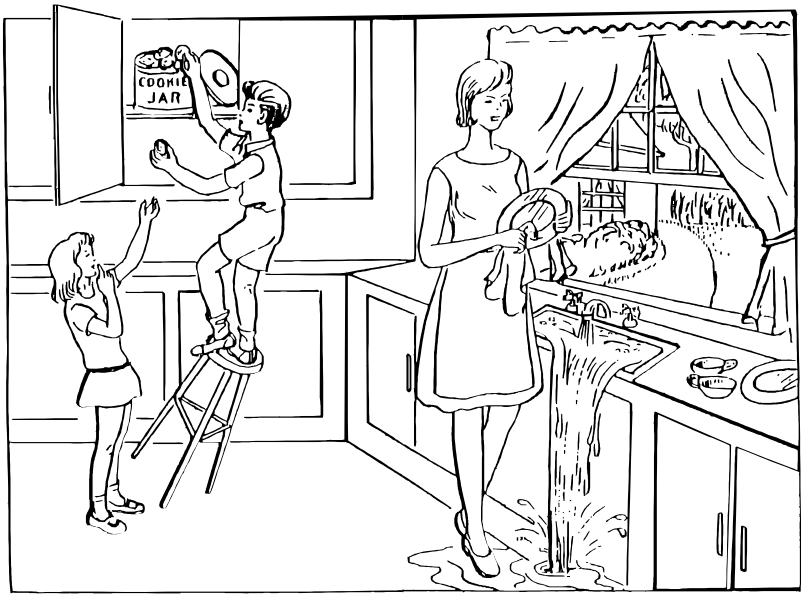
Appendix A (5/6): National Institute of Health Stroke Scale (NIHSS)



You know how.

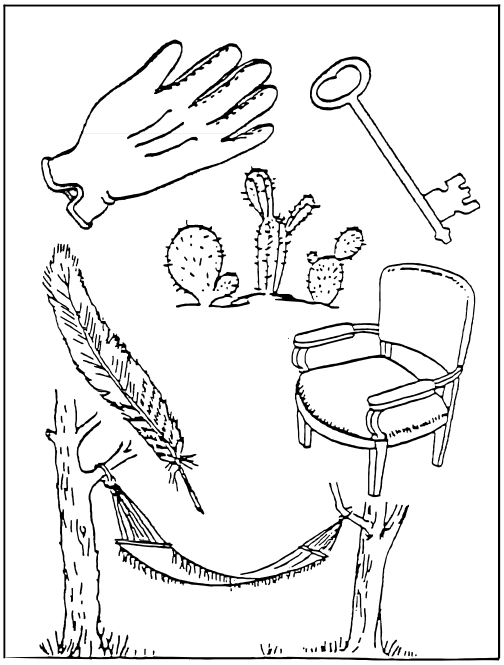
Down to earth.

I got home from work.

Near the table in the dining room.

They heard him speak on the radio last night.

Appendix A (6/6): National Institute of Health Stroke Scale (NIHSS)



MAMA

TIP – TOP

FIFTY – FIFTY

THANKS

HUCKLEBERRY

BASEBALL PLAYER

Appendix B: Modified Rankin Scale (mRS)

(Provided by the Internet Stroke Center — www.strokecenter.org)

MODIFIED RANKIN SCALE (MRS)

Patient Name: _____
Rater Name: _____
Date: _____

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

TOTAL (0–6): _____

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