Prognostic factors of functional outcome in acute ischemic stroke

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CHAPTER 1

General introduction

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Worldwide, stroke is the fourth most common cause of death behind disease of the heart, cancer and chronic lower respiratory disease.[1] Between 1998 and 2008 the annual stroke death rate decreased 35%, but still stroke is a leading cause of serious long-term disability.[1] Most patients suffer from physical disability, but one third of patients also suffers from psychiatric symptoms like depression.[2] In the Netherlands, the annual incidence of stroke is estimated to be 41.000, while about 300.000 persons live with the consequence of a stroke.[3,4] In 2011, stroke is the third most common cause of death in the Netherlands in men (5.2%), after pulmonary carcinoma (9.9%) and ischemic heart disease (5.8%). In women stroke is the most common cause of death (7.7%), before pulmonary carcinoma (5.2%) and carcinoma of the breast (4.6%).[5] In the past decades the mortality rate of stroke in the Netherlands has declined from approximately 10% for men and 15% for women in 1970 to approximately 5% and 8% in 2010.[5] Also, the mortality rate in the first year after a stroke has declined with approximately a quarter between 2000 and 2005, because of more adjusted treatment in the acute phase and more attention to stroke services.[6]

Despite this reduction in stroke mortality and a decrease in stroke incidence, due to primary and secondary prevention, an increase in the prevalence and burden of stroke is to be expected, considering the growth of the total elderly population and the general improvements in life expectancy.[7,8] Therefore, it is important to continue and to improve prevention strategies. Additionally, to reduce the burden of stroke it is also important to explore novel strategies that reduce disability after stroke, particularly development of novel treatment options and improvement of currently available treatments.

Revascularization therapy

The development of revascularization therapy with recombinant tissue plasminogen activator (tPA) has shown great benefit in improving outcome and reducing disability in patients with acute ischemic stroke.[9-13] In the first years of use, tPA treatment was registered as an effective treatment for acute ischemic stroke within 3 hours after symptom onset.[12] The chances of benefit from tPA treatment diminish as time elapses. Compared to controls the odds ratios of a favorable outcome for patients treated with tPA were 2.81 (1.75 – 4.50) for those treated within 90 minutes and 1.55 (1.12 – 2.15) for those treated within 91 and 180 minutes of symptom onset.[12] In 2010, a pooled analysis of tPA trials showed that tPA treatment also improves the odds of a favorable outcome when looking at a treatment interval until 270 minutes after symptom onset.[13] Beyond the limit of 270 minutes the benefits of tPA treatment may not outweigh the risks.[13] Overall, tPA treatment is the most effective when administrated in the first 90 minutes after symptom onset, but despite all efforts it is still only effective in a minority of patients. In the patient group treated within

90 minutes the number needed to treat (NNT) to achieve one excellent outcome (modified Rankin Scale 0-1, ***Table 1***) is about 5, within 91-180 minutes of stroke onset the NNT it is 9 and within 181-270 minutes it drops to 15. [13]

Table 1: Outcome measures following modified Rankin Scale (mRS)

Outcome measure	Corresponding score	Description
Excellent outcome	mRS 0-1	No symptoms/no significant disability
Good outcome	mRS 0-2	Independence with regard tot activities of daily living, unable to carry out all previous activities
Poor outcome	mRS 3-6	Dependence or death

The major risk associated with tPA treatment is the occurrence of symptomatic intracranial hemorrhage (SICH). Various trials report a significant higher risk of SICH in tPA treated patients compared to non-treated patients, for instance 6.4% versus 0.6% [9], 8.8% versus 3.4% [11] and 7.0% versus 1.1% [14]. However, the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST) showed that the daily risk of SICH in routine practice is slightly lower than in the trials mentioned. [15]

Because of the relatively short time window, the number of patients who actually receive tPA treatment and therefore potentially benefit, is small. Secondly, a number of contra-indications for tPA treatment limits the actual eligibility of many patients to receive this treatment. Elevated serum glucose levels, elevated blood pressure levels and use of anti-coagulation drugs are factors that increase the risk of complications of tPA treatment. [16] Efforts to increase the number of patients eligible for thrombolytic therapy are made, for instance by improving the triage pathway and the public awareness, in order to reduce onset-to-needle time. [17,18] Efforts are made to improve stroke treatment with new thrombolytics and endovascular treatment. [19,20]

Neuroprotection

Another therapeutic strategy to improve stroke outcome and reduce resulting disability is the development of neuroprotective agents. Neuroprotection refers to the effect of drugs or agents that prolong neuronal survival after ischemia. Compounds that block excitotoxicity have often been studied in this context. These agents are especially thought to protect the ischemic penumbra, a phenomenon which was first introduced by Astrup in 1981. [21] The penumbra is a zone with markedly reduced blood flow, with neurons that are functionally inactive but still viable. Saving the ischemic penumbra will lead to smaller infarct size and could lead to better recovery and functional outcome. In the absence of reperfusion or neuroprotection, the tissue in the ischemic penumbra undergoes also infarction.

The neurons in the penumbra can stay viable within a time window of several hours as demonstrated by positron emission tomography (PET) and magnetic resonance imaging (MRI). [22-24] After several hours of ischemia the neurons are irreversibly damaged as a consequence of a cascade of biochemical events, called the ischemic cascade, in which calcium ion-influx, intracellular calcium release and glutamate neurotoxicity play major roles. (***Figure 1***) [25,26] Reduction of blood flow will lead to depletion of energy stores, failure of the sodium/potassium-pumps and, consequently, to massive depolarization. This depolarization causes release of large amounts of presynaptic glutamate. Under physiological conditions glutamate causes no neuronal damage, but in the state of ischemia excessive amounts of glutamate are neurotoxic. The acute elevation of glutamate will lead to activation of N-methyl-d-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and this, in turn, causes neuronal calcium influx as well as release from intracellular calcium stores. Moreover, depolarization itself also causes an increase in intracellular calcium concentration. These elevated intracellular calcium concentrations cause irreversible damage by several mechanisms. Activation of proteases, lipases and caspases exerts detrimental effects on cellular function and membrane structure, ultimately leading to necrosis. Second, an increase in free radicals damages cell membranes. Finally, the resulting stimulation of intracellular processes increases oxygen demand and further worsening of the hypoxic state. [25,26]

Furthermore, the raised intracellular calcium concentrations and the increase of oxygen free radicals in ischemia trigger the expression of pro-inflammatory genes and thereby activate inflammatory processes. These inflammatory reactions have been suggested to contribute to the late stages of ischemic injury in worsening neurological outcome. [27,28]

Figure 1

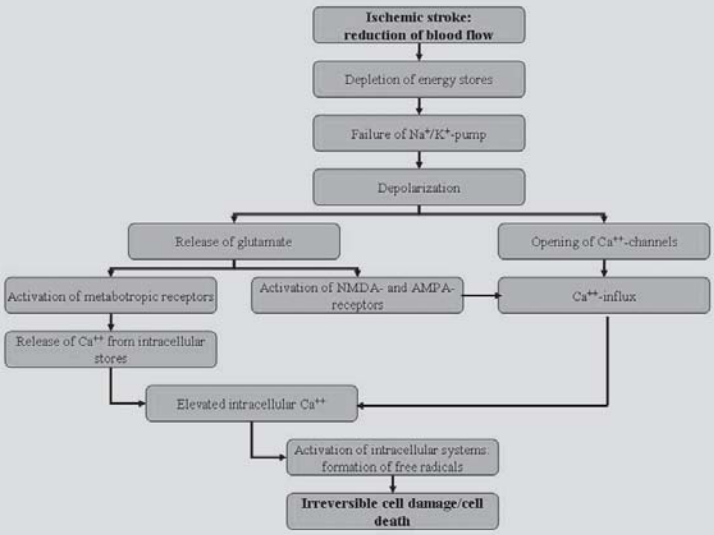

Figure 1: representation of the ischemic cascade in stroke

Several neuroprotective agents or therapies have been designed to try to salvage neurons within the penumbra. These therapies include calcium channel blockers, glutamate inhibitors and gamma-aminobutyric acid (GABA) agonists, free radical scavengers and hypothermia. Preclinical studies showed promising results, but all phase III clinical trials have failed to demonstrate positive clinical effects. [25,26,29-33] Various explanations for these disappointing results have been proposed.

A major cause are the methodological differences between animal models and clinical trials. In animal trials, the neuroprotective drugs are administered shortly before or after the ischemic event; in clinical trials these intervals are much larger. [25,30-32] It is hypothesized that in most clinical trials the administration would be too late to expect a meaningful neuroprotective effect. Another issue is the discrepancy between drug-dosing schedules. [25,30,31] In general, in clinical trials dosage regimens have been used that were lower and more variable than those used in animal experiments, to avoid possible adverse drug effects. A single dose may not provide sufficient neuroprotection, while prolonged administration may also cause adverse effects. For instance, NMDA-antagonist may produce a neuroprotective effect in the early phase, but blockage can be detrimental in the later phases. NMDA activation is necessary for neurite outgrow that may play a role in recovery. [34] Furthermore, heterogeneity of the study population plays a major role. In animal models strict physiological conditions with respect to blood pressure, temperature and oxygenation are maintained while the infarct type studied is mostly a cortical stroke by medial cerebral artery (MCA) occlusion. In the clinical trials these physiological conditions can not be standardized. In addition, patients suffer from other factors that influence prognosis, like age and co-morbidity. [25,30,31] Patients with cortical and lacunar strokes are often lumped together, but pathophysiological mechanisms between grey and white matter strokes may differ. Therefore, neuroprotective drugs could also act differently in these stroke subtypes. [35] Finally, in most animal models efficacy of the neuroprotective drug to be investigated was assessed by histological infarct size. In clinical trials, the effect of a neuroprotective drug is measured by using functional outcome scales. Infarct size has a poor correlation with outcome. Small infarcts in critical locations produce large deficits while large infarcts in less eloquent locations may produce little detectable function loss. [25,30,31]

Hypothermia is a promising alternative in neuroprotection, having already shown benefits in patients after cardiac arrest and perinatal asphyxia. [36] In animal models of cerebral ischemia it has consistently shown benefit. [36-38] However, translation to clinical cerebral ischemic stroke has been less successful, partly because of issues like patient comfort and complications. [36-38]

In summary, factors that might be responsible for the failure of the development of neuroprotective agents are: the methodologies used, insufficient information about therapeutic doses and the therapeutic time window available. Moreover, the animal models used in these studies do not mimic the human clinical situation, particularly not the effects of infarct size and localization, nor the contribution of co-morbidity. [25,29-32]

Combination therapies

Despite the disappointing results up to now, the prospect of neuroprotective agents remains tantalizing. Another research strategy is the combination of different treatments. As mentioned previously, several pathways in the ischemic cascade lead to increase of intracellular calcium and cell death. (Figure 1) A neuroprotective agent acts mostly on one specific pathway. A single neuroprotective agent may not be enough to result in a significant clinical impact, as measured by functional outcome scales like the modified Rankin Scale (mRS) and the Barthel Index (BI). Therefore, it would be interesting to explore treatment options using either a combination of two or more neuroprotective agents or a combination of reperfusion therapy with a neuroprotective agent. [25,29-32,39] In animal models, evidence has been found for a positive interaction when combining tPA with a neuroprotective drug. [39-42] Treatment with tPA increases the chance of reperfusion in the ischemic penumbra and preserves salvageable tissue. Therefore, it may extend the therapeutic window for neuroprotective therapies. [41-43] Co-administration of neuroprotective drugs to tPA treatment might also extend the therapeutic window for tPA treatment. [44,45] Finally, combination of various neuroprotective drugs that target different aspects of the ischemic cascade could produce a synergistic effect. [31] But new trials using a combination of experimental drugs can be methodologically complex.

To summarize: combination therapies may not only improve outcome in patients with acute ischemic stroke, but they may also increase the amount of patients eligible for tPA treatment.

Aims and scope of the thesis

Reperfusion with tPA is the intervention of choice in acute ischemic stroke treatment to improve outcome, but as discussed before it is only possible in a selected group of acute ischemic stroke patients. It has several risks and contra-indications. Another approach to improve outcome after ischemic stroke is neuroprotection, with the aim to reduce secondary brain damage in the ischemic penumbra. Several treatment strategies targeting neurotoxic mechanisms that accompany acute brain ischemia have been explored, but in clinical settings these therapies have largely failed to even approach the benefits that animal models had suggested. Preclinical studies also found evidence for positive treatment interactions when revascularization therapy and neuroprotective agents were combined.

In this thesis, the influence will be studied of two possible neuroprotective drug classes on functional outcome in tPA treated acute ischemic stroke patients. Furthermore, I will explore other prognostic factors that influence the therapeutic effects and safety of tPA treatment with regard to functional outcome and complications of revascularization therapy.

Chapter 2-4 describe the effects of possible neuroprotective agents and compounds on functional outcome. In Chapter 2 the effects on functional outcome of selective serotonin re-uptake inhibitors (SSRIs) that are being used prior to the ischemic stroke are examined.

SSRIs are thought to be neuroprotective through their effects on neuronal cell survival and the plasticity of brain function. [46-48] The potential neuroprotective effect of prior statin use on functional outcome is studied in Chapter 3. Statins are widely used for the primary and secondary prevention of cardiovascular disease. They prevent first as well as recurrent ischemic strokes mainly because of their lipid lowering effects. But they might have pleiotropic effects, amongst them neuroprotection in acute ischemic stroke. [49-51] Finally, the effects of serum uric acid concentrations on functional outcome are assessed (Chapter 4). Uric acid (UA) is a predictor for developing cardiovascular disease and stroke by mechanisms that are involved in the development and progression of atherosclerosis. It is also a powerful anti-oxidant and it could act as a free radical scavenger that reduces oxidative stress and protects the neurons in the ischemic penumbra. [52,53]

Admission hyperglycemia is common in patients with acute ischemic stroke. It is independently associated with poor functional outcome, in patients treated with and without intravenous tPA, regardless of a history of diabetes. [54-61] Previous studies in patients not treated with intravenous tPA found a dissociation of this harmful effect between lacunar and non-lacunar strokes, with a better outcome in hyperglycemic patients and lacunar stroke. [56,60] Chapter 5 explores the effects of hyperglycemia on functional outcome in tPA treated patients for both lacunar and non-lacunar strokes.

There is much controversy whether to treat ischemic stroke patients with intravenous tPA when using a vitamin K antagonist (VKA) with an suboptimal international normalized ratio (INR). [62,63] In the first part of Chapter 6 the relation between prior VKA use and a suboptimal INR with the occurrence of SICH and functional outcome is studied. The second part of Chapter 6 consists of a systematic review and meta-analysis of this subject. Finally, Chapter 7 presents a general discussion as well as the main conclusions of this thesis.

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1

CHAPTER 2

Effect of selective serotonin re-uptake inhibitors (SSRIs) on functional outcome in patients with acute ischemic stroke treated with tPA

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Abstract

Background

Selective serotonin re-uptake inhibitors (SSRIs) may have therapeutic potential in the treatment of ischemic stroke by effects on neuronal cell survival and the plasticity of brain processes. In the present study, we investigated whether prior treatment with a SSRI is associated with more favorable functional outcome in a cohort of patients with acute ischemic stroke treated with tissue plasminogen activator (tPA).

Methods

In a prospective observational cohort study of 476 acute ischemic stroke patients treated with tPA we investigated the relationship between prior SSRI treatment and functional outcome at 3 months. Ischemic stroke subtypes were defined according to the Oxfordshire Community Stroke Project Classification. Favorable outcome was defined as a modified Rankin Scale score ≤2.

Results

In the cohort of 476 patients, 22 (5%) patients used a SSRI at stroke onset. At 3 months, 217 (46%) patients had a favorable outcome of whom 9 (41%) on SSRI treatment and 208 (46%) not using SSRIs (p = 0.65). In a multivariable analysis SSRI treatment showed a trend to association with unfavorable outcome (OR 0.4, 95% CI 0.14-1.13, p = 0.08). In the 376 patients with cortical stroke, SSRI treatment was associated with a unfavorable outcome (OR 0.17, 95% CI 0.04-0.73, p = 0.017).

Conclusion

Our data suggest that in patients with acute ischemic stroke treated with tPA, prior SSRI use may be associated with a less favorable outcome, especially in cortical stroke.

Introduction

The last two decades there is an increasing use of selective serotonin re-uptake inhibitors (SSRIs) in the treatment of depression and other psychiatric disorders. Depression also occurs in approximately 40% of acute stroke patients and has been significantly linked to poor cognitive and physical recovery. [1,2] In patients who had a stroke, treatment with SSRIs after stroke improved cognitive and functional outcome. [3-5] In one study this effect was seen regardless of whether patients were initially depressed. [5] One way in which treatment with SSRIs may facilitate recovery of function in stroke patients is by improving mood and motivation to perform rehabilitation tasks. [3,5,6] Secondly, SSRIs could also have direct effects on neuronal cell survival and the plasticity of brain processes, for instance by modulation of neurotrophins, particularly brain-derived neurotrophic factor (BDNF). BDNF has a function as key regulator of neurite outgrowth, synaptic plasticity and the selection of functional neuronal connections. [7-9] Chronic administration of SSRIs enhances BDNF transcription. [10,11] Furthermore, SSRIs also have effects on cerebral energy homeostasis and ion channel function. Altogether these data suggests that SSRIs might have therapeutic possibilities in several neurological disorders including ischemic stroke. [12,13]

Two clinical studies evaluating the effect of fluoxetine on functional recovery of patients who had a stroke suggested an improvement in motor performance. [14,15] However, neither study included acute ischemic stroke patients. In the present study, we investigated whether prior treatment with a SSRI is associated with more favorable functional outcome in a cohort of patients with acute ischemic stroke treated with recombinant tissue plasminogen activator (tPA).

Methods

An ongoing prospective registry of patients with acute ischemic stroke receiving tPA treatment was started at the University Medical Centre of Groningen in April 2002. All patients registered between April 2002 and March 2009 were included in this study. tPA treatment was performed within a time window of 4.5 hours after onset of symptoms according to a protocol which has been described earlier. [16] The neurological deficit before tPA treatment was assessed according to the National Institute of Health Stroke Scale (NIHSS). Demographic and clinical information were recorded. Prior medication use, medication name and dosages were recorded for antiplatelet, lipid lowering and antidepressant drugs. During hospital stay and after discharge SSRI therapy was continued. We did not record how many patients using SSRI's before stroke, stopped the treatment in the following 3 months after discharge. Furthermore, we did not document whether patients naive to SSRI's started with a SSRI after discharge. Stroke subtypes were defined into lacunar infarcts (LACI) and cortical infarcts (PACI and TACI) according to the Oxfordshire Community Stroke Project Classification. [17]

Outcome

The modified Rankin Scale (mRS) was used to determine functional outcome and was measured 3 months after stroke onset. Outcome was dichotomized into favorable outcome (mRS 0-2), corresponding to independence with regard to activities of daily living, and unfavorable outcome (mRS 3-6), reflecting death or dependency. Functional outcome was compared between patients with and without prior treatment with SSRIs.

Statistics

Baseline characteristics for patients with or without prior treatment with SSRIs were compared. Mann Whitney U-test was used for continuous and ordinal variables without a normal distribution. Pearson's Chi Square test and Fisher's exact test were used for dichotomous variables. Variables within the univariable baseline characteristics for SSRI use and non SSRI use with a p value < 0.20 were selected as covariates for the multivariable logistic regression analysis. Regardless of the difference in baseline characteristics, age, NIHSS score and serum glucose level at presentation were entered in the multivariable analyses. These variables were considered to be possible confounders, since these variables are related to outcome in patients with acute ischemic stroke. Because of the aim of the study, SSRI therapy was also entered in the multivariable analysis. In the multivariable analysis with favorable outcome as dependent factor, variables with a p value > 0.20 were removed from the model.

All statistical analyses were performed using SPSS 16.0. Statistical significance was taken to be at two tailed level < 0.05. A binary logistic regression model was used for multivariable analysis, with adjustment for possible confounders, to calculate odds ratios with 95% confidence interval.

Results

Patient sample

A total of 478 patients with acute ischemic stroke were treated with tPA within the study period. Data on functional outcome at 3 months were missing for 2 patients and they were excluded. In the cohort of 476 patients, 32 (7%) patients were using antidepressant drugs of which 22 (5%) patients were on a SSRI. Of the 22 patients with SSRI treatment, 8 patients used paroxetine, 7 citalopram, 4 fluoxetine, 2 venlafaxine and 1 patient escitalopram. Baseline characteristics of the entire cohort, SSRI users and patient not on a SSRI are shown in ***Table 1***. Users of SSRIs were slightly younger than non users (64 vs 68 years), but this difference was not significant (p = 0.22). Women used more frequently a SSRI compared to men (73% vs 27%, p = 0.01). The presence of vascular risk factors at presentation and of infarct subtype did not significantly differ between the two groups.

Table 1: Baseline characteristics entire cohort and (non) SSRI users

	Entire cohort	SSRI user	Non SSRI user	
Characteristic	N=476	N=22	N=454	P
Mean age (SD), years	68 (14)	64 (17)	68 (14)	0.219•
Male	257 (54)	6 (27)	251 (55)	0.010
Mean NIHSS score (SD)*	13 (6)	12 (5)	13 (6)	0.407•
Mean time until treatment (SD), min*	163 (50)	149 (44)	163 (50)	0.142•
Stroke subtype				0.807#
LACI	77 (16)	5 (23)	72 (16)	
TACI	136 (29)	4 (18)	132 (29)	
PACI	240 (51)	12 (55)	228 (50)	
POCI	22 (5)	1 (5)	21 (5)	
Early ischemic changes on brain CT scan	191 (40)	9 (41)	182 (40)	0.939
Hypodensity area > 33% on brain CT scan	29 (6)	1 (5)	28 (6)	1.000#
Vascular risk factors				
Hypertension	220 (46)	10 (46)	210 (46)	0.941
Diabetes	62 (13)	5 (23)	57 (13)	0.188#
Hyperlipidemia	201 (42)	13 (59)	188 (41)	0.101
Smoking	139 (29)	9 (41)	130 (28)	0.216
Previous stroke/TIA	88 (19)	7 (32)	81 (19)	0.153
Prior use of antiplatelets	134 (28)	5 (23)	129 (28)	0.562
Prior use of statins	98 (21)	4 (18)	94 (21)	1.000#
Mean systolic blood pressure (SD), mm Hg*	154 (27)	151 (26)	154 (27)	0.659•
Mean diastolic blood pressure (SD), mm Hg*	82 (16)	81 (17)	83 (16)	0.803•
Mean glucose (SD), mmol/l	6.6 (1.9)	6.2 (1.4)	6.6 (1.9)	0.644•

*Values are numbers (%), unless otherwise indicated. P values are between SSRI users and non users. P-values calculated with Pearson's X2 test. # Fisher's exact test. • Mann-Whitney-U-test. *2 missing values (for non SSRI users). SD: standard deviation. LACI/TACI/PACI/POCI: lacunar infarction/total/partial anterior infarction/posterior circulation infarction.*

Functional outcome

The distribution of the modified Rankin Scale scores at 3 months after acute ischemic stroke was not significantly different for both groups. At 3 months, 217 (46%) patients had a favorable outcome. Nine (41%) patients on a SSRI had a favorable outcome in comparison to 208 (46%) patients not on a SSRI (p = 0.65). In a multivariable analysis with adjustment for confounders SSRI use shows a trend to association with unfavorable functional outcome (OR 0.40 with 95% CI 0.14-1.13 and p = 0.08) (***Table 2***). In contrast, younger age, lower NIHSS score at presentation and lower serum glucose levels were significantly associated with a favorable functional outcome.

Table 2: Multivariable analysis: association of SSRI therapy with favorable outcome

	Entire cohort (n=476)			Cortical stroke (n=376)		
Variables	**OR**	**95% CI**	**p**	**OR**	**95% CI**	**p**
SSRI therapy	0.40	0.14 – 1.13	0.083	0.17	0.04 – 0.73	0.017
Age, year	0.95	0.93 – 0.96	<0.001	0.95	0.93 – 0.96	<0.001
NIHSS score	0.81	0.77 – 0.85	<0.001	0.82	0.77 – 0.86	<0.001
Serum glucose level (mmol/L)	0.88	0.78 – 1.00	0.045			

Of the 376 patients with a cortical stroke (of which 16 patients were on a SSRI), 147 (39%) had a favorable functional outcome. Three (19%) of the patients on a SSRI and 144 (40%) of the non SSRI users had a favorable outcome with a trend to significance (p = 0.088). The final multivariable regression model showed an association between SSRI use and unfavorable outcome (OR 0.17 with 95% CI 0.04-0.73 and p = 0.017) (Table2). Younger age and lower NIHSS score at presentation were significantly associated with a favorable outcome.

Of the 77 patients with a lacunar stroke, 5 used a SSRI. Sixty (78%) patients had a favorable outcome, all of the 5 patients using a SSRI (100%) and 55 (76%) of the patients naive to SSRI treatment (p = 0.58). A multivariable analysis was not performed because of the small number of SSRI users with a lacunar stroke.

Discussion

To our knowledge, this is the first clinical study to investigate the effect of prior SSRI use on functional outcome in patients with acute ischemic stroke treated with tPA. Our results suggest that prior SSRI use is associated with a less favorable outcome (mRS >2), especially in patients with a cortical stroke. In contrast to our findings, two earlier clinical studies

investigating the neuroprotective effect of fluoxetine found an improved motor function following treatment with fluoxetine. [14,15] However, treatment with fluoxetine was started a few weeks to months after the stroke. Our patients were already treated with a SSRI before their stroke.

A deleterious effect of SSRIs used prior to the stroke might be explained by enhanced glutamate toxicity. Long term SSRI treatment promotes BDNF transcription and increases the BDNF concentration. [10,11,18] However, BDNF induces glutamate release and sensitivity, and glutamate in turn stimulates also BDNF production, resulting in a vicious circle. [19] Excessive sustained elevation of extracellular glutamate causes excitotoxicity, especially under conditions of reduced energy and oxygen availability as happens during acute ischemic stroke. [20,21]

Our study was performed in patients with acute ischemic stroke treated with tPA. We cannot exclude a possible interaction of SSRI use with tPA, because we have no control group of patients without tPA treatment. These could be an interesting area of further research, since no data exist on such possible interaction.

A weakness of our study is the small number of patients using an SSRI. Because of the small sample size, no sub-analysis based on individual SSRIs could be performed. The drug dosage regimens were different between the patients and we did not evaluate drug compliance.

Our patients used SSRIs because of previous depression. We have no information on the severity of their depression just prior to the stroke. There is recent literature suggesting intracerebral neuro-inflammatory changes in depression. [22] However, how this may influence functional outcome after stroke is not clear, especially when patients are already treated for their depression with SSRIs.

The worldwide increase in the number of patients with depression or mood disturbances and the widely use of SSRIs underlie the need for taking these factors into consideration when evaluating therapies for acute ischemic stroke.

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CHAPTER 3

Statin use and functional outcome after tissue plasminogen activator treatment in acute ischemic stroke

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Abstract

Background

Preliminary findings suggest that statins may have a neuroprotective effect in patients with acute ischemic stroke. This study investigated whether patients prior on statin therapy and treated with tissue plasminogen activator (tPA) for acute ischemic stroke have a better functional outcome than statin-naïve patients.

Methods

In a prospective observational cohort study of 476 acute ischemic stroke patients treated with tPA we investigated the relationship between prior statin use and functional outcome at 3 months, the occurrence of symptomatic intracerebral hemorrhage (SICH) and early in-hospital mortality. Ischemic stroke subtypes were defined according to the TOAST classification. Favorable outcome was defined as a modified Rankin Scale score ^ 2.

Results

Of the 476 patients included, 98 (20.6%) used a statin at stroke presentation. In the entire cohort, 45.6% of patients had a favorable outcome with no difference between patients with or without statin therapy (45.9 vs. 45.5%, p = 0.94). In the multivariable analysis, statin use was not associated with favorable outcome (OR = 1.1, 95% CI = 0.6–1.9, p = 0.87). In none of the different stroke subtype groups was statin use associated with favorable outcome. Finally, statin use was not an independent risk factor of SICH or of early in-hospital mortality.

Conclusion

Prior statin therapy in patients with acute ischemic stroke treated with tPA is not associated with a more favorable outcome, and this is independent of stroke subtype.

Introduction

In the last decade, statins were widely used in the primary and secondary prevention of cardiovascular disease. The protective effect of statins on atherogenesis is thought to be caused by lowering the low-density lipoprotein (LDL) cholesterol levels. The Stroke Prevention by Aggressive Reduction in Cholesterol Levels Study demonstrated beneficial effects on stroke prevention in patients with cerebrovascular disease.[1] Statins may also have vasoprotective and neuroprotective effects in acute ischemic stroke.[2-7] This non-cholesterol-dependent or so-called pleiotropic effect of statins may be caused by anti-inflammatory effects or effects on the nitric oxide system resulting in modification of endothelial function.[8-10] The possible neuroprotective effect is probably more evident in non-cardio-embolic stroke, e.g. atherothrombotic and lacunar infarcts.[1,11,12] However, statins and lower LDL cholesterol levels have also been associated with an increased risk of symptomatic intracerebral hemorrhage (SICH) in patients treated with tissue plasminogen activator (tPA).[13] A small retrospective study found an association between prior statin use and a better functional outcome in patients treated with tPA in acute ischemic stroke in comparison to patients without statins, but this result awaits confirmation.[2] The primary aim of this study was to investigate the effect of prior statin use on functional outcome in acute ischemic stroke patients treated with tPA. We also studied this possible effect in stroke subtypes. Finally, we assessed the effect of statin use on the occurrence of SICH and early in-hospital mortality.

Methods

Data of consecutive ischemic stroke patients receiving tPA were collected in a single-centre database from April 2002 until March 2009. Patients were treated with tPA within a time window of 4.5 h after onset of symptoms according to a protocol which has been described previously.[14] Prior medication use and doses were recorded for antiplatelet and lipid-lowering drugs. Drug compliance was not assessed. Based on clinical and neuro-imaging findings, stroke subtypes were classified according to the TOAST classification.[15] Infarction of undetermined origin and infarction due to other causes were taken together for analysis.

Outcome

Functional outcome at 3 months after onset of symptoms was measured using the modified Rankin Scale (mRS). The mRS was scored by a trained stroke nurse. Favorable outcome was defined as a mRS score of 0–2, corresponding to independence with regard to activities of daily living. The occurrence of SICH was defined according to the SITS-MOST criteria.[16] Early in-hospital mortality was defined as mortality within 7 days of hospital stay.

Statistical analysis

Differences in baseline characteristics between patients with and without treatment with statins were assessed with the Mann-Whitney U test for continuous and ordinal variables without a normal distribution and Pearson's X^2 test and Fisher's exact test for dichotomous variables. Variables within the univariate baseline characteristics for statin use or non-statin use with a p value < 0.20 were selected as covariates for a multivariable logistic regression analysis. Because of the aim of the study, statin use was also entered in the multivariable analysis, regardless of the univariate analysis. Since age, NIHSS score at presentation and glucose concentration at presentation are known variables related to outcome, these variables were also entered in the multivariable analysis regardless of the univariate analysis. In the final logistic regression model with favorable outcome as a dependent variable, adjusted variables with a p value > 0.20 were removed. The analysis was furthermore stratified into subgroups based on infarct subtype.

All statistical analyses were performed using SPSS version 16.0. Statistical significance was taken to be at a two-tailed level of < 0.05. In the multivariable analysis, a binary logistic regression was used with adjustment for possible confounders to calculate odds ratios with a 95% confidence interval.

Results

Patient sample

A total of 478 patients were studied. In this group, data on functional outcome were missing in 2 patients and these patients were excluded. Of the 476 included patients, 98 (20.6%) patients were using statins at stroke presentation. Baseline characteristics of statin users and patients not on a statin are shown in ***Table 1***. Statin users had significantly more vascular risk factors compared to non-statin users. Patients on statins also used significantly more antiplatelets at stroke onset. Stroke subtype was not significantly different between the two groups. Statin users had significantly lower plasma cholesterol and LDL levels.

Table 1: Baseline characteristics entire cohort (n=476)

	Statin user	Non statin user	
Characteristic	**N=98**	**N=378**	**p**
Age (mean ± SD), years	71 ± 11	67 ± 15	0.15[a]
Male	53 (54)	204 (54)	0.984
NIHSS score (mean ± SD)[b]	12 ± 6	13 ± 6	0.502[a]
Time until treatment (mean ± SD), min[b]	153 ± 50	165 ± 50	0.028[a]
Stroke subtype			
Atherotrombotic	34 (35)	99 (26)	0.095
Small vessel disease	10 (10)	33 (9)	0.650
Cardio-embolic	20 (20)	92 (24)	0.414
Other cause	1 (1)	18 (5)	0.143[c]
Unknown cause	33 (34)	136 (36)	0.671
Early ischaemic changes on brain CT scan	31 (31)	159 (42)	0.054
Hypodensity area > 33% on brain CT scan	9 (9)	20 (5)	0.151
Vascular risk factors			
Hypertension	59 (60)	161 (42)	0.002
Diabetes	23 (23)	38 (10)	0.001
Hyperlipidemia	64 (65)	137 (36)	<0.001
Smoking	21 (21)	118 (31)	0.058
Previous stroke/TIA	36 (36)	52 (13)	<0.001
Prior use of antiplatelets	59 (60)	75 (19)	<0.001
Systolic blood pressure (mean ± SD), mm Hg[b]	153 ± 26	154 ± 27	0.615[a]
Diastolic blood pressure (mean ± SD), mm Hg[b]	80 ± 14	83 ± 17	0.099[a]
Mean glucose (SD), mmol/l	6,7 ± 2,0	6,5 ± 1.9	0.222[a]
Total cholesterol (mean ± SD), mmol/L[d]	4.1 ± 1.1	5.1 ± 1.1	<0.001[a]
LDL (mean ± SD), mmol/l[e]	2.3 ± 0.9	3.4 ± 1.7	<0.001[a]

Values are numbers (%), unless otherwise indicated. P-values calculated with Pearson's X^2 test.
[a] Mann-Whitney U test. [b] 1missing value for both groups. [c] Fisher's exact test. [d] 12 missing for statin users, 46 for non-statin users. [e] 15 missing for statin users, 62 for non-statin users. SD: standard deviation.

Functional outcome entire cohort

After 3 months, 217 patients (45.6%) had a favorable outcome (mRS score ≤2). The number of patients with a favorable outcome was not significantly different between patients on statins and non-statin users (45.9 vs. 45.5%, p = 0.94). In the final multivariable analysis, with adjustment for the confounders age, NIHSS score, hypodensity area of more than 33% on brain CT scan, history of hypertension, diabetes mellitus, diastolic blood pressure and prior use of antiplatelet drugs, statin use was not associated with a favorable outcome (OR = 1.1, 95% CI = 0.6–2.0, p = 0.73; ***Table 2***).

Table 2: Final logistic regression model with predictors of favorable outcome

	Entire cohort (n=476)		
Variables	**OR**	**95% CI**	**p**
Statin therapy	1.11	0.61 – 2.01	0.73
Age, year	0.95	0.93 - 0.96	<0.001
NIHSS score	0.82	0.78 - 0.86	<0.001
Hypodensity area > 1/3 on brain CT scan	0.23	0.07 – 0.71	0.01
Hypertension	0.65	0.41 – 1.05	0.08
Diabetes Mellitus	0.53	0.26 – 1.05	0.07
Prior antiplatelet therapy	1.65	0.96 – 2.85	0.07
Diastolic blood pressure, mm Hg	1.01	1.00 – 1.03	0.08

Stroke subtype

The distribution of stroke subtype according to the TOAST classification and statin use is shown in ***Table 1***. Favorable outcome was not significantly different between statin users and patients not on statins in all subgroups according to the TOAST classification. Especially, for patients with atherothrombotic stroke a significant difference in functional outcome between statin use and non-use was not found (56 vs. 41%, p = 0.144; ***Table 3***). After correction for possible confounders, prior statin use was still not associated with favorable outcome.

Table 3: Favorable outcome for stroke subtype

Stroke etiology	Statin use	Non statin use	p
Atherotrombotic	19/34 (56)	41/99 (41)	0.14
Small vessel disease/Lacunar stroke	10/10 (100)	27/33 (82)	0.31[a]
Cardio-embolic	6/20 (30)	39/92 (42)	0.31
Other cause	10/34 (29)	65/154 (42)	0.19

Classification of etiology according to TOAST-classification. Undetermined cause and unusual cause are taken together under 'other cause'. Values are n/N (%), n = number of total N for subgroup. P-values calculated with Pearson's X^2 test.
[a] Fisher's exact test.

Early in-hospital mortality and occurrence of SICH

There was no difference in early in-hospital mortality between patients with statin use and without statin use (9 vs. 9%, p = 0.82). In the entire cohort, SICH was more often seen in patients using statins compared to patients not using statins (10 vs. 5%, p = 0.041). However, in the multivariable analysis, statin use was not independently associated with the occurrence of SICH (OR = 1.6, 95% CI = 0.57–4.37, p =0.38). Higher serum glucose levels, antiplatelet therapy, higher NIHSS score at presentation and a hypodensity area of more than 33% on brain CT scan were independent predictors of SICH.

Discussion

This study could not demonstrate that patients with prior statin use and treated with tPA for acute ischemic stroke had a better functional outcome at 3 months in the entire cohort and in the different subgroups based on stroke subtype. Moreover, statin use did not reduce early in-hospital mortality or increase the occurrence of SICH. Our study could not confirm the results of a previous similar observational study investigating this topic.[2] In this study, 145 patients treated with tPA were analyzed and prior statin use was an independent factor for good functional outcome after 3 months with an odds ratio of 5.3 (95% CI = 1.5–18.7, p = 0.027). This study used the same definition of favorable outcome (mRS score ≤2). An explanation for the discrepancies between the previous and our study may be differences in baseline characteristics of the two cohorts. Stroke severity was significantly higher in the study of Alvarez-Sabin et al.[2], while in our study there were more patients with a history of transient ischemic attack/stroke and less patients with a potential cardio-embolic stroke. These imbalances may have led to the difference in results. Another difference is the sample size of the two studies. The sample size of the study by Alvarez-Sabin et al.[2] was relative small and 95% confidence intervals were wide. Our sample size was about three times larger with substantially smaller confidence intervals and therefore the results of our study may be more reliable.

Early in-hospital mortality in our study did not differ between the two groups, which indicates that there is no short-term beneficial effect of statins, as suggested in a study by Marti-Fabregas et al..[5] In the univariate analysis, statin use was associated with increased risk of SICH, but in multivariable analysis this lost significance. Therefore, statin use is not an independent risk factor for SICH, which was also observed in other studies.[13,17] A previous study using astrocytes from rats suggested that statins may reduce the risk of SICH by down-regulating matrix metalloproteinase-9 activity. Up-regulation of matrix metalloproteinase-9 is stimulated by tPA and mediates a breakdown of the blood-brain barrier causing hemorrhagic transformation.[18] A possible protective effect of statins on tPA-related hemorrhagic complications needs further investigation in clinical studies.

Our study has limitations. First, this is an observational study with a relatively small number of patients, especially with regard to the subgroups and this limits statistical analysis. Second, in comparison to other studies, a large amount of patients in our study were classified as having 'unknown' stroke etiology according to the TOAST classification. Our hospital has a regional function in the treatment of stroke patients with tPA. After tPA treatment, some patients were transferred to hospitals in the region. Therefore, we did not have access to all the diagnostic findings of these patients, except for the functional outcome at 3 months. Third, all statins were considered together, although they may not share all effects. [19] We did not perform any sub-analysis based on individual drugs. However, the results mainly reflect the effect of simvastatin and atorvastatin used by 45.9 and 42.9% of the patients, respectively. Furthermore, we did not assess drug compliance. However, cholesterol and LDL plasma levels were significantly lower in statin users than in non-users, suggesting that there was sufficient compliance in the statin user group. Although cholesterol and LDL levels were low, in experimental studies finding a neuroprotective effect of statins, high dosages of statins were needed to achieve neuroprotection with consequently very low LDL plasma levels, which might not be the case in our study. [20] There was no withdrawal of statins in the acute phase of stroke or after hospitalization, which could explain the results. [21]

In conclusion, our study does not support the hypothesis that prior statin therapy in patients with acute ischemic stroke treated with tPA is associated with a more favorable outcome, independent of stroke subtype. Therefore, we question whether statins have a neuroprotective effect in acute ischemic stroke.

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CHAPTER 4

Lack of association between serum uric acid levels and outcome in acute ischemic stroke

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Abstract

Background

The prognostic value of serum uric acid (UA) in acute ischemic stroke is controversial. The aim of this study is to further analyze the relation between UA and outcome after acute ischemic stroke.

Methods

We analyzed UA levels in blood samples collected within 6 hours of stroke onset from patients included in the placebo arm of the US and Canadian Lubeluzole Ischemic Stroke Study (LUB-INT-9). We compared mean serum UA levels in patients with and without early neurological improvement (≥ 4 versus < 4 points improvement on NIHSS after 5 days) and in patients with good functional and poor functional outcome (mRS 0-2 versus mRS 3-6). Multivariable logistic regression analyses were performed to adjust for possible confounders.

Results

UA levels of 226 patients were available for analysis. Mean serum UA levels were not significantly higher in patients with than without early neurological improvement (0.33 mmol/L versus 0.30 mmol/L, p = 0.070). The difference between patients with good and patients with poor functional outcome was borderline statistically significant (0.34 mmol/L versus 0.31 mmol/L, p = 0.050). After adjustment for confounders, higher serum UA levels were neither associated with early neurological improvement OR (1.30, 95% CI 0.98-1.73, p = 0.069), nor with a good functional outcome (OR 1.09, 95% CI 0.72-1.65, p = 0.690).

Conclusion

We found no association between admission serum UA levels and both short- and long-term outcome in acute ischemic stroke.

Introduction

Uric acid (UA) is a predictor for developing cardiovascular disease and stroke by mechanisms involved in development and progression of atherosclerosis, but it remains controversial whether this is an independent effect of uric acid by itself or an epiphenomenon. It is also thought that the effect is linked to other factors that predispose cardiovascular disease and stroke, like hypertension and diabetes, which are associated with uric acid. [1,2] On the other hand, UA is a powerful anti-oxidant contributing to approximately two third of free radical scavenging in plasma. [3,4]

Oxidative stress in the acute phase of ischemic stroke increases brain injury. [3,5] Oxidative stress reflects an imbalance between potentially harmful oxidants and protective antioxidants and predisposes to local tissue damage, partly through excess free radical activity. In this perspective UA could act as a free radical scavenger reducing oxidative stress in patients with acute ischemic stroke and protect cells in the ischemic penumbra. In addition, an interventional study showed that administration of uric acid in healthy volunteers with low baseline serum concentrations increased the antioxidant capacity. [6] Therefore, UA might be neuroprotective in acute ischemic stroke.

A neuroprotective effect of UA has been shown in animal studies of focal brain ischemia. [7,8] However the prognostic value of serum uric acid in acute ischemic stroke in human remains controversial. Chamorro et al. found an inverse correlation between serum UA concentrations and early neurological improvement and final infarct size. [9] Two recent studies found that higher serum UA levels were independently associated with good functional outcome and smaller infarct volumes in patients treated with thrombolysis. [10,11] There are also reports of a detrimental effect of serum UA concentration on functional outcome. [12-16] For example, a large study by Weir et al. showed that increase in admission serum UA levels independently predicted worse outcome. [16] Although the exact mechanisms of this detrimental effect are still unknown, increased UA concentration may be associated with platelet adhesiveness with influence on thrombus formation or could become a pro-oxidant under certain circumstances. [5,17]

The aim of this study is to further analyze the relation between serum UA levels and outcome after acute ischemic stroke. We assessed the relation between UA and early neurological improvement at day 5 after stroke and with functional outcome at 3 months after stroke.

Methods

Data were obtained from the United States and Canadian Lubeluzole in Ischemic Stroke Study (LUB-INT-9), a multicenter, randomized, placebo controlled-trial investigating the efficacy and safety of lubeluzole in acute ischemic stroke patients. [18] Only data from the

placebo treated patients were used. Blood samples were collected within 6 hours of stroke onset and serum UA concentration was measured in mmol/L. Neurological status was examined by using the National Institute of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS). Classification of ischemic stroke subtypes was based on the TOAST-criteria and included small vessel disease, large vessel disease and cardio-embolic strokes [19]

Outcome

Early neurological improvement and functional outcome at 3 months after acute ischemic stroke were used as outcome measures. We defined early neurological improvement as ≥ 4 points improvement in score of NIHSS after 5 days of the initial event. Functional outcome was defined according to the modified Rankin Scale (mRS) and dichotomized into favorable outcome (mRS 0-2), corresponding to independence with regard to activities of daily living, and unfavorable outcome (mRS 3-6). To compare with previous studies, the relation with excellent outcome (mRS 0-1) was also assessed.

Statistics

Baseline characteristics for the entire cohort were calculated. Mann Whitney U-test was used for continuous and ordinal variables without a normal distribution. Pearson's Chi Square test was used for dichotomous variables. Variables within univariate analysis for serum UA levels < and ≥ 0.32 mmol/L (mean) with a p-value < 0.20 were selected as covariates for the multivariable logistic regression analysis. Regardless of the difference in baseline characteristics, age, NIHSS score and serum glucose level at presentation were entered in the multivariable analyses.

These variables were considered to be possible confounders, since these variables are related to outcome in patients with acute ischemic stroke. (20) Furthermore because of the association between UA and cardiovascular risk factors, a history of hypertension, coronary artery disease and previous stroke were also entered as covariates in the multivariable analysis. [1] In the multivariable analysis with early neurological improvement or favorable outcome as dependent factor, variables with a p value > 0.20 were removed from the model.

All statistical analyses were performed using PASW Statistics version 18.0. Statistical significance was taken to be at two tailed level < 0.05. A binary logistic regression model was used for multivariable analysis, with adjustment for possible confounders, to calculate odds ratios with 95% confidence interval.

Results

Patient sample

Out of the 353 patients in the placebo group of the LUB-INT-9 trial, only 233 patients were included because of availability of serum UA levels in these patients.

Another 7 patients were excluded because of lack of data on functional outcome at 3 months. Therefore, baseline characteristics were calculated for 226 patients (64% of total population) and are presented in ***Table 1***. Mean age of the cohort was 71 years (SD 12) with a slightly higher number of men than women (54 vs 46%). Mean serum UA level was 0.32 mmol/L (SD 0.10). Median NIHSS at presentation was 15 (IQR 11-19) and median NIHSS at day 5 was 12 (IQR 6-18). Almost two third of patients were diagnosed with hypertension (62%). 75 patients (33%) had a cardio-embolic stroke and 57 patients (25%) were diagnosed with large vessel disease stroke.

Eighty-seven patients (38%) showed early neurological improvement (improvement of NIHSS ≥ 4 points at day 5); 47 patients (21%) had a favorable outcome and 42 patients (19%) had an excellent outcome at 3 months (***Table 2***). A number of 120 patients were excluded because of no availability of serum UA levels. Of these 120 patients 7 had a hemorrhagic stroke and 5 had no data on functional outcome at 3 months available. The other 108 patients did not differ significantly in baseline characteristics from the included patients.

Table 1: Baseline characteristics

	Included population	Excluded population	
Characteristic	**(n = 226)**	**(n = 108)**	**p**
Male (%)	123 (54%)	61 (57%)	0.72
Mean age (SD), year	71 (12)	69 (13)	0.20[b]
Median NIHSS at presentation (IQR)	15 (11-19)	13 (10-19)	0.10[b]
Mean serum glucose level (SD), mmol/L[a]	8.0 (3.4)	7.9 (3.7)	0.33[b]
Mean diastolic RR (SD), mm Hg	84 (16)	82 (17)	0.36[b]
Mean serum uric acid level (SD), mmol/L	0.32 (0.10)	n.a.	
Vascular risk factors			
Hypertension (%)	140 (62%)	66 (61%)	0.88
Diabetes (%)	46 (20%)	28 (26%)	0.25
Atrial fibrillation (%)	58 (26%)	27 (25%)	0.90
Coronary artery disease (%)	74 (33%)	30 (28%)	0.36
Smoking (%)	57 (25%)	23 (21%)	0.43
Stroke subtype			
Cardio-embolic (%)	75 (33%)	36 (33%)	0.98
Large vessel disease (%)	57 (25%)	21 (20%)	0.24
Small vessel disease (%)	32 (14%)	12 (11%)	0.44
Unknown (%)	62 (27%)	39 (36%)	0.25

Values are number unless otherwise indicated. SD: standard deviation. IQR: inter-quartile ratio.
n.a.: Not available. [a] 17 missing. P-values calculated with Pearson's X^2 test. [b] Mann-Whitney U test

Table 2: Outcome variables

	Total population
Outcome	**(n = 226)**
Early neurological improvement (%)	87 (38%)[a]
Median NIHSS after 5 days (IQR)	12 (6-18)[a]
MRS 0-2 at 3 months	47 (21%)
MRS 0-1 at 3 months	42 (19%)

Values are number unless otherwise indicated.
IQR: inter-quartile ratio. [a] 13 missing,

Early neurological improvement

Mean serum UA concentration was not significantly higher in patients with than without early neurological improvement. (0.33 mmol/L versus 0.30 mmol/L, p = 0.070; ***Figure 1***).

In the univariate analysis, serum UA level was significantly associated with early neurological improvement (OR 1.35, 95% CI 1.02-1.79, p = 0.036). However, significance was lost after adjustment for confounders (OR 1.30, 95% CI 0.98-1.73, p = 0.069; ***Table 3***). In the final logistic regression model, male gender was associated with early neurological improvement (OR 1.77, 95% CI 1.00-3.12, p = 0.048).

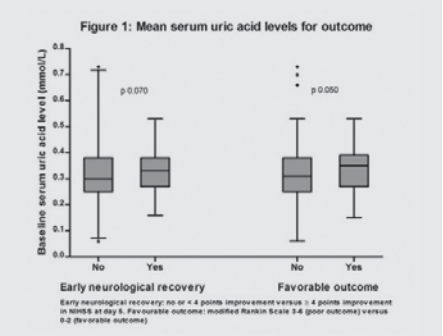

Table 3: Multivariable analysis: association of UA and early neurological improvement

	Early neurological improvement		
Variables	**OR**	**95% CI**	**p**
Serum uric acid level, mmol/L (per 0.1 mmol/L)	1.30	0.98 – 1.73	0.069
Gender, male	1.78	1.01 – 3.13	0.046

OR: odds ratio. CI: confidence interval. Hosmer-Lemeshow p = 0.244. Variables entered in the model: age, baseline NIHSS, serum glucose level, gender, history of coronary artery disease, history of previous stroke and large vessel disease as stroke etiology.

Functional outcome

A near significant difference was found in serum UA level between patients with favorable and patients with poor functional outcome (0.34 mmol/L versus 0.31 mmol/L, p = 0.050) (***Figure 1***). Regarding excellent functional outcome, serum UA level was significantly higher in patients with than in patients without excellent functional outcome (0.33 mmol/L versus 0.31 mmol/L, p = 0.032).

After adjustment for confounders serum UA level was no longer associated with a favorable outcome (OR 1.16, 95% CI 0.86-1.56, p = 0.356) or excellent outcome (OR 1.09, 95% CI 0.72-1.65, p = 0.690). In the final multivariate analysis lower age, lower baseline NIHSS, lower serum glucose levels, no previous history of diabetes and no large vessel stroke were associated with a favorable or excellent outcome (***Table 4***).

Table 4: Multivariable analysis: association of UA and favorable outcome

	Favorable outcome			Excellent outcome		
Variables	**OR**	**95% CI**	**p**	**OR**	**95% CI**	**p**
Serum uric acid level, mmol/L (per 0.1 mmol/L)	1.09	0.72 – 1.65	0.690	1.14	0.74 – 1.78	0.552
Age, year	0.95	0.92 – 0.98	0.002	0.98	0.92 – 0.98	0.002
NIHSS score	0.84	0.77 – 0.91	<0.001	0.78	0.77 – 0.91	<0.001
Serum glucose level	0.83	0.68 – 1.00	0.049	0.86	0.69 – 1.01	0.070
History of diabetes	0.20	0.04 – 0.97	0.046	0.20	0.04 – 0.94	0.042
Stroke etiology: large vessel disease	0.27	0.08 – 0.91	0.035	-	-	-

OR: odds ratio. CI: confidence interval. NIHSS: NIH stroke scale. Hosmer-Lemeshow p = 0.582/0.370. Variables entered in the model: age, baseline NIHSS, serum glucose level, gender, history of coronary artery disease, history of previous stroke and large vessel disease as stroke etiology.

Discussion

We found no association between serum UA levels and early neurological improvement at day 5 or favorable functional outcome at 3 months after the initial ischemic stroke.

In the literature, different findings for and against a neuroprotective effect of UA were found as is summarized in ***Table 5***. A study by Chamorro et al. found a 12% increase of the odds of good outcome at hospital discharge (mean 11 days after admittance) for each milligram per deciliter increase of serum UA.[9] In a recent study by Amaro et al. in patients with ischemic stroke also treated with reperfusion therapies, increased serum UA levels were independently associated with a mRS score of 0-1 at 3 months after the initial event.[10] In contrast, another study by Dawson et al. could not find an independent association of UA levels with functional outcome at 3 months.[12] Karagiannis et al. reported that elevated levels of UA are independently associated with early death after ischemic stroke (median 4 days after stroke, IQR 2-7).[13] Newman et al. found that elevated UA concentration is

Table 5: Summary of available literature

Characteristics				Uric Acid			Outcome
	Stroke-type	tPA	Patient number	Sample time UA	Mean serum UA (mmol/L)	Outcome measure	Effect
Chamorro et al	Ischemic	No	881	18.2 h ±15.5	0.31	Mathew score>75	Association between higher UA and good outcome
Amaro et al	Ischemic	Yes	317	17-42 h	0.33	mRS 0-1	Association between higher UA and good outcome
Dawson et al	Ischemic	No	852	<6 h	0.35	mRS 0-2	No association
Karagiannis et al	Ischemic/ Hemorrhagic	No	435	-	0.33	Mortality	Association between higher UA and mortality
Newman et al	Ischemic/ Hemorrhagic	No	140	<24 h after admittance	0.34	Event: New stroke, MI or vascular death	Association between higher UA and more vascular events
Seet et al	Ischemic	No	503	-	0.35	MRS 0-2	Association between low and high UA and poor outcome
Weir et al	Ischemic/ Hemorrhagic	No	3731	<48 h	0.31	Alive and at home	Association between higher UA and poor outcome
Logallo et al	Ischemic	Yes No	186 261 689	1.5 h tPA 1.9 h non-tPA 18.7h late	0.36 0.35 0.35	MRS 0-3 and NIHHS day 0-7	In tPA-treated patients association between higher UA and positive outcome, no association in non-treated patients
Miedema et al	Ischemic	No	226	<6 h	0.32	MRS 0-2 and NIHSS ≥4 improved at day 5	No association

tPA: tissue plasminogen antigen. UA: Uric acid. mRS: Modified Rankin Scale. MI: Myocardial infarct. NIHSS: National Institute of Health Stroke Scale.

independently associated with increased risk of future vascular events in patients with diabetes mellitus type 2.[14] Seet et al. found that both patients with relatively decreased and with elevated serum UA levels had poor functional outcome compared to patients with median serum UA levels one year after the initial event.[15] Weir et al showed that increased serum UA levels independently predicted worse outcome 3 months after stroke (alive in care or death).[16] Logallo et al found a beneficial effect of UA in patients treated with tPA, but they could not extend this to non-treated patients.[11] The difference in findings as described above is additionally complicated by the use of different outcome measures and also study size and population, consequently hamper comparing of the various results. Also timing of UA sampling is different between all studies, which could also be of influence on the results.

Looking at our study in comparison with the previous ones, we found also no neuroprotective effect of serum UA levels, neither in the early phase after the ischemic stroke nor in the late phase. A possible explanation for the protective effect of UA in the acute phase of ischemic stroke is thought to be explained by reducing oxidative stress in the ischemic penumbra and thereby reducing the definite infarct volume as has been found in earlier both animal and human studies.[8,10,21] We can not confirm this hypothesis with the results of our study. An explanation could be that the neuroprotective effect of serum UA is too small to result in significant difference in the larger outcome scales. However, even when we look at improvement of NIHSS at day 5 after the initial stroke, a more specific marker than the larger mRS score, no neuroprotective effect of serum UA was found. As mentioned, we also could not extend this effect to the outcome at 3 months. This is in contrast with the study by Amaro et al.[10] Also when we adjust our analysis to the same outcome measure (excellent outcome, mRS 0-1 at 3 months) serum UA level was not associated with excellent outcome (***Table 3***). An explanation for the difference in results for outcome at 3 months could be the more severe strokes in our cohort showed by higher baseline NIHSS and fewer patients with favorable outcome. In our cohort the median baseline NIHSS was 15 (IQR 11-19) and 47 patients (21%) with a mRS of 0-2 versus a median baseline NIHSS of 11 (IQR 6-18) and 153 patients (48%) with a mRS of 0-2 in their cohort. Amaro et al. used as outcome measure mRS of 0-1. In addition, the study by Amaro et al. was performed using patients also treated with alteplase. Animal studies have demonstrated a synergic neuroprotective effect in rats treated with alteplase and administration of UA.[7] Thus their finding of an association of serum UA levels and excellent outcome could also be influenced by the synergic effect of alteplase. This could also explain why Logallo et al found a neuroprotective effect in the alteplase treated patients and not in the larger cohort of non-treated patients.[21] At the moment, the URICO-ICTUS study is trying to evaluate the clinical efficacy of UA administration in acute ischemic stroke patients treated with alteplase within the 4,5 hour time window in a phase 3 randomized double-blind controlled trial.[22]

Another hypothesis could be that the neuroprotective effect in the acute phase of the initial stroke is counteracted by the expression of also more cardiovascular disease in these patients with higher serum UA levels, with also impact on the outcome scores.

Our study was limited by the lack of serial UA level measurements. We determined the baseline UA levels, therefore we could not assess whether UA levels decreased or increased in patients with early neurological improvement or good functional outcome. Furthermore, we could not adjust for serum creatinine levels or renal insufficiency, which both could be possible confounding factors by their association with elevated UA levels and influence on outcome. In addition, we did not adjust for the use of diuretics, which also could influence serum UA levels. No data of infarct size after the initial stroke were available and thus we could not compare infarct size with serum UA levels. It would be interesting to further investigate this subject for instance with size of perfusion/diffusion weighted MRI-lesions in relation to serum UA levels. Finally, our sample size was relative small compared to previous studies on this subject. Although our study could not identify an association between serum UA levels and short/long term outcome, a small positive effect could be missed due to the small sample size.

In conclusion, serum UA levels were not associated with early neurological improvement at day 5 and favorable functional outcome at 3 months after the initial ischemic stroke. Our results do not support the neuroprotective hypothesis of serum UA.

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CHAPTER 5

Admission hyperglycemia and outcome after intravenous thrombolysis: is there a difference among the ischemic stroke-subtypes?

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Submitted

Abstract

Background

The prognostic influence of hyperglycemia in acute ischemic stroke has been well established. While in cortical stroke there is a strong association between hyperglycemia and poor outcome, this relation is less clear in lacunar stroke. It is unclear if this discrepancy is present among patients treated with intravenous tissue plasminogen activator (tPA).

Methods

In two prospectively collected cohorts of patients treated with intravenous tPA for acute ischemic stroke, we investigated the effect of hyperglycemia (serum glucose level >8 mmol/L) on functional outcome and occurrence of symptomatic intracranial hemorrhage (SICH) in lacunar and non-lacunar stroke. Poor functional outcome was defined as modified Rankin Scale score 3-6 at 3 months.

Results

Of the 857 patients included, 161 patients (19%) had lacunar stroke and 696 (81%) non-lacunar stroke. The prevalence of hyperglycemia did not differ between stroke subtypes (21% versus 19%, p = 0.58). In multivariate analysis hyperglycemia was associated with poor outcome in lacunar strokes (OR, 2.61; 95% CI, 0.98-6.02; p = 0.056) and significantly associated with poor outcome in non-lacunar strokes (OR, 1.59; 95% CI, 1.03-2.57; p = 0.033). The incidence of SICH was not different between hyperglycemic and normoglycemic patients in either stroke subtype.

Conclusion

In acute ischemic stroke patients treated with intravenous tPA, hyperglycemia is an independent predictor of poor functional outcome in both patients with non-lacunar stroke and lacunar stroke.

Introduction

Admission hyperglycemia is common in patients with acute ischemic stroke and occurs among all stroke subtypes. [1] Hyperglycemia is independently associated with poor functional outcome in patients with acute ischemic stroke. In patients treated with intravenous recombinant tissue plasminogen antigen (tPA), hyperglycemia was associated with lower recanalization rates and was a strong predictor of symptomatic intracerebral hemorrhage (SICH), regardless of a history of diabetes. [2-9] In addition, admission hyperglycemia was independently associated with mortality. [2,6,9]

While in cortical stroke there is a strong association between hyperglycemia and poor outcome, this relation is less clear in lacunar stroke. Two previous studies even report a favorable effect of moderate hyperglycemia in lacunar stroke. [4,8] It is unclear if this discrepancy is present among patients treated with intravenous tissue plasminogen activator (tPA). We aimed to investigate the effect of admission hyperglycemia on functional outcome and the risk of symptomatic intracranial hemorrhage (SICH) in patients with lacunar and non-lacunar strokes treated with intravenous tPA.

Methods

Data were obtained from two stroke centers in the Netherlands and Belgium. Both centers have an ongoing prospective registry of consecutive patients with acute ischemic stroke treated with intravenous tPA treatment. The registry was started at the University Medical Centre of Groningen (UMCG) in April 2002. All patients registered between April 2002 and October 2012 were included in this study. In the Universitair Ziekenhuis Brussel (UZB), the registry was started in March 2009. In both hospitals tPA treatment was performed within a time window of 4.5 hours after onset of symptoms according to a protocol which has been described earlier. [10]

Stroke severity before administration of tPA was assessed according to the National Institute of Health Stroke Scale (NIHSS). Stroke subtype was classified as lacunar stroke (LACI = lacunar infarct) or non-lacunar stroke (PACI = partial anterior circulation infarct and TACI = total anterior circulation infarct) according to the Oxfordshire Community Stroke Project Classification. [11] Patients with posterior circulation infarction (POCI = posterior circulation infarct) were excluded.

Demographic and clinical information was recorded, including cardiovascular risk factors and serum glucose concentration. Hyperglycemia was defined as >8 mmol/L (≈ 144 mg/dl) in accordance with previous studies on this subject. [3,8,9]

Outcome

The modified Rankin Scale (mRS) was used to determine functional outcome at 3 months after stroke onset. Functional outcome was dichotomized into poor outcome meaning dependence or death (mRS 3-6) and favorable outcome (mRS 0-2), corresponding to independence with regard to activities of daily living.

The occurrence of symptomatic intracranial hemorrhage (SICH) was defined according to the Safe Implementation of Treatment in Stroke (SITS) criteria defined as a neurological deterioration (NIHSS ≥ 4 points) within 36 hours following tPA treatment with a parenchymal hematoma on brain CT-scan compatible with clinical symptoms. [12]

Statistics

Baseline characteristics for patients stratified by stroke subtype were compared. Mann Whitney U-test was used for continuous and ordinal variables without a normal distribution. Pearson's Chi Square test and Fisher's exact test were used for dichotomous variables. A binary logistic regression model is used for multivariable analysis, with adjustment for possible confounders, to calculate odds ratios with 95% confidence interval. A history of diabetes is entered in the multivariable analysis and an interaction between hyperglycemia and a history of diabetes was tested. All statistical analyses were performed using IBM SPSS Statistics 20. Statistical significance is taken to be at two tailed level < 0.05.

Results

Patient sample

Nine hundred and ninety patients were treated with tPA during the study period. From this group 876 patients (88%) were treated in the UMCG and 114 patients (12%) in the UZB. Three month mRS scores of 53 patients were missing (5% of total population). Furthermore, 9 patients were excluded because of missing data on stroke subtype and 71 patients (7%) with POCI were excluded.

We included a total of 857 patients consisting of 161 patients (19%) with lacunar stroke and 696 patients (81%) with non-lacunar stroke. In the non-lacunar stroke group, 449 patients (65%) had PACI and 247 patients (35%) TACI.

The baseline characteristics are presented in ***Table 1***. No difference in baseline serum glucose levels were found between the two stroke subtypes (6.8 mmol/L versus 6.7 mmol/L, p = 0.58) and the occurrence of admission hyperglycemia, did not differ between the subtypes (21% versus 19%, p = 0.58).

Table 1: Baseline characteristics of the study cohort of 857 patients with acute ischemic stroke receiving intravenous tPA treatment

	Lacunar stroke	Non-lacunar stroke	
Characteristic	**N=161**	**N=696**	**p-value**
Male (%)	88 (55%)	363 (52%)	0.57
Mean age (SD), years	66 (15)	70 (14)	0.001[e]
Median NIHSS at presentation (IQR)[a]	6 (5-9)	13 (8-17)	<0.001[e]
Mean systolic RR (SD), mmHg[b]	161 (24)	153 (29)	<0.001[e]
Mean diastolic RR (SD), mmHg[b]	85 (15)	81 (18)	0.001[e]
Mean serum glucose level (SD), mmol/L	6.8 (1.9)	6.7 (2.2)	0.58[e]
Hyperglycemia (>8mmol/L)	33 (21%)	129 (19%)	0.58
Vascular risk factors			
Hypertension (%)	77 (52%)	351 (50%)	0.55
Diabetes (%)	30 (19%)	103 (15%)	0.23
Hypercholesterolemia (%)	105 (65%)	389 (56%)	0.031
Atrial fibrillation (%)	15 (9%)	158 (23%)	<0.001
Smoking (%)[c]	58 (37%)	168 (25%)	0.003
History of previous stroke (%)[d]	35 (23%)	124 (20%)	0.42
Use of antiplatelet drugs (%)[d]	53 (35%)	217 (35%)	0.96
Outcome			
mRS 3-6 at 3 months	36 (22%)	390 (56%)	<0.001
SICH	3 (2%)	40 (6%)	0.042

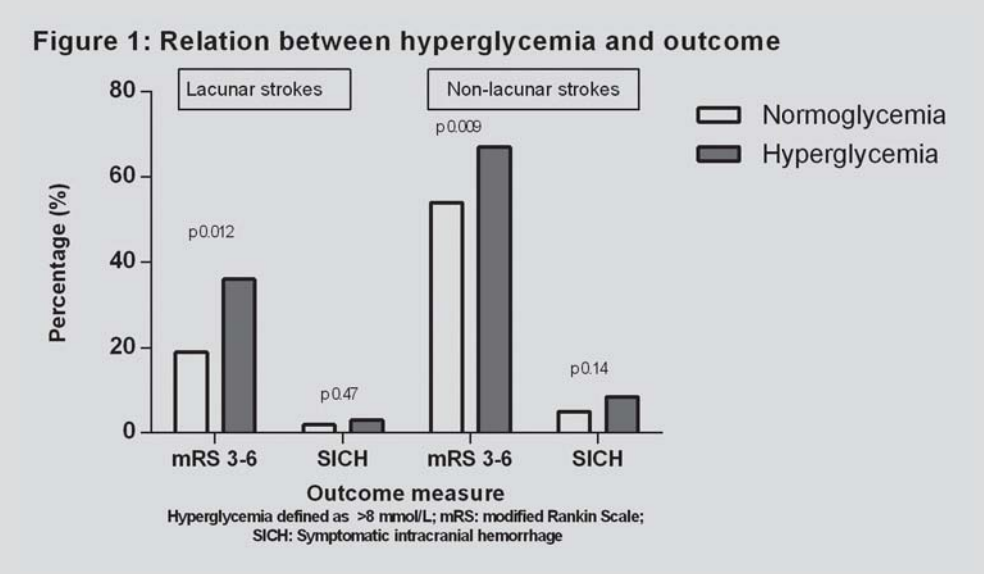
Values are number unless otherwise indicated. IQR, inter-quartile range, mRS, modified Rankin Scale; SD, standard deviation; SICH, symptomatic intracranial hemorrhage according to the SITS criteria. a 2 missing, b 102 missing, c 19 missing, d 92 missing. P values calculated with Pearson's χ^2-test, unless otherwise indicated. e Mann-Whitney U-test

Functional outcome

In total, 426 patients (50%) had a poor outcome (mRS 3-6). The occurrence of poor outcome was significantly different between patients with lacunar and non-lacunar strokes (22% versus 56%, p = <0.001).

Univariate analysis showed that patients with lacunar stroke and normoglycemia had less frequently poor outcome than those with hyperglycemia (19% vs. 36%, respectively; p = 0.030) (***Figure 1***). After adjustment for possible confounders in multivariate analysis, admission hyperglycemia was near to a significant association with poor functional outcome (OR, 2.61; 95% CI, 0.98-6.02; p = 0.056).

In non-lacunar stroke, univariate analysis also showed a significant difference between normoglycemic and hyperglycemic patients with regard to poor outcome (54% vs. 67%; p = 0.007) (Figure 1). The association between admission hyperglycemia and poor outcome was confirmed in multivariate analysis taking possible confounders into account (OR, 1.59; 95% CI, 1.03-2.57; p = 0.033). Older age and higher baseline NIHSS score were



associated with poor functional outcome in both stroke subtypes. Testing for the interaction term including hyperglycemia and diabetes did not change the results in the multivariate models.

Symptomatic intracranial hemorrhage

Forty-three patients (5%) suffered from SICH. The incidence of SICH was significantly lower in lacunar stroke than in non-lacunar stroke (1.9% vs. 5.7%; p = 0.042).

In lacunar stroke, univariate analysis revealed no difference in occurrence of SICH between normoglycemic and hyperglycemic patients (2% versus 3%, p = 0.50) (***Figure 1***). Because of the low occurrence of SICH in lacunar stroke patients, multivariate analysis could not be performed. Univariate analysis in non-lacunar stroke patients showed a non-significant higher incidence of SICH in patients with hyperglycemia compared to patients with normoglycemia (8% versus 5%, p = 0.28) (***Figure 1***).

Discussion

In both lacunar and non-lacunar stroke, admission hyperglycemia was associated with poor functional outcome after intravenous tPA treatment. In patients with non-lacunar stroke, hyperglycemia predicted poor functional outcome, independently of other predictors for unfavorable outcome. A similar effect was observed in patients with a lacunar stroke, though less pronounced. The incidence of SICH was not significantly different between hyperglycemic and normoglycemic patients in either stroke subtype.

The mechanisms by which hyperglycemia augments ischemic brain injury are not fully understood and several mechanisms are proposed: induction of acidosis by anaerobic glycolysis enhancing free radical production in the ischemic penumbra; increase of blood-brain barrier permeability; increase of coagulation processes and decreased fibrinolytic activity; and induction of vascular changes with a pro-vasoconstrictive, pro-thrombotic and pro-inflammatory effects which compromise the collateral circulation and enhance reperfusion injury. [13,14]
Our findings differ from two previous studies in patients not treated with thrombolytic therapy, showing a differential effect of hyperglycemia with regard to the ischemic stroke subtype. [4,8] It was suggested that the favorable functional outcome in patients with lacunar stroke and moderate hyperglycemia can be related to the absence of an ischemic penumbra in most lacunar strokes, as hyperglycemia-related mechanisms are thought to be harmful to the salvage of the penumbra. [5] A correlation was found between lactate levels in the cerebrospinal fluid and functional outcome in patients with non-lacunar stroke, but not in patients with lacunar stroke. [15] Increased lactate production in the hyperglycemic state may fuel astrocytes and thus facilitate axonal survival in the white matter in lacunar stroke. (8) We hypothesize that lacunar stroke patients receiving thrombolytic therapy are exposed to aggravated reperfusion injury, which can counterbalance the protective effects of hyperglycemia in these patients. Therefore, hyperglycemia may exacerbate reperfusion injury through enhanced oxidative stress and a stronger inflammatory response. [13] Moreover, exposure of reperfused tissue to tPA may amplify neuronal damage as neurotoxic effects of tPA have been shown in animal models. [16] Taken together, these mechanisms may lead to increased infarct size and poor functional outcome in lacunar stroke, especially in case of reperfusion after tPA.

Trials investigating the effects of glucose lowering in acute ischemic stroke show mostly disappointing results. Overall, no improvement of functional outcome was found [17], but large trials on this subject are ongoing. Based on the results of our study, it may not be necessary to differentiate the management of hyperglycemia between stroke subtypes.

In line with previous studies, a trend toward a higher incidence of SICH was observed among patients with non-lacunar stroke and admission hyperglycemia.[2,6] This effect was not observed in the lacunar stroke patients, but this can be explained by the very low absolute number of SICH in this stroke subtype.

Our study has several limitations. Despite the quite large sample size, the subgroup of patients with lacunar stroke is relatively small, which may negatively influence the statistical power of our findings. Further, information on serial glucose measurements or glycosylated hemoglobin concentrations was not available. Finally, the relation between hyperglycemia and infarct growth was not studied by follow-up imaging.

In conclusion, we found admission hyperglycemia to be an independent predictor of poor functional outcome in patients with acute ischemic stroke treated with intravenous tPA. Contrary to patients not receiving thrombolytic therapy, the negative effect of hyperglycemia on outcome was present in both non-lacunar and lacunar stroke, although less pronounced in the latter.

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CHAPTER 6

Thrombolytic therapy, warfarin use and international normalized ratio in acute ischemic stroke

PART 1

6

Prior anticoagulant therapy, subtherapeutic international normalized ratio and thrombolytic therapy in acute ischemic stroke

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Content

Due to the increased risk of symptomatic intracerebral hemorrhage (sICH) among patients using vitamin-K antagonist (VKA) prior to stroke, an international normalized ratio (INR) of >1.7 is considered as a contraindication for treatment with intravenous recombinant tissue plasminogen antigen (tPA).[1] However, few studies have investigated the relation between INR levels and sICH after tPA and the results of these are conflicting.[2-4] One study showed a higher incidence of sICH among VKA users with an INR of less than 1.7 treated with intravenous tPA.[4] A cohort of intra-arterial tPA treatment and another series of both intravenous and intra-arterial tPA treatment, found no increased risk in patients using VKA.[2,3] We studied the relationship between VKA use, elevated INR and outcome after tPA treatment.

In a prospective observational study of 524 ischemic stroke patients treated with tPA, we investigated the relationship between prior VKA- use, an elevated (INR), sICH and favorable outcome after 3 months, defined as a modified Rankin scale score (mRS) 0-2. Data were analyzed separately for VKA users and non-VKA users and for normal INR (≤1.1) and elevated INR (1.2 – 1.7).

Of the 524 patients, 22 (4%) used VKA. An elevated INR was present in 59 (11%) patients. No differences were found between VKA users and non-VKA users in occurrence of sICH (9% vs. 6%, p=0.36) and favorable outcome (41% vs. 48%, p=0.50). Elevated INR was non-significantly associated with sICH (12% vs. 5%, p=0.09) but significantly associated with less favorable outcome (50% vs. 35%, p=0.03). Multivariable analyses demonstrated that elevated INR was associated with worse outcome and an increased risk of sICH. VKA treatment alone (with or without elevated INR) was only associated with increased risk of sICH (***Table 1***).

Table 1: OR of multivariable analysis

OR for sICH and favorable outcome (mRS 0-2)						
	sICH and VKA use*			Favorable outcome and VKA use**		
	OR	95% CI	P	OR	95% CI	P
VKA use	5.72	1.06 – 30.75	0.042	0.86	0.28 – 2.71	0.80
	sICH and INR ≥ 1.2*			Favorable outcome and INR ≥ 1.2**		
	OR	95% CI	P	OR	95% CI	P
INR ≥ 1.2	3.17	1.19 – 8.45	0.021	0.47	0.23 – 0.99	0.046

** corrected for baseline NIHSS, antiplatelet use and systolic blood pressure. ** corrected for baseline NIHSS, age, serum APTT and serum glucose level. CI: confidence interval. sICH: symptomatic intracranial hemorrhage. VKA: vitamin K-antagonist. OR: odds ratio. mRS: modified Rankin Scale. INR: international normalized ratio.*

Our study suggests that prior VKA use with a normal or elevated INR before intravenous tPA was independently associated with an increased risk of sICH, but not with a poor functional outcome. To summarize caution should be taken to treat patients, who have an elevated INR, spontaneously or due to VKA, with tPA.

PART 2

6

Thrombolytic therapy for ischemic stroke in patients using warfarin: as systematic review and meta-analysis

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Abstract

Background

It is uncertain whether thrombolytic therapy is safe in patients with acute ischemic stroke who are treated with warfarin and have a subtherapeutic international normalized ratio (INR) at stroke onset.

Methods

The authors performed a systematic review of the literature and included studies that assessed the relation between prior warfarin use with subtherapeutic INR and outcome after intravenous or intra-arterial thrombolytic therapy in acute ischemic stroke. Outcome measures were symptomatic intracranial hemorrhage (SICH), modified Rankin scale score 0-2 and mortality. Second, the authors performed a meta-analysis of the included studies.

Results

Seven studies with 3631 patients were included. 240 (6.6%) patients used warfarin before stroke onset. The risk of SICH was increased in the warfarin group (OR 2.6; 95% CI 1.1 to 5.9. p 0.02). There was no significant difference, however, in functional outcome (OR 0.9; 95% CI 0.6 to 1.2, p 0.32) or death from all causes (OR 1.2; 95% CI 0.9 to 1.8).

Discussion

The risk of SICH after thrombolytic therapy is increased in patients using warfarin with subtherapeutic INR levels. The authors found no evidence of an increase in death from all causes or worsening of functional outcome in warfarin treated patients.

Introduction

The treatment of acute ischemic stroke with recombinant tissue plasminogen activator (tPA) improves functional outcome. (5) A feared and often devastating complication of tPA treatment is the occurrence of symptomatic intracranial hemorrhage (SICH). An important clinical consideration for stroke physicians is to identify which patients are at risk for developing SICH after tPA treatment. Since the introduction of tPA, several predictors for good functional outcome and SICH have been elucidated on which current guidelines are based. [6-9] There is much controversy whether to treat ischemic stroke patients using warfarin with suboptimal international normalized ratio (INR). While the American Heart Association guidelines suggest treating patients with an INR <1.7, [1] the European Stroke Organisation guidelines discourage the use of tPA in patients treated with warfarin regardless of the INR. [10]

A number of cohort studies investigated the safety of tPA in this subgroup of warfarin treated patients, but the results were conflicting. [2-4,11-14] Some investigators found an increased risk of SICH and/or poor clinical outcome, whereas others found no important differences regarding clinical outcome and risk of SICH. Many of these studies had insufficient power because of small sample sizes.

The aim of this study was to perform a systematic review and meta-analysis of studies that investigated the relation between prior warfarin use with subtherapeutic INR and outcome after thrombolytic therapy in patients with acute ischemic stroke.

Methods

Literature search

We searched in Medline and Embase for the MeSH terms 'isch(a)emic stroke' and 'thrombolysis' or 'tissue plasminogen activator' or 'thrombolytic therapy' and 'warfarin' or 'vitamin K antagonist'. We selected only studies that were written in English. Based on the abstracts we included the relevant studies. References of the included studies were thoroughly searched to find potentially relevant citations.

Eligibility criteria

We used the following inclusion criteria: [1] Studies comparing acute stroke patients with and without prior warfarin therapy treated with intravenous tPA or/and intra-arterial thrombolytic therapy. [2] Available outcome measures: modified Rankin scale (mRS) score at 3 months, SICH and/or death from all causes. SICH had to be defined as an intracranial hemorrhage (parenchymal hemorrhage or hemorrhagic transformation) with neurological deterioration. We excluded case reports, non-comparative case series and studies that only treated patient with mechanical thrombectomy.

Data collection

Available baseline characteristics such as age, National Institutes of Health Stroke Scale (NIHSS) score, INR values and treatment modality (intravenous and/or intra-arterial thrombolysis) were extracted from the studies by two investigators (IM and MU). In case of disagreement, consensus was obtained by discussion with the other authors. When available, data about recanalization rates (TIMI (thrombolysis in myocardial infarction score) grade 2-3 or TIBI (thrombolysis in brain ischemia) grade 4-5) after treatment were also obtained. Results from our own centre, were also added to the meta-analysis. [15]

Statistical analysis

For the meta-analysis, data from the included studies were collected and ORs for the three different outcome measures were calculated using the Mantel-Haenszel method with fixed effect models. Heterogeneity between the studies was assessed with the I2 statistic. In case of significant heterogeneity ($p<0.05$), random effect models were used. Sensitivity analyses were performed with exclusion of studies with slightly different definitions of SICH/ mortality. The statistical analyses were performed with Review Manager 5.1 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011).

Results

Literature search

Based on our search strategy, we found 147 studies in the literature, of which nine were potentially relevant after screening the abstracts/titles. [2-4,11-14,16,17] Two studies were excluded for the meta-analysis because of small case series with a non-comparative design [2,14] and one study was excluded because patients were only treated with mechanical thrombectomy. [17] The total number of patients in the pooled dataset was 3631, of whom 240 (6.6%) used warfarin before stroke onset. The characteristics of the studies are presented in ***Table 1***. Most studies were retrospective analyses of prospectively collected data. One study primarily compared elevated INR with normal INR instead of warfarin/no warfarin use. [16] Most studies excluded patients with INR levels >1.7. One study reported nine tPA treated patients with an INR >1.7, and none of these patients developed SICH. [16] Patients with warfarin therapy were older and had more severe strokes in most studies. SICH was defined as an increase in NIHSS score of four or more points and a parenchymal hemorrhage within 36 h after treatment, except for one study which did not specify the degree of neurological deterioration on the NIHSS. [12] Two studies did not assess functional outcome with the mRS, [4,16] and one study assessed functional outcome at discharge. [12] The remaining studies reported 3 months functional outcome, defining favorable outcome as mRS score of 0-2. Kim et al [3] published mortality rates at 3 months. The other studies only reported in-hospital mortality. Two studies assessed recanalization rates using conventional angiography, MR angiography or transcranial Doppler. Both studies did not find a significant difference in recanalization rates between the two groups (***Table 2***).

Table 1: Characteristics of the included studies.

First Author	Study Design	N	Comparison (N / N)	Median INR (Warfarin vs no warfarin)	Median NIHSS (Warfarin vs no warfarin)	Mean age (Warfarin vs no warfarin)	Treatment modality
Kim (3)	RS analysis PS cohort	179	Warfarin vs no warfarin 28 / 151	1.14 vs 0.99	17 vs 16.5	69.2 vs 68.5	IV tPA +/- IA urokinase
Vergouwen (12)	RS analysis PS cohort	1739	Warfarin vs no warfarin 125/1614	1.2 vs 1.0	14 vs 12	79 vs 74 (median)	IV tPA
Ibrahim (13)	RS of phase 2 trial	284	Warfarin vs no warfarin 9 / 180	-	15 vs 15.8 (Mean)	72 vs 68.3	IV tPA
Prabhakaran (4)	RS analysis PS cohort	107	Warfarin vs no warfarin 13/94	1.21 vs 1.03	15 vs 14	80.6 vs 67.6	IV tPA
Brunner (16)	RS analysis PS cohort	688	Elevated vs normal INR 29 / 659	1.5 vs ? *	13 vs 11	73 vs 72	IV tPA
Seet (11)	not clearly described	212	Warfarin vs no warfarin 14 / 198	1.15 vs 1.0	13 vs 13	79 vs 74	IV tPA
Miedema (15)	RS analysis PS cohort	524	Warfarin vs no warfarin 22 / 502	1.4 vs 1.0	13 vs 11	74 vs 68	IV tPA

** in this study 8 patients had an INR > 1.7 (1.8, 1.9, 2.0, 2.2 and 2.9). RS: retrospective. PS: prospective. INR: international normalized ratio. Vs: versus. IV: intravenous. IA: intra-arterial. tPA: tissue plasminogen activator. NIHSS: National Institute of Health Stroke Scale.*

Table 2: Outcome of the included studies

First Author	All ICH (% Warfarin vs no warfarin)	Symptomatic ICH (% Warfarin vs no warfarin)	mRS 0-2 (% Warfarin vs no warfarin)	Mortality (% Warfarin vs no warfarin)	Recanalisation (% Warfarin vs no warfarin)
Kim (3)	37 vs 31.5	14.8 vs 8.1*	51.9 vs 40.7	18.5 vs 20.7‡	92 vs 85
Vergouwen (12)	16.8 vs 11.1	8 vs 5.7†	26.8 vs 31.3	19.2 vs 16.1§	-
Ibrahim (13)	-	0 vs 5*	55.6 vs 53.1		55.6 vs 38.2
Prabhakaran (4)	38.5 vs 5.3	30.8 vs 3.2*	-	15.4 vs 9.6§	-
Brunner (16)	5.6 vs 7.7	0 vs 4.4*	-	8.3 vs 8.8§	-
Seet (11)	43 vs 14	36 vs 6*	29 vs 52	36 vs 19§	-
Miedema (15)	-	9.1 vs 5.6*	40.9 vs 48.3	4.5 vs 8.2§	-

*ICH: intracranial hemorrhage. mRS: modified Rankin Scale. * Symptomatic ICH according to the Safe Implementation of Treatments in Stroke (SITS) definition. † Symptomatic ICH according to the National Institute of Neurological Disorders and Stroke (NINDS) definition. ‡ mortality at 3 months. § In hospital mortality.*

Outcome and meta-analysis

Symptomatic ICH

In two studies, prior warfarin therapy was significantly associated with SICH.[4,11] In the other five studies, there was no significant difference between incidence of SICH in patients with and without warfarin therapy, although 95% CIs of most studies were wide. The risk of any ICH after tPA in patients with prior warfarin was substantially higher in two studies, whereas in the other five studies the rates were rather similar in both groups (***Table 2***).

The meta-analysis demonstrated a significant degree of heterogeneity between the studies (***Figure 1A***). In the pooled analysis, prior warfarin use was significantly associated with SICH (OR 2.6; 95% CI 1.1 to 5.9. p=0.02). Excluding the study from Vergouwen et al[12] who used a slightly different definition of SICH, the same significant association was yielded (OR 3.0; 95% CI 1.1 to 8.4, p=0.03).

Functional outcome

Five studies reported 3-month functional outcome with the mRS from which the number of patients with a favorable outcome (mRS 0-2) could be extrapolated (***Table 2***).[3,11-13,15] None of the studies showed a significant relation between prior warfarin use and good outcome after tPA. There was no significant heterogeneity between the studies (***Figure 1B***). The pooled OR from the meta-analysis did not demonstrate a significant relation between favorable functional outcome and prior warfarin use (OR 0.9; 95% CI 0.6 to 1.2, p=0.32).

Mortality

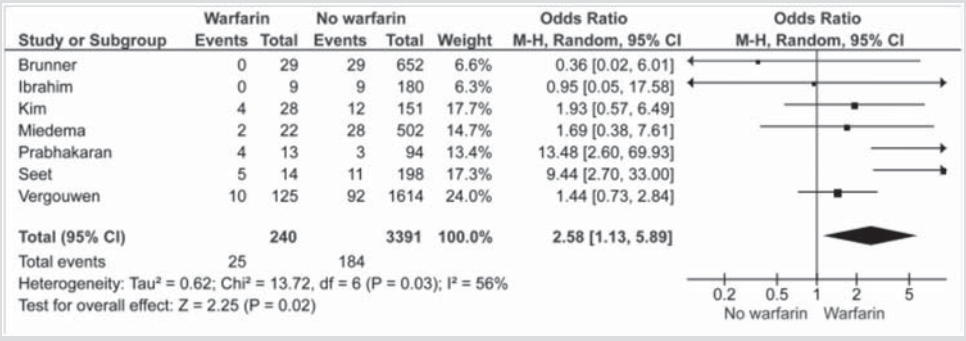
One study did not report data about mortality; Kim et al[3] reported 3-month mortality and the other five studies assessed the in-hospital mortality (***Table 2***). No differences were found in the in-hospital mortality between patients with and without prior warfarin therapy (OR 1.2; 95% CI 0.9 to 1.8, p=0.28) (***Figure 1C***). Excluding the study of Kim et al[3] that reported mortality at 3 months did not alter the results (OR 1.3; 95% CI 0.9 to 1.9, p=0.28).

Discussion

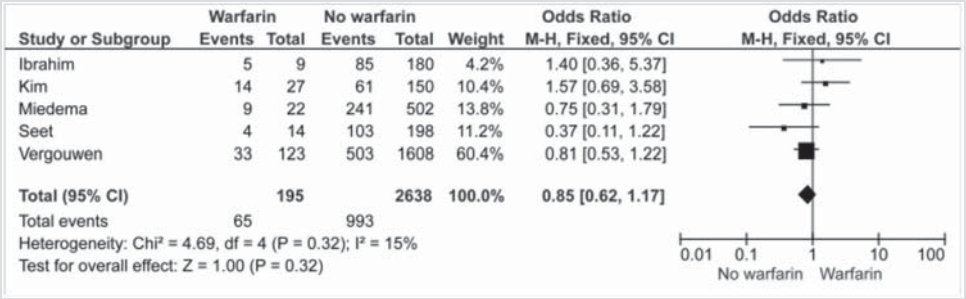
The main finding of this meta-analysis was an increased risk of SICH in patients with acute ischemic stroke and prior warfarin therapy with a subtherapeutic INR after treatment with intravenous and/or intra-arterial thrombolysis. The risk of SICH was more than two times higher in patients on warfarin therapy compared with those without. However, there was no evidence that prior warfarin therapy was associated with (un)favorable outcome or an increase in death from all causes. Most of the included studies concerned patients treated with intravenous tPA, and few studies were done in patients receiving endovascular treatment. A reanalysis of the MERCI (Mechanical Embolus Removal in Cerebral Ischemia) and multi-MERCI trials[17] demonstrated that mechanical thrombectomy in patients with

Figure 1: Meta-analysis of outcome in patients with and without warfarin treated with thrombolysis
ICH: intracranial hemorrhage, mRS: modified Rankin Scale.

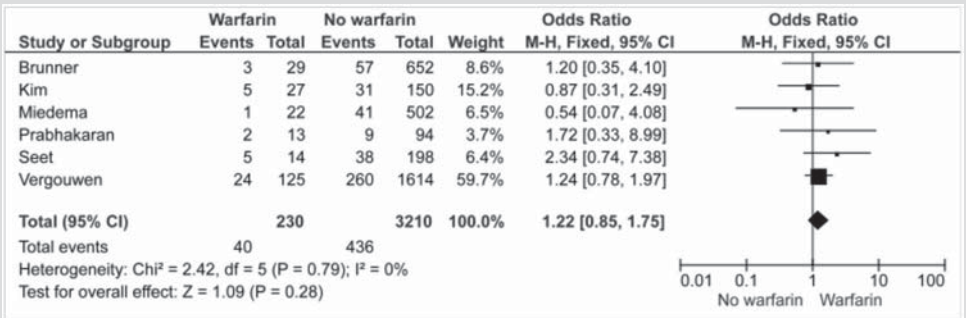
(A) Symptomatic ICH random effect models



(B) Favorable functional outcome (mRS 0-2), fixed effect models



(C) Mortality, fixed effect models



acute ischemic stroke and prior warfarin use with an INR larger than 1.7 was also associated with SICH.

During acute cerebral ischemia the blood brain barrier becomes damaged, which can lead to spontaneous hemorrhagic transformation. [18] Disruption of the blood brain barrier is influenced by several proteolytic enzymes, such as matrix metalloproteinases (MMP). [19] In acute ischemic stroke, tPA treatment is associated with an up-regulation of MMP-9 and may therefore enhance the risk of SICH. [20] Reperfusion also enhances the risk of SICH. [21] Animal experiments have demonstrated that warfarin increases the risk of hemorrhagic transformation in acute ischemia, which was highly dependent on reperfusion. [22] So, a combination of reperfusion injury, up-regulation of MMP-9 and strong anticoagulant effect of warfarin might explain the observed increased risk of SICH. The same effect has been observed in patients treated with antiplatelets before tPA treatment. [8,23]
On the other hand, a common problem after thrombolytic therapy is the occurrence of early re-occlusion. Some have hypothesized that recanalization rates with warfarin may be better than without warfarin, although this idea was not confirmed by two of the included studies (***Table 2***). [3,13] A topic of interest is whether newer anticoagulants, such as dabigatran etexilate, also increase the risk of SICH. We are only aware of a single case report of an acute stroke patient on dabigatran treated with intravenous tPA who did not develop hemorrhagic complications. [24]

This systematic review has limitations. First, there were some small differences in the definition of SICH, which could influence the SICH rates. Moreover, the meta-analysis regarding SICH revealed a significant heterogeneity between the studies. Most studies however used a definition of intracranial hemorrhage with an NIHSS score increase of four or more points. In the sensitivity analysis, excluding the study that used another definition of SICH, the results remained the same; therefore, it seems unlikely that these differences influenced the final results. Second, we could not perform multivariate analyses with adjustment for possible confounders. Patients with warfarin were older and, as a consequence, may have had more often leucoaraiosis. Both age and leucoaraiosis may also have contributed to an increased risk of SICH. [25] Patients with warfarin had also more often cardio-embolic strokes. It is known that cardio-embolic strokes are associated with larger diffusion weighted imaging lesions, which is also a strong predictor of SICH after intravenous tPA. [9] Other possible confounders, such as early ischemic changes on brain CT or blood pressure values, [26] were not included in our analyses. These imbalances can confound the relation between prior warfarin and SICH. Third, we did not have data about the INR levels from patients with and without SICH. It could be possible that there is a cut-off point for the INR at which the risk of SICH increases. Results from our centre suggest that an elevated INR >1.2 was associated with SICH. [15] Vergouwen et al. found no association between the INR level and occurrence of SICH. [12] Fourth, only two studies documented recanalization rates. It would be interesting to evaluate the degree of

recanalization in patients with and without prior warfarin in a larger sample size. Fifth, it may be possible that, due to publication bias, the risks of hemorrhagic complications after thrombolytic therapy in patients with warfarin treatment were underestimated.

In summary, this study suggests that patients on warfarin therapy, but with subtherapeutic INR, have an increased risk of SICH after thrombolytic therapy. However, we did not observe an association between prior warfarin therapy and a worse functional outcome or increase in death from all causes. Further studies should focus at which INR cut-off score the risk of SICH increases.

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CHAPTER 7

General discussion and conclusion

Summary and integrated discussion

In the last two decades, the development of revascularization therapy with intravenous recombinant tissue plasminogen antigen (tPA) has greatly improved the outcome in acute ischemic stroke patients, but this therapy is only available for a selected group of patients and even in eligible patients, it is underused. [1-5] But treatment with intravenous tPA has also safety concerns with several contra-indications and an increased risk of intracranial hemorrhage. [1,3,12] In addition to revascularization therapy, neuroprotective strategies need to be developed to improve functional outcome by acting on the ischemic penumbra, but results from randomized controlled trials in humans have been uniformly disappointing. [6-11]

Neuroprotection

In **Chapter 2 and 3** the potential neuroprotective effects of selective serotonin re-uptake inhibitors (SSRIs) and statins (HMG-CoA reductase inhibitors) were investigated. For both drugs, pleiotropic effects have been found in pre-clinical studies. [13-18] Based on these preclinical findings, the idea arose that these drugs could be neuroprotective in acute ischemic stroke. In human clinical cohorts conflicting results were reported. [16,19-26] We studied the relation between these drugs and functional outcome in an observational cohort study with a prospectively collected dataset of patients, whose acute ischemic stroke was treated with intravenous tPA within 4.5 hours of symptom onset.

In patients, the use of SSRIs, started in the first weeks till months after the ischemic stroke, has been shown to be associated with improved functional outcome, possibly by pleiotropic effects on brain plasticity. [19,20] Chronic use of SSRIs enhances the concentration of brain-derived neurotrophic factor (BDNF) which plays a part in neurite outgrowth, synaptic plasticity and the selection of functional neuronal connections. [27-29] Contrary to these findings, we found (**Chapter 2)** a detrimental effect on functional outcome in patients who used a SSRI prior to stroke onset. This effect was strongest in patients with a cortical stroke. The discrepancy with previous results could be explained by the effects of elevated BDNF in chronic SSRI users which also increases the release and sensitivity of glutamate. [30] Glutamate plays a major role in excitotoxicity in de acute phase of ischemic stroke. [11] Therefore, SSRIs may be more regenerative than neuroprotective, with improved functional outcome when prescribed in the late phase of ischemic stroke, while in the acute phase it could be harmful to patients treated with intravenous tPA. Additional research to explore these various potential effects is warranted.

A large meta-analysis on the efficacy of statins in animal stroke models supports the neuroprotective effect of statin. These drugs appear to influence infarct volume (reduction of 25%) as well as improvement of neurological recovery (improvement up to 20%). [22] In patients treated with intravenous tPA, one study showed a beneficial effect of statins on

functional outcome [25], but other studies could not replicate this. A detrimental effect of statins was even suggested, with a higher risk of symptomatic intracranial hemorrhage (SICH). [23-26,31,32] This latter finding remains unexplained although it is sometimes cross-connected to serum lipid levels. [23,26,32] As described in **Chapter 3**, we found neither a beneficial nor a detrimental effect of prior statin use on functional outcome. Our results do not support a neuroprotective effect of statins in acute ischemic stroke patients treated with intravenous tPA. In line with our results, a large pooled analysis of 11 databases including 4000 patients, confirmed that prior statin use was neither associated with functional outcome nor with intracranial hemorrhage, but it may be considered as an indicator of baseline cardiovascular characteristics that are associated with a less favorable course. [33] In contrast, an other large recent observational multicenter study that included 2000 patients found statin use in the acute phase of stroke after intravenous thrombolysis to be associated with favorable short- and long term outcome. [34] In this trial the subgroup of statin users differed significantly from the no-statin group with regard to several prognostic baseline factors, like stroke severity. Thus, the verdict on the issue is still open, awaiting the randomized controlled trials that at this moment are lacking.

In **Chapter 4**, the effects of serum uric acid concentrations on functional outcome after acute ischemic stroke have been discussed. On the one hand uric acid supports the development and progression of atherosclerosis; on the other hand it acts as a powerful anti-oxidant. [35-37] Uric acid can be administrated safely in human subjects, thus increasing anti-oxidant capacity. [38] In animal models administration of uric acid has been proven to be neuroprotective. [39,40] In clinical cohort studies a neuroprotective effects of uric acid was suggested, while other cohort studies reported an association with poor outcome. [41-48] We investigated the effect of serum uric acid concentrations on functional outcome in a cohort of prospectively collected patients with acute ischemic stroke who were not treated with intravenous tPA. We failed to demonstrate a neuroprotective effect of uric acid. One hypothesis could be that the neuroprotective effect in the acute phase of the initial stroke is obscured by preexistent more severe cardiovascular disease in these patients with higher serum uric acid levels. This would impact final outcome.

For all three potentially neuroprotective agents investigated in **Chapters 2, 3 and 4** of this thesis we failed to identify actual neuroprotective effects. For the SSRIs we even found a relation with poor functional outcome. As described earlier, several factors may be held responsible for these disappointing results. Some of them need to be addressed here.
There are large difference between the methodology and results from animal experiments and trials in humans. In animal studies histological stained infarct volume is most commonly used as the primary end-point for a neuroprotective effect. In human studies, neuroprotection is most commonly assessed by clinical functional outcome. The neuroprotective effect on the ischemic penumbra in human patients may be too small to result in a significant difference in the 'crude' outcome scales that are being used. Functional

imaging modalities like diffusion/perfusion MRI may ultimately yield significant differences, without effects on functional outcome, and thus no clinical consequences. Secondly, the pleiotropic effects of the agents investigated may not be only beneficial, but they may also be harmful, protective effects being counteracted by detrimental effects. Thirdly, in the entire spectrum of neuroprotective research in ischemic stroke the vast array of methodological differences between animal and human clinical studies have been considered to be responsible for the lack of translational success of neuroprotective agents. [49,50] It has been questioned whether the most efficacious agents were selected for clinical trials. [49] Proposed methodological differences are dosage regimens, time of administration of the agent, route of administration and availability to the target area. [50,51]

In this thesis, only observational cohort studies were performed, no actual intervention trials. A population of patients who already used the drugs of interest at the onset of stroke was studied. This mimics to some extent the setting of the pre-clinical animal trials, where drug administration is usually started pre-stroke or early after stroke initiation. although from a methodological point of view major differences still exist with these pre-clinical trials. The observational nature of the studies in this thesis is far from ideal for the clinical investigation of neuroprotective agents, but based on observational data, one would hope to find at least an association with functional outcome.
With regard to statins results are conflicting. Recently an association with favorable short- and long term functional outcome. [34] The only way to answer this question is to perform a large clinical trial. But still, even if a neuroprotective effect of statin use will be found, we expect it to be small, as it has already been extensively researched in observational studies, which yielded mainly neutral results.

Neurotoxicity
Hyperglycemia is a known risk factor for the development of SICH and poor outcome in patients with acute ischemic stroke. In patients with acute stroke who were not treated with thrombolysis, a differential effect of admission hyperglycemia on outcome was found, depending upon stroke subtype: poor outcome in non-lacunar stroke and a more favorable outcome in lacunar stroke with moderate hyperglycemia. [52,53] **Chapter 5** examines whether this finding could be reproduced in patients treated with intravenous tPA. It appeared that hyperglycemia was associated with poor functional outcome in both lacunar and non-lacunar stroke. Possibly, in patients with lacunar stroke treated with intravenous tPA the exposure to reperfusion injury aggravated by hyperglycemia is the same as in patients with non-lacunar stroke. These effects may counterbalance the suggested protective effects of hyperglycemia in lacunar stroke patients. No significant difference in occurrence of SICH between hyperglycemic and normoglycemic patients in either stroke subtype was found, probably because of the low number of SICH in the study population.

Safety of tPA treatment

The safety and efficacy of tPA treatment has been proven in various large randomized controlled trials, but tPA treatment remains associated with an increased risk of intracranial and systemic bleeding complications. [1-5,12] Several factors clearly enhance the risk of intracranial hemorrhage, like age and stroke severity. Data on the safety of tPA treatment in patients with prior use of vitamin K antagonists and subtherapeutic INR are contradictory. [54,55] These patients are considered eligible for intravenous thrombolysis but may have an increased risk of bleeding complications. In our prospective observational study on the risk of symptomatic intracranial hemorrhage (SICH) in patients treated with intravenous tPA and prior use of vitamin K antagonists and/or elevated INR presented in the first part of **Chapter 6**, we found that prior treatment with vitamin K antagonists at the moment of acute ischemic stroke, with a normal or slightly elevated INR (<1.7), was associated with an increased risk of SICH. However, there was no association of prior use of vitamin K antagonists and/or elevated INR with poor functional outcome. In the second part of **Chapter 6**, a systematic review of the current literature and a meta-analysis of this subject was performed, combining results of our own observational study with those of other observational cohort studies. This meta-analysis demonstrated that prior use of vitamin K antagonists is significantly associated with the risk of intracranial hemorrhage. But no association with functional outcome and in-hospital mortality could be found. Recently, the results of a large observational study including more than 20.000 patients on this topic have been published. [56] No increased risk was found of SICH among patients with acute ischemic stroke and prior use of vitamin K antagonists before treatment with thrombolysis. Although these results represent the largest experience on safety of thrombolysis in patients with prior use of vitamin K antagonists, this study should be interpreted with caution. Differences in methods and patient selection need to be considered in explaining the discrepancy among the studies. [57,58] Therefore, despite the heterogeneity between the various studies in the meta-analysis and despite the finding that prior use of vitamin K antagonists does not influence the actual functional outcome at three months after the acute ischemic stroke, the increased risk of intracranial hemorrhage makes it important to be careful with intravenous thrombolysis in patients with prior use of vitamin K antagonists and a subtherapeutic INR.

Patients who used vitamin K antagonists prior to acute ischemic stroke are likely to suffer more often cardio-embolic strokes, due to their underlying conditions which required vitamin K antagonists in the first place. As known from the literature, cardio-embolic strokes are associated with larger infarct size; they constitute a risk factor for SICH. [59,60] Therefore, the assumed association of higher SICH rates in these patients may be confounded by the higher incidence of cardio-embolic stroke, in addition to the effects of vitamin K antagonists itself and the combination of vitamin K antagonists with thrombolysis, as discussed in **Chapter 6**.

Future perspectives

In acute ischemic stroke rapid restoration of blood flow by thrombolysis, with resupply of oxygen and glucose is currently the most effective form of neuroprotection. In the last decades, over 1000 other neuroprotective agents have been tested in pre-clinical stroke trials.[49] Around 200 clinical trials investigating the effects of neuroprotective agents have been performed or are still ongoing, nearly all yielding disappointing results.[49] The observational studies in this thesis could not demonstrate a neuroprotective effect of the compounds studied. In the perspective of this large amount of negative trials, the question arises whether it is justified to continue further research on this topic. Future research should be well designed and well targeted. To better translate promising pre-clinical neuroprotective agents to clinical application, bridging the methodological differences between animal and human studies is necessary. In the design of pre-clinical experiments and trials, population type (age, co-morbidity), ischemic territory, duration of occlusion and dosage regimens are examples of factors that should be addressed.[51,61] Furthermore, outcome parameters should be carefully considered, with functional outcome, rather than infarct size as the main parameter. Even with these changes, it is questionable whether the translation of animal ischemic stroke models into the clinical setting will be successful.

A new treatment modality that addresses neuroprotection in patients with acute ischemic stroke is hypothermia, which is known to improve outcome in patients with post-anoxic encephalopathy. This therapy showed no beneficial effects in traumatic encephalopathy. At the moment trials assessing the possible therapeutic effect of hypothermia in combination with revascularization therapy are ongoing.[62]

Identification of patients who will obtain most benefit from intravenous tPA treatment or are at high risk for the development of complications remains a crucial goal. This thesis confirmed that admission hyperglycemia is associated with poor outcome in patients treated with intravenous tPA irrespective of infarct subtype. The next logical step would be to investigate the role of intervention with glucose lowering strategies in such patients. At this moment glucose lowering intervention studies have been unable to provide evidence that tight glycemic control improves functional outcome in patients with acute ischemic stroke.[63,64] Timing of glucose management, ideally before reperfusion is complete and therefore before treatment with tPA, maintaining tight glucose levels as well as preventing hypoglycemia, are issues that require further research on this topic. The ideal intervention should balance safety and benefit, considering that hypoglycemia has also a potential detrimental effect on tissue in the already vulnerable ischemic penumbra.

This thesis showed that prior use of vitamin K antagonists in patients with acute ischemic stroke treated with intravenous thrombolysis is associated with an increased risk of SICH. As conflicting data have recently been published[56], further research on this topic is needed. It should not only focus on early occurrence of SICH in vitamin K antagonists users and long term functional outcome, but it should also include serial INR data to consider at which cut-off point for INR patients are at high-risk of SICH development. This cut-off

point for INR could then be implemented in the current acute ischemic stroke and thrombolysis guidelines. The American Heart Association's current guidelines suggest a cut-off point of INR <1.7, which is not in line with the European Stroke Organisation guidelines that discourage the use of thrombolysis in all patients using vitamin K antagonists. [55,65] With the upcoming use of new oral anticoagulants like dabigatran etexilate and rivaroxiban, the effects and risks of these drugs in patients with acute ischemic stroke treated with thrombolysis need to be re-assessed.

Main conclusions

The field of neuroprotection in acute ischemic stroke is an relevant subject of research with promising results from pre-clinical trials. Unfortunately, in the clinical setting these successes can not be reproduced. The data presented in this thesis also do not support a neuroprotective effect of selective serotonin re-uptake inhibitors, statins and serum uric acid. It is questionable whether the development of better adjusted pre-clinical and clinical trials with neuroprotective agents in general would easily overcome these limitations. Until that time treatment with intravenous thrombolysis remains the most effective therapy for acute ischemic stroke. However, selection of patients to reduce the risks of hemorrhagic complications is a major issue. Use of vitamin K antagonists, elevated INR and hyperglycemia are associated with the occurrence of SICH and with poor functional outcome, respectively. The widespread use of vitamin K antagonists and the high incidence of admission hyperglycemia in patients with acute ischemic stroke prioritizes these factors for further research. But from the broader perspective of neuroprotection and safety of tPA treatment, the question remains whether influencing only a single factor would improve overall outcome in patients with acute ischemic stroke, as acute ischemic stroke has multiple causes and occurs in often frail patients with extensive co-morbidity.

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7

CHAPTER 8

Nederlandse samenvatting

Nederlandse samenvatting

Het herseninfarct is een vaak voorkomend ziektebeeld met frequent overlijden tot gevolg. Ondanks de afnemende incidentie in de afgelopen jaren en daling van mortaliteit, is de verwachting dat de prevalentie en kosten in de komende jaren juist toenemen mede door groei van de oudere populatie bij een langere levensverwachting. De ontwikkeling van revascularisatie-therapie met recombinant tissue plasminogen activator (tPA, verder genoemd trombolyse) heeft reeds geleid tot een verbetering van de uitkomst en vermindering van beperkingen na een herseninfarct. Het effect van trombolyse is het meest duidelijk bij vroegtijdige toediening van het medicijn, waarbij de meeste effectiviteit, met een number needed to treat (NNT) van 5, is aangetoond wanneer het wordt toegediend in de eerste 90 minuten na aanvang van de verschijnselen. Het belangrijkste risico van trombolyse is de ontwikkeling van een symptomatische intracraniële bloeding. Vanwege het relatief korte tijdsbestek waarin de behandeling effectief is, komt er in de praktijk slechts een deel van de patiënten in aanmerking voor deze therapie. Daarnaast bepalen ook een aantal contra-indicaties de toepasbaarheid van trombolyse in de dagelijkse praktijk, zoals een verhoogde glucose waarde, gebruik van anti-stollingsmedicatie en een verhoogde bloeddruk.

Een andere strategie om de uitkomst na een herseninfarct te verbeteren is de ontwikkeling van neuroprotectiva. Neuroprotectie verwijst naar een positief effect van medicatie of andere stoffen op de neuronale overleving na cerebrale ischemie. Na het acute moment van ischemie begint er een ischemische cascade waarbij activatie van diverse stoffen, waaronder ionen en neurotransmitters, uiteindelijk leidt tot irreversibele schade aan de neuronen en daarmee celdood. In de afgelopen jaren zijn verschillende neuroprotectieve stoffen onderzocht. Preklinische studies lieten hierbij vaak veelbelovende resultaten zien, maar de vertaling naar klinische fase III onderzoeken lijkt niet goed te lukken. Factoren die hoogstwaarschijnlijk hierin een rol spelen zijn bijvoorbeeld methodologische verschillen en verschillen in doseringen en tijdstip van toedienen van de stoffen. Daarnaast simuleert de setting van diermodellen met een herseninfarct waarschijnlijk onvoldoende de klinische setting bij de mens. Ondanks deze tegenvallende resultaten, blijft het concept van neuroprotectie veelbelovend klinken. Daarom wordt toch verder onderzoek gedaan naar de mogelijkheden van neuroprotectie, deels met gebruik van combinatie van verschillende therapieën waarbij gedacht wordt dat niet alleen de uitkomst na een herseninfarct kan verbeteren, maar mogelijk ook het aantal patiënten wat in aanmerking komt voor trombolyse zou kunnen toenemen.

In dit proefschrift is het effect onderzocht van een aantal mogelijke neuroprotectieve stoffen op de klinische uitkomst bij patiënten met een doorgemaakt herseninfarct behandeld met trombolyse. Ook is gekeken naar andere prognostische factoren die de therapeutische effecten en veiligheid van trombolyse zouden kunnen verbeteren.

In Hoofdstuk 2 en 3 werd het potentiële neuroprotectieve effect van selective serotonine re-uptake inhibitors (SSRI's) en HMG-CoA reductase inhibitors (statines) onderzocht. In een observationele cohort studie van prospectief verzamelde patiëntgegevens werd de relatie onderzocht tussen het gebruik van één van de medicamenten met de functionele uitkomst van patiënten met een acuut herseninfarct behandeld met trombolyse. Eerder onderzoek had laten zien dat het starten van een SSRI in de eerste weken tot maanden na een acuut herseninfarct geassocieerd was met een betere uitkomst. Het bleek echter dat de functionele uitkomst van patiënten met een herseninfarct die reeds vooraf aan het herseninfarct een SSRI gebruikten juist slechter was, met name bij patiënten met een corticaal infarct. Het gebruikt van een SSRI lijkt dus geen neuroprotectief effect te hebben. De mogelijke verklaring hiervoor wordt besproken in Hoofdstuk 2. Het toedienen van statines heeft in diermodellen een neuroprotectief effect laten zien. Er werd zowel een kleiner infarct volume als een verbetering van neurologisch herstel gezien. Patiëntgebonden studies hebben een enkele maal een neuroprotectief effect aangetoond, maar regelmatig werd dit ook niet gevonden. In de studie beschreven in Hoofdstuk 3 werd ook geen effect gevonden van het gebruik van een statine op de uitkomst na een herseninfarct behandeld met trombolyse. In de recente literatuur blijft er veel discussie bestaan over het mogelijke neuroprotectieve effect van statines doordat er tegenstrijdige onderzoeksresultaten gepubliceerd worden. Het ontbreekt nog aan een grote gerandomiseerde studie om het vraagstuk op te lossen.

Urinezuur is één van de meest krachtige natuurlijke anti-oxidanten en zou daarom een neuroprotectief effect hebben, zoals ook aangetoond in diermodellen. Urinezuur is echter ook geassocieerd met een hoger risico op ontwikkeling en progressie van atherosclerose. In Hoofdstuk 4 werd het effect van de concentratie urinezuur in het bloed op de uitkomst na een herseninfarct onderzocht. Het bleek dat er geen neuroprotectief effect van urinezuur kon worden aangetoond. Een verklaring hiervoor zou kunnen zijn dat de mogelijk geringe beschermende effecten van urinezuur worden overschaduwd door de meer uitgesproken cardiovasculaire comorbiditeit bij deze patiënten.

Hyperglycemie is geassocieerd met een slechtere uitkomst en een hoger risico op het ontwikkelen van een symptomatische intracraniële bloeding na een herseninfarct. Bij patiënten met een acuut herseninfarct niet behandeld met trombolyse werd eerder juist een positief effect van een milde hyperglycemie beschreven bij patiënten met een lacunair infarct. Hoofdstuk 5 beschrijft de studie waarin gekeken werd of dit ook gold voor infarcten behandeld met trombolyse. Het bleek echter dat hyperglycemie bij trombolyse in zowel lacunaire als corticale infarcten geassocieerd was met een slechtere uitkomst.

Het is onduidelijk of het veilig is om een patiënt die orale anticoagulantia gebruikt en een subtherapeutische international normalized ratio (INR) heeft te behandelen met trombolyse. De richtlijn van de American Heart Association suggereert om patiënten met een INR <1.7

te behandelen, de richtlijn van de European Stroke Organisation ontmoedigt het gebruik van trombolyse bij alle patiënten die orale anticoagulantia gebruik, onafhankelijk van de INR. Verschillende cohort studies hebben de veiligheid van trombolyse in combinatie met gebruik van anticoagulantia onderzocht, maar de gevonden resultaten waren tegenstrijdig: een toegenomen risico op bloeding en slechte uitkomst tegenover geen aantoonbaar verschil. Vaak betrof het een kleine onderzoekspopulatie. In Hoofdstuk 6 werd geprobeerd meer duidelijkheid hierover te verkrijgen. Het bleek dat gebruik van orale anticoagulantia met een normale of licht verhoogde INR (<1.7) was geassocieerd met een verhoogd risico op het ontwikkelen van een bloeding na trombolyse. Er werd echter geen relatie met de functionele uitkomst gevonden. Deze resultaten werden vervolgens gecombineerd met eerdere resultaten van andere studies in een meta-analyse. In de meta-analyse werd tevens een verhoogd risico op het krijgen van een bloeding na trombolyse gevonden, maar opnieuw geen relatie met de uitkomst na het herseninfarct. Er werd geconcludeerd dat voorzichtigheid geboden is bij het trombolyseren van patiënten met gebruik van orale anticoagulantia en een INR <1.7, maar dat verder onderzoek nodig is om onder andere het exacte afkappunt van de INR waarboven het risico toeneemt te bepalen.

Onderzoek naar neuroprotectie bij herseninfarcten is een interessant onderwerp met veelbelovende resultaten in diermodellen. In de klinische setting kunnen deze resultaten helaas niet goed worden gereproduceerd. De studies in dit proefschrift naar SSRI´s, statines en serum concentraties urinezuur hebben ook geen neuroprotectief effect van deze stoffen kunnen aantonen. Tot op heden is behandeling met trombolyse de meest effectieve therapie voor patiënten met een acuut herseninfarct. Een goede selectie van patiënten om zo het risico op een bloedingscomplicatie of een slechtere uitkomst te verminderen is belangrijk. De aanwezigheid van een hyperglycemie ten tijde van de behandeling met trombolyse is geassocieerd met een slechtere uitkomst na het herseninfarct, ongeacht het type herseninfarct. Het gebruik van orale anticoagulantia met een normale tot licht verhoogde INR is geassocieerd met een hoger risico op een intracraniële bloeding, zonder dat dit de uitkomst beïnvloedt. De hoge incidentie van hyperglycemie en het frequente gebruik van orale anticoagulantia bij patiënten met een herseninfarct maakt verder onderzoek naar deze factoren noodzakelijk. Vanuit een breder perspectief blijft het de vraag of beïnvloeding van slechts een enkele factor de uitkomst na een herseninfarct voldoende zal kunnen verbeteren om een klinisch significant effect te kunnen meten; de oorzaak van een herseninfarct is vaak multi-factorieel bepaald en het betreft meestal kwetsbare patiënten met uitgebreide co-morbiditeit.

8

Dankwoord

Curriculum vitae

List of publications

Dankwoord

Dit proefschrift was nooit verschenen zonder de hulp, begeleiding en steun van velen. Graag wil ik een aantal van hen specifiek benoemen.

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Curriculum vitae

Irene Miedema is op 5 juli 1985 geboren in Franeker. In 2003 deed zij eindexamen gymnasium op het Christelijk Gymnasium Beyers Naudé te Leeuwarden. Aansluitend is zij begonnen met de studie Geneeskunde aan de Rijksuniversiteit Groningen waar zij in 2006 haar Bachelor behaalde. In 2009 behaalde zij cum laude haar Master Geneeskunde. Tijdens de verschillende co-schappen ontstond de interesse in de neurologie en zij volgde uiteindelijk haar laatste co-schap als semi-arts op de afdeling Neurologie en Kinderneurologie van het Universitair Medisch Centrum Groningen. Aansluitend verrichte zij haar wetenschappelijke stage waar onder begeleiding van Gert-Jan Luijckx de basis voor dit proefschrift werd gelegd. In oktober 2009 is Irene gestart met de opleiding tot Neuroloog in het Universitair Medisch Centrum Groningen met als opleider Prof. Dr. H.P.H. Kremer. Zij hoopt in 2016 de opleiding tot Neuroloog af te ronden.

List of publications

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