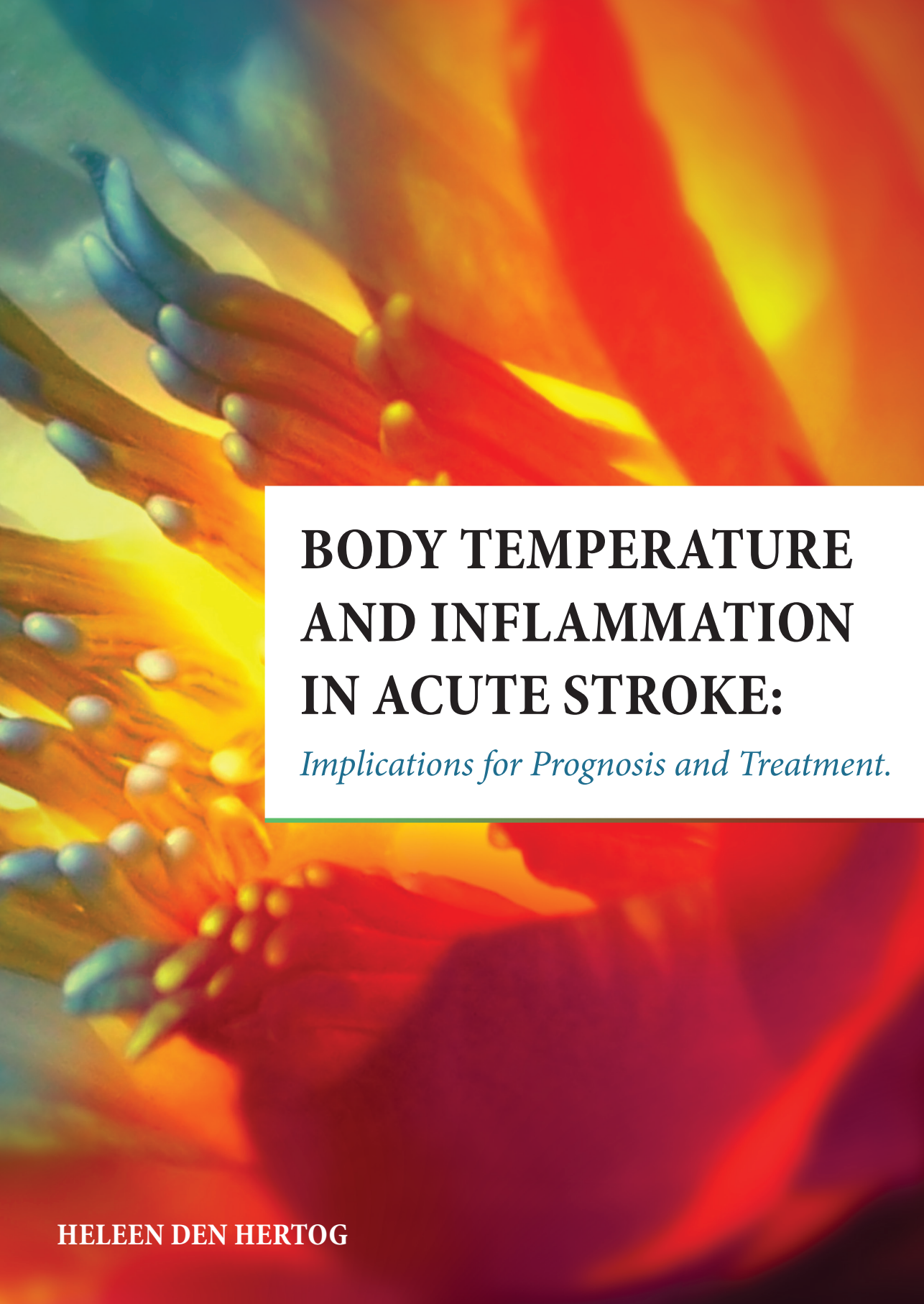

BODY TEMPERATURE AND INFLAMMATION IN ACUTE STROKE:

Implications for Prognosis and Treatment.

HELEEN DEN HERTOG

Body temperature and inflammation in acute stroke:

implications for prognosis and treatment

Heleen den Hertog

ACKNOWLEDGEMENT

Research described in this thesis was supported by a grant of the Netherlands Heart Foundation (grant number 2002B148). Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged. Financial support for the publication of this thesis was kindly provided by the Department of Neurology, GlaxoSmithKline BV, Servier and Boehringer Ingelheim.

ISBN: 978-90-8559-579-3
Cover Design: Patrick Hessels
Design and layout of the thesis: Optima Grafische Communicatie and Heleen den Hertog
Printed by: Optima Grafische Communicatie

Body Temperature and Inflammation in Acute Stroke:
implications for prognosis and treatment

Lichaamstemperatuur en ontsteking in de acute fase van een beroerte:
implicaties voor de prognose en de behandeling

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. H.G. Schmidt
en volgens het besluit van het College van Promoties.

De openbare verdediging zal plaatsvinden op
donderdag 12 november 2009 om 15.30 uur

door

Heleen den Hertog

geboren te Utrecht

PROMOTIECOMMISSIE

Promotor	prof.dr. P.J. Koudstaal
Overige leden	prof.dr. D.J.G.M. Duncker prof.dr. J.D. Laman prof.dr. E.W. Steyerberg
Copromotoren	dr. D.W.J. Dippel dr. H.B. van der Worp

"Alone we can do little, together we can do so much."

Helen Adams Keller

Aan mijn ouders

Aan Michiel

CONTENTS

Chapter 1

General introduction

Safe, cheap, and broadly applicable therapies for acute stroke are urgently needed. Stroke ranks second as a cause of death worldwide and is the main cause of disability in high-income countries. In the Netherlands alone, more than 37.000 patients are admitted to hospital for acute stroke each year.[1] As the incidence of stroke rises exponentially with age, the demographic change in world's population will increase its socio-economic impact.

Strokes are either ischemic or hemorrhagic. Treatment of ischemic stroke and intracerebral hemorrhage has remained unsatisfactory. Stroke unit care has been proven effective for all stroke patients, with an absolute risk reduction of death of 3% and long-term dependency of 5%.[2] In patients with ischemic stroke, treatment with recombinant tissue-plasminogen activator (rt-PA) reduces the number of patients with poor outcome at three months by about 9%[3,4], but the short time window for administration (4.5 hours) and the associated bleeding risk restrict treatment with rt-PA to a minority of patients. Surgical decompression improves outcome in a very small selected group of patients aged up to 60 years who deteriorate because of space-occupying edema within 48 hours of stroke onset.[5] Aspirin, started within 48 hours of symptom onset, is probably effective across the entire range of patients with ischemic stroke, but the benefit is small, with a number needed to treat of 79 to prevent death or dependency in a single patient.[6]

ETIOLOGY OF STROKE

Ischemic stroke accounts for about 80% of all strokes and results from a transient or permanent reduction of cerebral blood flow caused by occlusion of a cerebral artery or arteriole. Stroke registries usually fail to identify a definite cause of ischemic stroke in up to a third of patients, depending on the quality, completeness, and rapidity of the work-up, but also on the definition of "cause".[7] The most common causes are atherothrombosis and embolism from the heart.[8] Atherothrombotic stroke can be subdivided into large vessel disease, including the carotid and vertebral arteries, medium vessel disease, and small vessel disease of the small penetrating arteries perfusing the brain stem and the deep structures of the cerebral hemispheres. The most common sites of atheroma are artery branch points, especially in the distribution of the internal carotid artery, curvature and confluence.[9] Atherothrombotic stroke is caused by either embolism from the carotid or vertebral artery to a distal smaller calibre intracranial vessel or local intracranial thrombotic arterial occlusion. In practice, it may be very difficult to differentiate between these two, and in many patients various lesions at different sites are found.

Emboli may arise from the heart, most frequently due to atrial fibrillation. Less common are recent myocardial infarction, dilated cardiomyopathy, infective endocarditis, prosthetic valves, and emboli from the right-sided circulation with subsequent passage through a patent foramen ovale.[9] Stroke can also rarely result from inadequate cerebral

blood flow due to a severe fall in blood pressure, for instance after cardiac arrest, or from other rare disorders, syndromes, and diseases such as the moyamoya syndrome, moyamoya disease, hypercoagulable disorders, fibromuscular dysplasia, dissection, and vasculitis.[9]

Hemorrhagic stroke represents about 20% of all strokes and is caused by rupture of a blood vessel in the brain. There are two main types of hemorrhagic strokes: intracerebral hemorrhage (15%) and subarachnoid hemorrhage (5%). The latter is beyond the scope of this thesis. Intracerebral hemorrhages are most frequently caused by hypertension or amyloid angiopathy. Other causes of intracerebral hemorrhage include coagulation disorders, arteriovenous and other vascular malformations, vasculitis, and primary or metastatic brain tumors.[9]

PATHOPHYSIOLOGY OF ACUTE STROKE

In order to establish new potential treatment targets, detailed knowledge of the natural course of the disease is required. In both ischemic stroke and intracerebral hemorrhage, a complex cascade of pathophysiological events that evolve over time and space may contribute to tissue damage.

Underlying mechanisms of damage during and following ischemic stroke

The extent of ischemic damage depends on both the severity and duration of cerebral ischemia. In the core of the ischemic area, cerebral blood flow is reduced to 0-10 ml/100 g brain tissue per minute.[10] This will result in necrosis of neurons and also of supporting cellular elements (glial cells) within a few minutes. The infarct core is surrounded by the so-called ischemic penumbra, which may be considered as ischemic tissue that is functionally impaired and at risk of infarct, but has the potential to be salvaged by reperfusion. In the penumbra, processes including excitotoxicity, radical formation, lipid peroxidation, and inflammation may lead to cell damage and cell death.[11]

Although there is ample evidence that the penumbra exists in patients with ischemic stroke[12-14], the extent and temporal dynamics of this area are less well defined and probably vary between individuals.

Ischemic stroke and inflammation

There is substantial evidence that inflammation is important in the pathophysiology of ischemic stroke, particularly after reperfusion. Inflammation plays a pivotal role in tissue recovery, but it may also have unfavorable effects. Within a few minutes to hours of reduction of cerebral blood flow, proinflammatory genes are upregulated, including those for transcription factors, heat shock proteins, cytokines, chemokines and adhesion mol-

ecules.[15] Locally produced cytokines including interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), produced by microglia, astrocytes, endothelial cells, and neurons, mediate these inflammation processes.[16, 17] As a consequence, leucocytes are activated, and stimulate the release of chemokines, and upregulation of complementary adhesion molecules on cerebral microvessels and circulating leucocytes.[17] In acute ischemic stroke, inflammation may augment tissue damage by disruption of the blood-brain barrier, release of cytotoxic agents, activation of the complement system, edema resulting from endothelial cell injury and leukocyte-mediated injury, microvascular thrombosis and increased body temperature.[18] The inflammatory reaction may last for several days. The general hypothesis is that the acute inflammatory reaction is detrimental, while the chronic phase inflammation is essential for repair and regeneration.

Underlying mechanisms of damage during and following intracerebral hemorrhage

The pathological consequences of intracerebral hemorrhage include the immediate injury and delayed secondary processes. The former results from physical disruption of adjacent tissue and mass effect. Mechanical forces during hematoma formation or chemical toxicity from the clot might result in necrosis. The hematoma increases in size in about a third of the patients during the first 24 hours.[19] Early hematoma growth may be a dynamic process, with continued bleeding or rebleeding occurring at multiple sites.[19]

Perihematomal brain edema develops immediately after intracerebral hemorrhage and peaks several days later.[20] The mechanisms of how edema contributes to tissue damage are not well understood. Previously, swelling of the tissue was thought to lead to ischemia of the surrounding brain parenchyma.[21] These ischemic areas were believed to trigger an inflammatory reaction, producing vasogenic edema. However, more recent studies showed that the blood flow to these areas was unchanged.[22, 23] It is now thought that end-products of coagulation activate the inflammatory cascade which in turn leads to vasogenic edema, cytotoxic edema, and disruption of the blood-brain barrier, and thereby amplifyies the damage caused by the hematoma.[24] It is also possible that the hematoma itself actively induces pathways that lead to tissue damage. These pathways include thrombin produced during clot formation and iron released from hemoglobin.[25, 26]

Intracerebral hemorrhage and inflammation

There is growing evidence that inflammation is involved in the pathophysiology of intracerebral hemorrhage as well. In animal studies, intracerebral hemorrhage initiated a microglial response, followed by activation of systemic neutrophils and other leucocytes.[27] Molecules related to adhesion of leucocytes to the endothelium are upregulated. Consequently, leucocytes migrate across the blood-brain barrier to the site of injury.[27] In addition, the complement system may also play a role in tissue injury after intracerebral hemorrhage.[28]

INFLAMMATORY PARAMETERS AND PROGNOSIS AFTER STROKE

In patients with first-ever stroke, case fatality ranges between 12% within the first seven days to 19% in the first three months, and is highest in intracerebral hemorrhage.[29, 30] Between 50% and 70% of patients regain functional independence after a stroke.[31]

The patient's age, pre-stroke health status, stroke type and indicators of stroke severity (conscious level, lesion volume and severity of neurological impairments) determine the likelihood of survival and good functional recovery.[32-34] Recurrent stroke[33], secondary complications related to the stroke including the occurrence of infections[35] and venous thrombosis[36] and physiological parameters such as level of serum glucose, blood pressure, and oxygen[37-39] have been reported to be related to outcome after stroke. In addition, the cause of stroke may affect the prognosis and the likelihood of recurrent events.[40, 41]

The central role of inflammation in the pathogenesis of neuronal damage following acute stroke, suggests a prognostic role of inflammatory parameters such as body temperature and C-reactive protein (CRP).

Identifying new prognostic factors may have a two-fold aim. It may be useful, both for the prediction of functional outcome and stimulating research into the underlying pathogenetic mechanisms and development of new, more targeted, medical treatments for acute stroke.

MEASURING OUTCOME AFTER ACUTE STROKE

Most phase III stroke trials have used the degree of dependency or death as their main outcome measures. Commonly used outcome scales for assessing dependency are the modified Rankin Scale (mRS)[42] and Barthel Index (BI)[43]. The BI is an activity of daily living scale and measures the degree of disability on 10 different items (see appendix). The cumulative score ranges from 0 to 20, with 20 indicating no disability and 0 indicating complete dependence. The mRS quantifies dependency using an ordinal hierarchical grading from 0 (no symptoms) to 5 (severe disability). Sometimes 6 (death) is added to facilitate statistical analysis and interpretation (see appendix).

In previous stroke trials, both the mRS and BI have been collapsed to a binary outcome of favorable versus unfavorable. This approach of dichotomizing an ordinal outcome scale has several disadvantages. It may not correspond with everyday clinical practice. Most treatment strategies tested in acute stroke trials are not expected to be completely curative, but to lead to improvement. Therefore, it is also informative to show that treatment moves patients from severe to moderate disability or from moderate disability to complete recovery, and not only to demonstrate differences in the numbers of patients with a good or poor functional outcome. Furthermore, dichotomization may limit statistical power. Typically, the sample size calculation of a phase III stroke trial assumes that

each patient in the placebo group has a 50% probability of poor outcome at 3 months. In practice, there will be substantial prognostic heterogeneity because of differences in baseline variables between patients. If this is not taken into account, the chance of finding a true treatment effect may be reduced.

Because of these disadvantages of dichotomization, new approaches to outcome analysis have been proposed and tested in acute stroke trials.[44-48] These novel outcome analyses consider the full range of the ordinal outcome scale and lead to a single and meaningful estimate of the treatment effect. One of these new approaches is the so-called "sliding dichotomy".[46] Analysis by sliding dichotomy allows each patient's baseline prognosis to be taken into account. The outcome of an individual patient is thus regarded as favorable or unfavorable depending on what would have been expected based on the severity of stroke and other prognostic factors.

A PROMISING TREATMENT MODALITY IN ACUTE STROKE

The development of therapies for acute stroke has proven to be a difficult and a challenging task, reflecting the complexity of the pathophysiology and clinical aspects of this heterogeneous disorder.

Subfebrile temperature or fever is present in about a third of patients on the first day after stroke onset.[49, 50] The pathophysiology of this increase in body temperature is not completely understood. On the one hand, increased body temperature may be a natural consequence of brain infarction. However, animal studies have suggested that high body temperatures may increase the damage induced by cerebral ischemia.[51] An association between increased body temperature and poor outcome has also been shown in patients with acute stroke.[49, 50, 52-59] The odds of poor outcome were doubled for every degree increase in body temperature measured within 12 hours of stroke onset.[50] Therefore, reduction of body temperature and prevention of fever might be a promising approach in treatment to improve functional outcome after stroke. The background and existing evidence of this treatment modality will be discussed in Chapter 2.

Based on the existing evidence, we started the Paracetamol (Acetaminophen) In Stroke (PAIS) trial.[60] In this trial, we assessed whether early treatment with high-dose paracetamol improves functional outcome in patients with acute stroke. The majority of the studies described in this thesis are based on the PAIS trial.

AIMS AND OUTLINE OF THE THESIS

This thesis focuses on body temperature and temperature-lowering therapy in acute stroke. A secondary aim is to further expand the knowledge on inflammation in relation to prognosis and treatment in acute stroke.

Chapter 2 gives a general review of the literature concerning temperature-lowering therapy and discusses the existing evidence in terms of feasibility and effectiveness of this treatment strategy in acute stroke. Chapter 3 focuses on the effect of pharmacological temperature-lowering therapy by paracetamol in acute stroke. For the PAIS trial, we decided to change the planned analysis for the primary outcome measure from a fixed dichotomy of the mRS to a sliding dichotomy analysis. Chapter 3.1 describes the protocol change. The main results of the PAIS trial are presented in Chapter 3.2. In Chapter 3.3, the prognostic role of body temperature in acute stroke is further explored. Besides its antipyretic and analgesic properties, high-dose paracetamol may have indirect effects. Chapter 3.4 describes the effects of high-dose paracetamol on blood pressure. Chapter 4.1 evaluates the prognostic value of CRP on clinical outcome in patients with acute stroke. Since levels of inflammatory markers may be partially genetically determined, we examined haplotypes representing common variations in the CRP gene in relation to levels of CRP in acute ischemic stroke in Chapter 4.2

In Chapter 5, a first step into further research in acute stroke aimed at integrating basic and clinical science is presented.

The implications of the studies described in this thesis on patient care and future research are discussed in Chapter 6. Finally, the results of the studies are summarized in Chapter 7.

REFERENCES

1. Vaartjes I. Hart-en vaatziekten in Nederland 2008. *Nederlandse Hartstichting*, 2008.
2. Govan L, Weir CJ, Langhorne P. Organized Inpatient (Stroke Unit) Care for Stroke. *Stroke* 2008 Epub.
3. Hacke W, Kaste M, Bluhmki E et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; 359:1317-1329.
4. Wardlaw JM, Zoppo G, Yamaguchi T et al. Thrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev* 2003; 3:CD000213.
5. Hofmeijer J, Kappelle LJ, Algra A et al. Surgical decompression for space-occupying cerebral infarction (the Hemicraniectomy After Middle Cerebral Artery infarction with Life-threatening Edema Trial [HAMLET]): a multicenter, open, randomised trial. *Lancet Neurol* 2009; 8:326-333.
6. Sandercock PA, Counsell C, Gubitz GJ et al. Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev* 2008; 3:CD000029.
7. Amarenco P, Bogousslavsky J, Caplan LR et al. Classification of stroke subtypes. *Cerebrovasc Dis* 2009; 27:493-501.
8. Bousser MG, Amarenco P, Chamorro A et al. Rationale and design of a randomized, double-blind, parallel-group study of terutroban 30 mg/day versus aspirin 100 mg/day in stroke patients: the prevention of cerebrovascular and cardiovascular events of ischemic origin with terutroban in patients with a history of ischemic stroke or transient ischemic attack (PERFORM) study. *Cerebrovasc Dis* 2009; 27:509-518.
9. Warlow C, van Gijn J, Sandercock P. Stroke, practical managment. 2008.
10. Heiss WD. Flow thresholds of functional and morphological damage of brain tissue. *Stroke* 1983; 14:329-331.
11. Mergenthaler P, Dirnagl U, Meisel A. Pathophysiology of stroke: lessons from animal models. *Metab Brain Dis* 2004; 19:151-167.
12. Heiss WD. Ischemic penumbra: evidence from functional imaging in man. *J Cereb Blood Flow Metab* 2000; 20:1276-1293.
13. Kidwell CS, Alger JR, Saver JL. Evolving paradigms in neuroimaging of the ischemic penumbra. *Stroke* 2004; 35:2662-2665.
14. Wintermark M, Reichhart M, Thiran JP et al. Prognostic accuracy of cerebral blood flow measurement by perfusion computed tomography, at the time of emergency room admission, in acute stroke patients. *Ann Neurol* 2002; 51:417-432.
15. Barone FC, Feuerstein GZ. Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* 1999; 19:819-834.
16. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. *J Neuroimmunol* 2007; 184:53-68.
17. Emsley HC, Smith CJ, Gavin CM et al. An early and sustained peripheral inflammatory response in acute ischaemic stroke: relationships with infection and atherosclerosis. *J Neuroimmunol* 2003; 139:93-101.
18. del Zoppo GJ, Becker KJ, Hallenbeck JM. Inflammation after stroke: is it harmful? *Arch Neurol* 2001; 58:669-672.
19. Mayer SA, Brun NC, Begtrup K et al. Recombinant activated factor VII for acute intracerebral hemorrhage. *N Engl J Med* 2005; 352:777-785.
20. Zazulia AR, Diringer MN, Derdeyn CP et al. Progression of mass effect after intracerebral hemorrhage. *Stroke* 1999; 30:1167-1173.

21. Yang GY, Betz AL, Chenevert TL et al. Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. *J Neurosurg* 1994; 81:93-102.
22. Siddique MS, Fernandes HM, Wooldridge TD et al. Reversible ischemia around intracerebral hemorrhage: a single-photon emission computerized tomography study. *J Neurosurg* 2002; 96:736-741.
23. Mayer SA, Lignelli A, Fink ME et al. Perilesional blood flow and edema formation in acute intracerebral hemorrhage: a SPECT study. *Stroke* 1998; 29:1791-1798.
24. Zhang X, Li H, Hu S et al. Brain edema after intracerebral hemorrhage in rats: the role of inflammation. *Neurol India* 2006; 54:402-407.
25. Xi G, Wagner KR, Keep RF et al. Role of blood clot formation on early edema development after experimental intracerebral hemorrhage. *Stroke* 1998; 29:2580-2586.
26. Huang FP, Xi G, Keep RF et al. Brain edema after experimental intracerebral hemorrhage: role of hemoglobin degradation products. *J Neurosurg* 2002; 96:287-293.
27. Wang J, Dore S. Inflammation after intracerebral hemorrhage. *J Cereb Blood Flow Metab* 2007; 27:894-908.
28. Yang S, Nakamura T, Hua Y et al. The role of complement C3 in intracerebral hemorrhage-induced brain injury. *J Cereb Blood Flow Metab* 2006; 26:1490-1495.
29. Dennis MS, Burn JP, Sandercock PA et al. Long-term survival after first-ever stroke: the Oxfordshire Community Stroke Project. *Stroke* 1993; 24:796-800.
30. Hardie K, Hankey GJ, Jamrozik K et al. Ten-year survival after first-ever stroke in the perth community stroke study. *Stroke* 2003; 34:1842-1846.
31. Hardie K, Hankey GJ, Jamrozik K et al. Ten-year risk of first recurrent stroke and disability after first-ever stroke in the Perth Community Stroke Study. *Stroke* 2004; 35:731-735.
32. Hankey GJ, Jamrozik K, Broadhurst RJ et al. Long-term disability after first-ever stroke and related prognostic factors in the Perth Community Stroke Study, 1989-1990. *Stroke* 2002; 33:1034-1040.
33. Hankey GJ, Spiesser J, Hakimi Z et al. Rate, degree, and predictors of recovery from disability following ischemic stroke. *Neurology* 2007; 68:1583-1587.
34. Lewis SC, Sandercock PA, Dennis MS. Predicting outcome in hyper-acute stroke: validation of a prognostic model in the Third International Stroke Trial (IST3). *J Neurol Neurosurg Psychiatry* 2008; 79:397-400.
35. Aslanyan S, Weir CJ, Diener HC et al. Pneumonia and urinary tract infection after acute ischaemic stroke: a tertiary analysis of the GAIN International trial. *Eur J Neurol* 2004; 11:49-53.
36. Andre C, de Freitas GR, Fukujima MM. Prevention of deep venous thrombosis and pulmonary embolism following stroke: a systematic review of published articles. *Eur J Neurol* 2007; 14:21-32.
37. Baird TA, Parsons MW, Phanh T et al. Persistent poststroke hyperglycemia is independently associated with infarct expansion and worse clinical outcome. *Stroke* 2003; 34:2208-2214.
38. Leonardi-Bee J, Bath PM, Phillips SJ et al. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke* 2002; 33:1315-1320.
39. Rowat AM, Dennis MS, Wardlaw JM. Hypoxaemia in acute stroke is frequent and worsens outcome. *Cerebrovasc Dis* 2006; 21:166-172.
40. Kolominsky-Rabas PL, Weber M, Gefeller O et al. Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study. *Stroke* 2001; 32:2735-2740.
41. Petty GW, Brown RD, Whisnant JP et al. Ischemic stroke subtypes : a population-based study of functional outcome, survival, and recurrence. *Stroke* 2000; 31:1062-1068.
42. van Swieten JC, Koudstaal PJ, Visser MC et al. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988; 19:604-607.

43. Mahoney FI, Barthel DW. Functional Evaluation: The Barthel Index. *Md State Med J* 1965; 14:61-65.
44. Bath PM, Gray LJ, Collier T et al. Can we improve the statistical analysis of stroke trials? Statistical reanalysis of functional outcomes in stroke trials. *Stroke* 2007; 38:1911-1915.
45. Joffe MM, Greenland S. Standardized estimates from categorical regression models. *Stat Med* 1995; 14:2131-2141.
46. Murray GD, Barer D, Choi S et al. Design and analysis of phase III trials with ordered outcome scales: the concept of the sliding dichotomy. *J Neurotrauma* 2005; 22:511-517.
47. Saver JL, Gornbein J. Treatment effects for which shift or binary analyses are advantageous in acute stroke trials. *Neurology* 2009; 72:1310-1315.
48. Whitehead J. Sample sizes calculations for ordered categorical data. *Stat Med* 1996;15:1065-1066.
49. Castillo J, Davalos A, Marrugat et al. Timing for fever-related brain damage in acute ischemic stroke. *Stroke* 1998; 29:2455-2460.
50. Reith J, Jorgensen HS, Pedersen PM et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996; 347:422-425.
51. Ginsberg MD, Busto R. Combating hyperthermia in acute stroke: a significant clinical concern. *Stroke* 1998; 29:529-534.
52. Azzimondi G, Bassein L, Nonino F et al. Fever in acute stroke worsens prognosis. A prospective study. *Stroke* 1995; 26:2040-2043.
53. Boysen G, Christensen H. Stroke Severity Determines Body Temperature in Acute Stroke. *Stroke* 2001; 32:413-417.
54. Ernon L, Schrooten M, Thijs V. Body temperature and outcome after stroke thrombolysis. *Acta Neurol Scand* 2006; 114:23-28.
55. Hajat C, Hajat S, Sharma P. Effects of poststroke pyrexia on stroke outcome : a meta-analysis of studies in patients. *Stroke* 2000; 31:410-414.
56. Hindfelt B. The prognostic significance of subfebrility and fever in ischaemic cerebral infarction. *Acta Neurol Scand* 1976; 53:72-79.
57. Jorgensen HS, Reith J, Pedersen PM et al. Body temperature and outcome in stroke patients. *Lancet* 1996; 348:193.
58. Schwarz S, Hafner K, Aschoff A et al. Incidence and prognostic significance of fever following intracerebral hemorrhage. *Neurology* 2000; 54:354-361.
59. Wang Y, Lim LL, Levi C et al. Influence of admission body temperature on stroke mortality. *Stroke* 2000; 31:404-409.
60. den Hertog HM, van der Worp HB, van Gemert HM et al. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial: a multicentre, randomised, placebo-controlled, phase III trial. *Lancet Neurol* 2009; 8:434-440.

Chapter 2

Introduction to temperature-lowering therapy

Temperature-lowering therapy in acute ischemic stroke

Background, feasibility, and effectiveness

Heleen M den Hertog[1], H Bart van der Worp[2], H Maarten A van Gemert[3], Diederik WJ Dippel[1]

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rudolph Magnus institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Neurology, Meander Medical Center, Amersfoort, The Netherlands

Based on *Expert Rev Neurotherapeutics* 2007; 7:155-164

ABSTRACT

Increased body temperatures are common in the acute phase of stroke. Experimental and clinical studies have suggested that increased body temperatures are related to poor outcome. In animal studies of focal cerebral ischemia, early hypothermia consistently reduced infarct volume. Based on these findings, several phase II clinical trials have been performed to study physical methods to reduce body temperature in patients with acute stroke. The feasibility and safety of these methods have not yet been established with sufficient certainty. Pharmacological lowering of body temperature may be an attractive alternative approach. In guidelines for the treatment of acute stroke, antipyretics are generally recommended to reduce fever, although their effect on functional outcome is unknown. There is currently no evidence from randomized trials to support routine use of physical or pharmacological strategies to lower body temperature in acute stroke. Large randomized clinical trials are needed to study the effect of both physical and pharmacological temperature-lowering therapy on functional outcome after stroke.

abstract

INTRODUCTION

The incidence of stroke in Europe and North America ranges from 200 to 300 per 100.000 inhabitants.[1] As the world's population continues to age, stroke will become an increasingly important health problem. The large numbers of affected patients call for effective treatments that are easy to administer, cost-effective, and do not exceed health care budgets. Unfortunately, treatment of acute ischemic stroke and intracerebral hemorrhage has remained unsatisfactory. Aspirin, administered within 48 hours of stroke onset, is available for almost all patients but reduces the risk of disability or death by only 1%.[2] Intravenous thrombolysis with recombinant tissue-plasminogen activator (rt-PA) administered within three hours increases the probability of a good outcome by 10%, but only a limited number of patients will benefit from this treatment because of the small therapeutic time window and various contra-indications for treatment. No other treatment strategies have been proven effective in acute ischemic stroke.[3]

One approach to investigate new treatment concepts is to influence physiologic parameters that are known to be associated with outcome after stroke, including serum glucose levels[4] blood pressure[5], oxygen saturation[6], and body temperature.[7-12.]

Body temperature is increased in many patients with acute ischemic stroke. In this Chapter, I will explore the evidence from experimental and observational studies that higher body temperatures in the first hours after stroke are associated with poor functional outcome. Increased body temperature may be a direct consequence of brain infarction or a result of accompanying infections. Animal studies have suggested that elevated body temperatures increase the damage induced by cerebral ischemia and that hypothermia improves histological and functional outcome. For this reason, early temperature-lowering therapy may protect brain tissue from damage incurred by cerebral ischemia. This is a concept that holds promise for neurotherapeutic interventions.

In this Chapter, I will summarize the results of both animal and clinical studies of temperature-lowering therapy in acute ischemic stroke. I will provide a critical analysis of the conflicting results of trials of hypothermia in patients after cardiac arrest and in patients with severe traumatic brain injury.

One of the challenges is to find an intervention that is safe and easy to administer. This may not necessarily be physical temperature reduction. Pharmacological lowering of body temperature may be an attractive alternative approach. However, most known agents, such as antipyretics, have a rather small effect on body temperature and it may be questioned whether this could translate into a worthwhile effect on outcome. Studies that evaluate the effect of temperature-lowering therapy on functional outcome in acute ischemic stroke are scarce. In the last 10 years, experimental studies and clinical observations have shown an association between increased body temperature and poor outcome in acute stroke. Has the evidence in favor of a causal relationship between body temperature and outcome become strong enough to provide a rationale for phase III

studies? Or is the evidence as it stands a sufficient argument to combat fever and elevated body temperature on a regular basis, without further data from clinical trials?

INCREASED BODY TEMPERATURE AND STROKE SEVERITY: CAUSE, CONSEQUENCE, OR BOTH?

Increased body temperature after ischemic stroke may be a direct consequence of the brain damage inflicted by the stroke. Some animal experiments have indeed shown a spontaneous increase in body temperature following cerebral ischemia.[13,14] In the early phase of ischemia and reperfusion, inflammation processes ensue in the ischemic core and in the penumbra, where perfusion is compromised but tissue is still viable.[15,16] Numerous pro-inflammatory genes are upregulated, including those for transcription factors, heat shock proteins, cytokines, chemokines and adhesion molecules. The classic pro-inflammatory cytokines interleukin-1β (IL-β), interleukin-6 (IL-6), and Tumor Necrosis Factor (TNF) produced by microglia, astrocytes, endothelial cells, and neurons, mediate these inflammation processes. They activate leucocytes, chemokines are released, and complementary adhesion molecules on cerebral microvessels and circulating leucocytes are upregulated.[8,17] It has been suggested that increased body temperature is a result of this inflammatory reaction. In patients with acute ischemic stroke, a positive correlation was observed between increased plasma IL-6 levels and body temperature.[18,19]

Increased body temperature may not be only a consequence of cerebral ischemia; already in 1990, a controlled study with 21 rats first suggested an adverse effect of hyperthermia on histopathological outcome after global ischemia.[20]

It has been suggested that an increased body temperature in the early phase after stroke worsens ischemic damage by several mechanisms. First, the imbalance in the ischemic tissue between energy supply and demand may increase with higher body temperatures, as the metabolic rate of the brain increases.[21] In a cat model of global cerebral ischemia, an enhanced degree of intracellular acidosis and an impaired recovery of cerebral ATP and phosphocreatinine levels was observed when a body temperature of 40 °C was induced one hour before ischemia and maintained for 1.5-2 hours.[22] After global cerebral ischemia, rats with a body temperature of 39°C had a less complete recovery of ATP levels and adenylate energy charge in both cortical and subcortical regions compared to rats with lower body temperatures.[23]

Secondly, the release of glutamate increases with rising body temperature, which is associated with poor outcome in ischemic stroke. In a microdialysis study of 20-minute forebrain ischemia, normothermic rats (37°C) showed a 21-fold increase in glutamate levels during ischemia, whereas the increase of glutamate at 39°C was 37-fold.[24] In rats subjected to 2-hour focal cerebral ischemia at a body temperature of 39°C, glutamate

release in the penumbral cortex averaged 31-fold above baseline, compared with 6.5 fold elevations in rats with normal body temperatures.[25]

Thirdly, increased body temperature may result in an increased production of free radicals. In rats, oxygen radical production in the cortex during early recirculation after global cerebral ischemia was influenced by brain temperature.[26] In rats with brain temperatures of 39°C, free radical production was increased 4 to 5 fold. In rats with brain temperatures of 36°C, free radical production was 2 to 3 times increased. In rats with brain temperatures of 30°C, free radicals were not elevated at all.

Fourthly, the extent of injuries to the blood brain barrier by ischemia is modulated by body and brain temperature. A mild to moderate increase in body temperature leads to massive extravasation of protein tracers in ischemic tissue.[27-29]

It is therefore conceivable that increased body temperatures following stroke are not only a consequence of brain infarction, but increase ischemic damage as well. Timely temperature-lowering therapy may therefore protect brain tissue from the effects of cerebral ischemia.

CLINICAL EFFECTS OF INCREASED BODY TEMPERATURE AFTER STROKE IN MAN

Body temperature is increased in a considerable number of patients with acute stroke. Within the first six hours after onset, the proportion of patients with elevated body temperatures (over 37.5°C) has been reported to vary from 4 to 25%, but this may increase to about 33% at 24 hours (Table 1).

The correlation between body temperature (measured rectally or in the bladder) and brain temperature has been studied in 8 patients with traumatic brain injury.[30] Deep brain temperature was found to be 1-2°C higher than rectal and bladder temperatures. Differences were greatest when the rectal or bladder temperatures were above 38°C.[30] As temperatures in patients with acute stroke are usually assessed with rectal, bladder, or tympanic thermometry, the brain temperature in these patients may have been underestimated.

Several studies have investigated the relation between body temperature and outcome after stroke.[7-11] In an early retrospective study of 110 patients admitted within 24 hours of stroke onset, body temperatures between 37.5°C and 38°C during the first days

Table 1: Body temperature in patients with acute stroke.

Author (reference)	Number of patients	Stroke type	Body temperature on admission > 37.5°C	Time of admission
Reith et al	390	Isch. / Hem.	25 %	< 6 hours
Castillo et al	260	Isch.	3.8 % / 37 %	< 6 hours/ <24 hours
Azzimondi et al	183	Isch. / Hem.	15 %	< 24 hours
Boysen et al	647	Isch. / Hem.	5.4 %	< 6 hours

after stroke were associated with poor outcome.[9] However, potential confounders, such as age, infarct size, and infection were not taken into account. In a prospective study of 260 patients with ischemic stroke, body temperatures over 37,5°C within the first 72 hours were associated with increased mortality, and similar temperatures within the first 24 hours were associated with poor outcome and larger infarct volume independent of age and infections.[8] In a prospective study of 183 patients with ischemic stroke admitted within 6 hours of stroke onset, a relation between body temperatures over 37,5°C within 7 days after admission and mortality at 30 days or 3 months was found.[7] This relation was independent of age, level of consciousness, and blood glucose levels. A retrospective study of 509 patients with ischemic or hemorrhagic stroke showed a significant relation between increased body temperatures on admission and mortality in ischemic stroke patients only.[11] This relation was independent of clinical variables associated with stroke severity, such as incontinence and level of consciousness. In a retrospective study of 251 patients with supratentorial intracerebral hemorrhage, almost every patient (91%) had increased body temperature (>37.5°C) within the first 72 hours.[12] The duration of increased body temperatures was associated with poor outcome. This association was independent of the initial score on the Glasgow Coma scale and hematoma volume. In a prospective study of 390 unselected patients with ischemic and hemorrhagic stroke admitted within 6 hours of onset, a highly significant association between body temperature and outcome was observed (Figure 1). The association was independent of

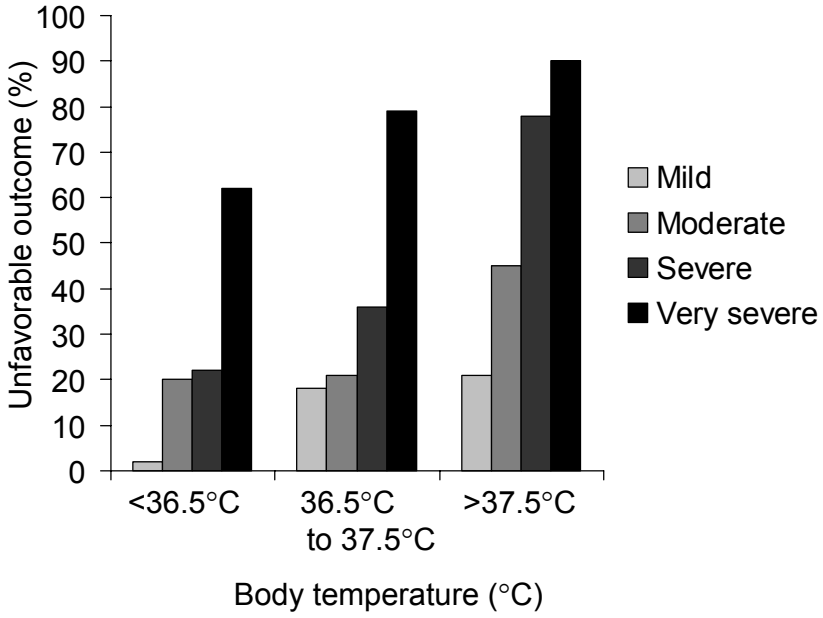

Figure 1: Relationship between poor outcome and body temperature on admission in patients with acute stroke, for different initial stroke severities. Data from Reith et al.[10]

initial stroke severity, age, gender, and the stroke risk profile (Figure 1).[10] For each degree Celsius increase in body temperature the relative risk of poor outcome rose by a factor of 2.2. This association was still present after 5 years of follow-up.[10] This implies that temperature-lowering therapy may have long-term effects on outcome in patients with acute stroke. The relation between body temperature on admission and poor outcome declined with time from stroke onset to admission, but was still significant and clinically relevant in patients admitted between 6 and 12 hours from stroke onset.[31] A 1°C increase in body temperature doubled the mortality risk. A similar trend was observed with regard to functional outcome in survivors, but this did not reach statistical significance.

In contrast to the studies above, no association between body temperature on admission and outcome was found in a prospective study of 725 patients with ischemic or hemorrhagic stroke admitted within 6 hours of stroke onset.[32] However, body temperature at 10 to 12 hours after stroke onset was related to poor outcome, despite treatment with paracetamol in all patients with temperatures above 37°C. The lack of an association between admission body temperatures and outcome may be explained by the fact that rather insensitive methods of statistical analysis were used (Spearman correlation and comparison of median modified Rankin Scale (mRS) scores with a non-parametric test).

To summarize, body temperature may be a strong independent prognostic factor of outcome in acute stroke. The time window of this association may be limited to the first 12 to 24 hours from stroke onset. This suggests that the relationship with poor outcome is not confounded by the occurrence of secondary infections, such as pneumonia or urinary tract infection, because these usually appear later in the course of the disease.

EFFECT OF THERAPEUTIC HYPOTHERMIA IN ACUTE STROKE: ANIMAL MODELS

Since the 1950's, hypothermia has been tested as a protective strategy in animal models of focal cerebral ischemia. Rosomoff first demonstrated the efficacy of profound hypothermia for this condition.[33] Several controlled animal studies suggested a protective effect of mild to moderate hypothermia (30-35°C) in rat focal cerebral ischemia models.[34,35] These studies showed that mild hypothermia resulted in a decrease in infarct volume in comparison with normothermia, provided that treatment was started within one hour after the induced cerebral ischemia.[34,35] The effect of hypothermia was larger in animal models with transient middle cerebral artery occlusion than in animal models with permanent occlusion. Furthermore, the protective effect was influenced by the duration of hypothermia.[34] A study in rats with embolic middle artery occlusion showed that trombolytic therapy following 2 hours of mild hypothermia could not improve the effect of hypothermia alone.[36]

For deep hypothermia (<30°C) the results are conflicting. Three studies with rats showed a reduction in infarct volume without adverse effects when the body temperature was

lowered below 30°C.[37-39] In contrast, two studies in monkeys, cats, and dogs reported an increase in infarct volume when body temperature was lowered below 30°C.[40,41]

As far as we know, only one animal study has been performed to test the effect of paracetamol on body temperature and infarct volume in rats. Treatment with paracetamol (250 mg/kg i.p.) significantly reduced body temperature (38.2 °C ± 0.4°C) compared with vehicle-treated animals (39.3 °C ± 0.3 °C), but only a trend towards a reduction in infarct volume was observed (Figure 2).[42]

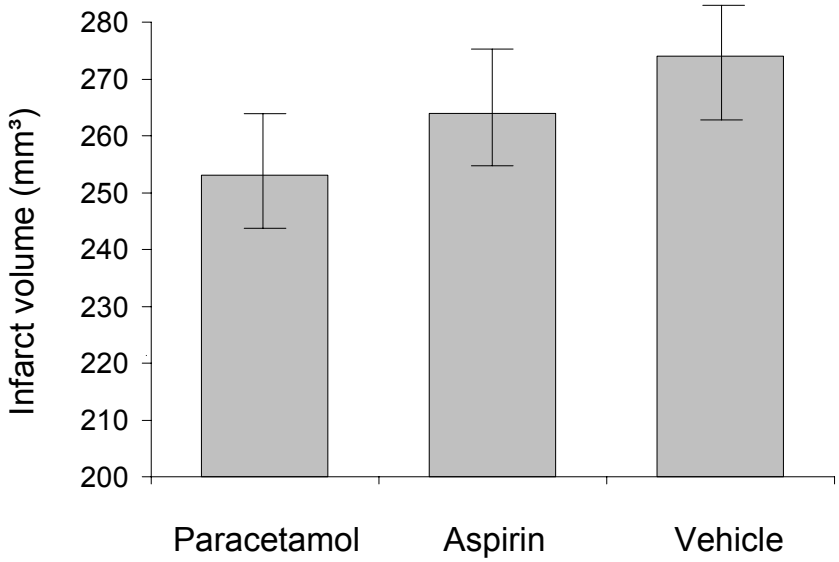

Figure 2: Effects of aspirin and paracetamol on infarct volume in rats. Data from Legos et al.[42]

EFFECT OF TEMPERATURE-LOWERING THERAPY: CLINICAL STUDIES

Based on the beneficial effects of hypothermia demonstrated in animal models, clinical studies of physical and chemical cooling were started. These studies were not only conducted in patients with acute ischemic stroke, but also in patients with severe traumatic brain injury, and in patients with global cerebral ischemia after cardiac arrest.

Temperature lowering therapy after traumatic brain injury and cardiac arrest

Encouraging results from large randomized trials of hypothermia in patients with cardiac arrest are now available. Two randomized trials showed that lowering body temperature to 32-34°C for 12-24 hours in comatose survivors of cardiac arrest improved neurological outcome. In one of the trials, survival with a good outcome was nearly doubled and in the other it was increased by 40%.[43-45] A recent meta-analysis concluded that mild therapeutic hypothermia improves short-term neurological recovery and survival in

patients resuscitated after cardiac arrest of presumed cardiac origin.[45] Further research to determine the long-term prognosis, quality of life, and the feasibility of the cooling methods was considered warranted.

Mild hypothermia has been used in traumatic brain injury for over 50 years. Several experimental studies were promising.[46-48] No clinical randomized clinical trials were performed until the last two decades. Although small randomized studies reported encouraging results,[49-56] the pivotal larger trial could not confirm the benefit of hypothermia.[57] A recent Cochrane review of 12 randomized controlled trials with a total of 1061 patients found no evidence for a beneficial effect of hypothermia in patients with traumatic brain injury.[58] Hypothermia increased the risk of pneumonia and other potentially harmful side effects such as hypotension and cardiac arrhythmia in patients with traumatic brain injury; hypothermia in traumatic brain injury should therefore not be used outside controlled trials.

In the largest published trial with 392 patients with traumatic brain injury, hypothermia (33°C) did not improve outcome measured by disability, vegetative state or death.[52] However, a subgroup analysis revealed that younger patients (<45 years) who had hypothermia on admission and were assigned to treatment had significantly better outcome then patients with normothermia on admission and assigned treatment. A recent published multi-center trial focusing on this subgroup randomized 215 patients with severe traumatic brain injury to long-term hypothermia (5 days) or short-time hypothermia (2 days).[59] The target temperature was 33°C. Long-term hypothermia significantly improved outcome compared to short-time hypothermia. However, the results of this study should be interpreted with caution, as this study used a pseudo-randomization procedure (alternate day), and the study did not incorporate a control group without hypothermia. Another study focusing on this subgroup is underway.[58]

An interesting question is why cooling is efficacious in patients with cardiac arrest, but not in patients with major traumatic brain injury. One obvious explanation is the amount of non-ischemic damage that plays a role in the latter condition. Furthermore, earlier studies in patients with major head trauma were very small single center studies. It is possible that the intervention was applied differently in different centers. Differences in time from trauma to start of treatment and differences in the duration of treatment and rewarming may also account for the discrepancy in the response to hypothermia.

Temperature-lowering therapy in acute stroke: physical temperature reduction

Several uncontrolled phase II studies and one randomized controlled phase II study have been performed to study the feasibility and the safety of different physical methods to reduce body temperature in patients with acute stroke.

In an open study in patients with acute ischemic stroke treated with trombolysis, cooling to 32°C for 12-72 hours with cooling blankets appeared feasible and safe.[60] In an

uncontrolled study of 50 patients with a severe middle cerebral artery infarct, body temperature could be reduced to 32 to 33°C with the use of cooling blankets, alcohol, and ice bags under complete anesthesia.[61] The most frequent complications were thrombocytopenia (70%), bradycardia (62%), and pneumonia (48%). The high frequency of complications may be explained by the severity of infarction. Because of the uncontrolled design of this study, one cannot draw firm conclusions about the complication rate on the one hand and effectiveness of the intervention on the other. An uncontrolled study with 25 patients with middle artery infarction with elevated intracranial pressure showed that hypothermia (33°C) for 48-72 hours significantly reduced intracranial pressure.[62] In a case series 25 patients with middle cerebral artery infarction and elevated intracranial pressure were treated with hypothermia (33°C). The authors observed a subsequent decrease in intracranial pressure. Whether this could be attributed to the hypothermic treatment remains to be investigated in controlled studies. The COOL-AID study, a randomized controlled study, used endovascular cooling instead of surface cooling.[63] Reduction in body temperature to 33°C was compared to standard medical treatment in 40 patients with acute ischemic stroke. The target temperature was reached in a mean of 77± 44 minutes. Shivering was suppressed by warming blankets and sedatives. Only 5 of 18 patients did not reach their target temperature.

The target body temperature in all of these studies was 32-33°C. In all of these studies, patients had to be sedated and intubated for artificial ventilation. This reduces the number of patients suitable for this way of treatment. The large reduction in body temperature increases the risk of complications, such as low blood pressure, pulmonary complications, infections, cardiac arrhythmia, and coagulation disturbances. The many complications that occurred in the study of Schwab may be explained by the fact that these patients all had severe middle cerebral artery infarcts. In the other studies safety concerns were less prominent.[61]

Three uncontrolled phase II studies have been performed with less extreme target temperatures. In a study with 73 patients with acute ischemic or hemorrhagic stroke, target temperature was 35,5°C.[64] Anesthesia was not required. Shivering in this study was treated with intravenous pethidine. In another study, temperature reductions of 1°C were maintained until 24 hours after onset of ischemic stroke.[65] The escalating treatment consisted of paracetamol, cooling blankets and sponging with 70 % alcohol. Shivering was prevented with continuous infusion of low dose midazolam (1-3 mg / hour). In a third study, a 1-2°C body temperature reduction was reached within 3.3 hours with a water-perfused cooling matrass in 16 of the 18 patients with ischemic or hemorrhagic stroke.[66] The patients received pethidine to avoid shivering.

In the Nordic Cooling Stroke Study[67], a multicenter randomized controlled trial in patients with ischemic or hemorrhagic stroke, surface cooling was started within 6 hours of stroke onset and continued for 9 hours. The target temperature was 35°C. Unfortunately,

the trial was terminated because of slow recruitment after the inclusion of 48 patients against a target recruitment of 1000 patients (U.J. Weber, personal communication).

In most physical temperature reduction studies, the nadir was reached within one to six hours from start of treatment. Taking into account the development of the ischemic penumbra[15], this may not be sufficient. However, some studies have shown a relation between body temperature after 12 hours of stroke onset and outcome, suggesting that even hypothermia reached within 24 hours after onset could be beneficial. At this moment there are no completed randomized controlled phase III trials of physical temperature reduction in acute stroke. Well-designed, controlled studies of several intensities of hypothermia, aimed to assess feasibility and safety, are still warranted. On the other hand the risk of complications and the high cost of this mode of treatment imply that there is a need for a simple medical intervention that may reduce body temperature to lesser extent, but is cheap and safe.

In studies of physical temperature reduction, shivering is a serious problem that interferes with treatment. Shivering is a physiological reaction that consists of repetitive contractions of muscles, in order to generate heat. The effect of pethidine to reduce shivering is not unambiguous.[68] Meperidine, buspirone[69] and dexmedetomidine have been shown to reduce the shivering threshold through unknown mechanisms.[70]

Temperature-lowering therapy in acute stroke: antipyretic drugs

Pharmacological lowering of body temperature may be an effective and safer alternative approach to cooling. In guidelines for acute stroke treatment antipyretics are generally recommended to reduce fever.[71] However, the effect on of antipyretic therapy on functional outcome after stroke is still unknown.

Paracetamol: mechanism of action

Paracetamol is one of the most commonly used antipyretic drugs. It is usually well tolerated and has almost no side effects. In spite of its wide use, the mechanism of action of paracetamol has not fully been elucidated. Paracetamol is a potent inhibitor of prostaglandin production within the central nervous system. Evidence has accumulated that prostaglandins are the proximal mediators of fever. They are synthesized in the brain by cyclooxygenases (COXs). There are two isoforms of COX, named COX-1 and COX-2[72], which differ in their expression patterns. COX-1 is expressed in all tissues and is not affected by inflammation mediators, whereas COX-2 is expressed in brain, kidney, testicles and lung tissue and is up-regulated by pro-inflammation mediators.[73,74] Recently, a new isoform of COX has been postulated, provisionally named COX-3, which is mainly produced in the brain. It has been suggested that this isoform, and not COX-2, plays a major role in the brain in fever production.

Paracetamol easily penetrates the brain. The reduction of prostaglandin synthesis by paracetamol can be explained by its competitive inhibition of arachidonic acid for the active site on COX.[75] This presumably accounts for its antipyretic properties. The properties of paracetamol are different from those of typical NSAIDs, as it potently reduces pain and fever but has very little effect on inflammation. This is one to the reasons the existence of COX-3 has been postulated[76], supported by experimental studies showing a very low sensitivity of COX-1 and COX-2 to paracetamol.[77]

Paracetamol: phase II studies

Five phase II studies have been performed to study the effect of paracetamol on lowering body temperature in patients with acute stroke. In two of these studies this effect was also compared with that of other antipyretic drugs. A randomized pilot study in 42 patients showed that treatment with paracetamol in a daily dose of 4000 mg resulted in a substantial reduction in the number of patients with a body temperature over 37.5°C. The size of the temperature reduction was not reported.[78] In a partly blinded randomized study of 39 patients, a daily dose of 3900 mg paracetamol lowered body temperature by 0.2°C. No effect on functional outcome was observed.[79] Our group conducted two pilot studies. Both studies were double-blind, randomized, placebo-controlled trials to study the effect of paracetamol on body temperature in patients with acute ischemic stroke confined to the carotid territory. In a first trial, 76 patients were treated with either 3000 mg or 6000 mg paracetamol or placebo.[80] Treatment with high-dose paracetamol resulted in a reduction in body temperature of 0.4°C at 24 hours even in normothermic and subfebrile patients. This effect was confirmed in a second trial.[81] In this study, patients with acute ischemic anterior circulation stroke were treated with daily doses of 2400 mg ibuprofen, 6000 mg paracetamol, or placebo for 5 days. Ibuprofen had no significantly effect on body temperature. Treatment with paracetamol resulted in a 0.3°C reduction in body temperature at 24 hours compared to placebo.[81] A pooled analysis of the data from both studies showed that a significant decrease of body temperature occurred within 4 hours after start of treatment with high-dose of paracetamol.[82] We did not observe an increased rate of (masked) infections in with paracetamol-treated patients in both trials.

In an uncontrolled study, 132 patients admitted with acute ischemic stroke were treated with either 1000 mg paracetamol as a suppository or 500 mg intravenous acetylsalicylic acid when body temperature rose above 37.5°C.[83] In case body temperature was still above 37.5°C at six hours, treatment was continued with 4000 mg paracetamol a day. Patients who were treated with paracetamol tended to be more often hypothermic (<36.5°C) and less often hyperthermic compared to patients who received acetylsalicylic acid, but these results were not statistically significant. In this study, a one-third lower daily dose of paracetamol was used than in our studies [80;81]. Secondly, the effect of antipyretic agents on body temperature was dichotomized as normal or elevated, which results in loss of power. Thirdly, the study did not incorporate a control group.[84]

In summary, treatment with a daily dose of 6000 mg paracetamol results in a small, but potentially worthwhile reduction in body temperature in patients with acute ischemic stroke. Ibuprofen had no significant effect on lowering body temperature. In studies with high-dose paracetamol, the nadir was reached within 4 hours from start of treatment. As mentioned earlier, this may be too late to be beneficial.[15] However, several studies have shown a relation between body temperature and outcome after 12 hours of stroke onset. Further large randomized clinical trials are needed to investigate whether the temperature reduction by this dose of paracetamol improves outcome in acute stroke.

In May 2003, we started with a large double-blind, randomized, placebo–controlled, multicenter trial of a high dose paracetamol in patients with acute stroke.[85] In total, 2500 patients with acute ischemic or hemorrhagic stroke will be included. Treatment with high-dose paracetamol (6 g/day) or placebo will be started within 12 hours after the onset of symptoms and continued for 3 days. Exclusion criteria are a body temperature lower than 36°C or higher than 39°C, a history of liver disease, alcohol abuse, liver enzymes increased above twice the upper limit of normal, allergy to paracetamol, and significant pre-stroke impairment. The primary outcome measure is a dichotomized score on the mRS (0-2: good outcome, 3-6: poor outcome) at 3 months.

Other antipyretic drugs

In 1998, a randomized placebo controlled trial of metamizol has been started.[86] Treatment with metamizol (6000mg intravenous) or placebo was started within 48 hours after the onset of symptoms, and continued for 3 days. Metamizol is a pyrazolon derivative and considered a non-steroidal anti-inflammatory drug. The results of this trial have not been published.

EXPERT OPINION

Body temperature is a predictor of outcome in acute stroke. The period in which an increased body temperature is associated with poor outcome may be limited to the first 12 to 24 hours from stroke onset. Animal studies of focal cerebral ischemia have shown promising results of interventions aimed at lowering body temperature. Mild hypothermia is associated with a more favorable neurological outcome in patients resuscitated after cardiac arrest. However, no therapeutic effect of hypothermia was observed in patients with severe traumatic brain injury. The uncontrolled studies in acute ischemic stroke that have been conducted so far have not provided sufficient evidence of safety and feasibility of physical cooling. We need randomized phase II studies with several cooling devices. These studies should explore the relation between intensity of treatment, temperature reduction, and adverse events, such as arterial hypotension, infections, and cardiac arrhythmias.

Pharmacological lowering of body temperature may be a safe alternative to physical temperature reduction. Although antipyretics are widely recommended for the treatment of elevated body temperatures in stroke patients, there is insufficient or no evidence of any effect on outcome. Treatment with a daily dose of 6000 mg paracetamol resulted in a 0.3°C reduction in body temperature in patients with acute ischemic stroke. This small effect of paracetamol on body temperature may seem insignificant at first sight, but may prove to be clinically relevant. In the Copenhagen Stroke Study, the relative risk of poor outcome rose by a factor of 2.2 (95% CI 1.4 to 3.5) with each degree Celcius increase in body temperature. Based on these data, a decrease of 0.3°C induced by paracetamol may lead to a 10% relative risk reduction of poor outcome in patients with acute stroke.

FIVE YEAR VIEW

Physical temperature reduction

Although the potential benefit of physical temperature reduction in acute stroke has been pointed out by many experts, more rigorous phase II studies need to be launched, followed by phase III randomized controlled trials.

Pharmacological temperature reduction

Within five years the PAIS study will have been completed. This study will provide evidence on the effect of pharmacological reduction of body temperature with high dose paracetamol on functional outcome in patients with acute stroke. If such an effect can be ascertained, a simple and cheap treatment will be readily available for patients with acute stroke.

KEY ISSUES

- In animal models, mild hypothermia started shortly after the onset of focal cerebral ischemia resulted in smaller infarct size.
- In patients with acute stroke, increased baseline body temperature is associated with poor outcome. This association may be limited to the first 12 to 24 hours from stroke onset.
- Mild therapeutic hypothermia improves short-term neurological recovery and survival in patients resuscitated after cardiac arrest. There is no evidence of a beneficial effect of hypothermia after traumatic brain injury.

- No phase III randomized controlled trials of physical cooling in acute stroke have been completed. Feasibility and safety are not sufficiently established and serious side effects such as hypotension, infections, and cardiac arrhythmias have been reported. More randomized phase II studies with various cooling devices are needed.
- Treatment with a daily dose of 6000 mg paracetamol results in a small but potentially worthwhile reduction in body temperature in patients with acute ischemic stroke. Further large randomized clinical trials are needed to study whether this reduction in body temperature leads to improved functional outcome.

REFERENCES

1. Thorvaldsen P, Davidsen M, Bronnum-Hansen H et al. Stable stroke occurrence despite incidence reduction in an aging population: stroke trends in the danish monitoring trends and determinants in cardiovascular disease (MONICA) population. *Stroke* 1999; 30:2529-2534.
2. International Stroke Trial Collaborative Group. The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. *Lancet* 1997; 349:1569-1581.
3. Hacke W, Kaste M, Skyhoj OT et al. Acute treatment of ischemic stroke. European Stroke Initiative (EUSI). *Cerebrovasc Dis* 2000; 3:22-33.
4. Baird TA Parsons MW, Phanh T et al. Persistent poststroke hyperglycemia is independently associated with infarct expansion and worse clinical outcome. *Stroke* 2003; 34:2208-2214.
5. Leonardi-Bee J, Bath PM, Phillips SJ et al. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke* 2002; 33:1315-1320.
6. Rowat AM, Dennis MS, Wardlaw JM. Hypoxaemia in acute stroke is frequent and worsens outcome. *Cerebrovasc Dis* 2006; 21:166-172.
7. Azzimondi G, Bassein L, Nonino F et al. Fever in acute stroke worsens prognosis. A prospective study. *Stroke* 1995; 26:2040-2043.
8. Castillo J, Davalos A, Marrugat J et al. Timing for fever-related brain damage in acute ischemic stroke. *Stroke* 1998; 29:2455-2460.
9. Hindfelt B. The prognostic significance of subfebrility and fever in ischaemic cerebral infarction. *Acta Neurol Scand* 1976; 53:72-79.
10. Reith J, Jorgensen HS, Pedersen PM et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996; 347:422-425.
11. Schwarz S, Hafner K, Aschoff A et al. Incidence and prognostic significance of fever following intracerebral hemorrhage. *Neurology* 2000; 54:354-361.
12. Wang Y, Lim LL, Levi C et al. Influence of admission body temperature on stroke mortality. *Stroke* 2000; 31:404-409.
13. Kato H, Araki T, Kogure K. Role of the excitotoxic mechanism in the development of neuronal damage following repeated brief cerebral ischemia in the gerbil: protective effects of MK-801 and pentobarbital. *Brain Res* 1990; 516:175-179.
14. Kuluz JW, Gregory GA, Han Y et al. Fructose-1,6-bisphosphate reduces infarct volume after reversible middle cerebral artery occlusion in rats. *Stroke* 1993; 24:1576-1583.
15. Baron JC. How healthy is the acutely reperfused ischemic penumbra? Cerebrovasc Dis 2005; Suppl 2:25-31.
16. Memezawa H, Smith ML, Siesjo BK. Penumbral tissues salvaged by reperfusion following middle cerebral artery occlusion in rats. Stroke 1992; 23:552-559.
17. Emsley HC, Tyrrell PJ. Inflammation and infection in clinical stroke. *J Cereb Blood Flow Metab* 2002; 22:1399-1419.
18. Vila N, Castillo J, Davalos A et al. Proinflammatory cytokines and early neurological worsening in ischemic stroke. *Stroke* 2000; 31:2325-2329.
19. Vila N, Reverter JC, Yague J et al. Interaction between interleukin-6 and the natural anticoagulant system in acute stroke. *J Interferon Cytokine Res* 2000; 20:325-329.
20. Dietrich WD, Busto R, Valdes I et al. Effects of normothermic versus mild hyperthermic forebrain ischemia in rats. *Stroke* 1990; 21:1318-1325.
21. Wood SC, Gonzales R. Hypothermia in hypoxic animals: mechanisms, mediators, and functional significance. *Comp Biochem Physiol B Biochem Mol Biol* 1996; 113:37-43.

22. Chopp M, Welch KM, Tidwell CD et al. Effect of mild hyperthermia on recovery of metabolic function after global cerebral ischemia in cats. *Stroke* 1988; 19:1521-1525.
23. Ginsberg MD, Sternau LL, Globus MY et al. Therapeutic modulation of brain temperature: relevance to ischemic brain injury. *Cerebrovasc Brain Metab Rev* 1992; 4:189-225.
24. Ginsberg MD, Busto R. Combating hyperthermia in acute stroke: a significant clinical concern. *Stroke* 1998; 29:529-534.
25. Takagi K, Ginsberg MD, Globus MY et al. Effect of hyperthermia on glutamate release in ischemic penumbra after middle cerebral artery occlusion in rats. *Am J Physiol* 1994; 267:H1770-H1776.
26. Globus MY, Busto R, Lin B et al. Detection of free radical activity during transient global ischemia and recirculation: effects of intraischemic brain temperature modulation. *J Neurochem* 1995; 65:1250-1256.
27. Dietrich WD, Busto R, Halley M et al. The importance of brain temperature in alterations of the blood-brain barrier following cerebral ischemia. *J Neuropathol Exp Neurol* 1990; 49:486-497.
28. Dietrich WD, Halley M, Valdes I et al. Interrelationships between increased vascular permeability and acute neuronal damage following temperature-controlled brain ischemia in rats. *Acta Neuropathol (Berl)* 1991; 81:615-625.
29. Huang ZG, Xue D, Preston E et al. Biphasic opening of the blood-brain barrier following transient focal ischemia: effects of hypothermia. *Can J Neurol Sci* 1999; 26:298-304.
30. Henker RA, Brown SD, Marion DW. Comparison of brain temperature with bladder and rectal temperatures in adults with severe head injury. *Neurosurgery* 1998; 42:1071-1075.
31. Jorgensen HS, Reith J, Pedersen PM et al: Body temperature and outcome in stroke patients. *Lancet* 1996; 348:193.
32. Boysen G, Christensen H. Stroke severity determines body temperature in acute stroke. *Stroke* 2001; 32:413-417.
33. Rosomoff HL. Hypothermia and cerebral vascular lesions. II. Experimental interruption of the middle cerebral artery during hypothermia. *J Neurosurg* 1956; 13:244-255.
34. Miyazawa T, Tamura A, Fukui S et al. Effect of mild hypothermia on focal cerebral ischemia. Review of experimental studies. *Neurol Res* 2003; 25:457-464.
35. Raimondi AJ, Clasen RA, Beattie EJ et al. The effect of hypothermia and steroid therapy on experimental cerebral injury. *Surg Gynecol Obstet* 1959; 108:333-338.
36. Meden P, Overgaard K, Pedersen H et al. The influence of body temperature on infarct volume and thrombolytic therapy in a rat embolic stroke model. *Brain Res* 1994; 647:131-138.
37. Baker CJ, Onesti ST, Barth KN et al. Hypothermic protection following middle cerebral artery occlusion in the rat. *Surg Neurol* 1991; 36:175-180.
38. Baker CJ, Onesti ST, Solomon RA. Reduction by delayed hypothermia of cerebral infarction following middle cerebral artery occlusion in the rat: a time-course study. *J Neurosurg* 1992; 77:438-444.
39. Onesti ST, Baker CJ, Sun PP et al. Transient hypothermia reduces focal ischemic brain injury in the rat. *Neurosurgery* 1991; 29:369-373.
40. Michenfelder JD, Milde JH. Failure of prolonged hypocapnia, hypothermia, or hypertension to favorably alter acute stroke in primates. *Stroke* 1977; 8:87-91.
41. Steen PA, Milde JH, Michenfelder JD. The detrimental effects of prolonged hypothermia and rewarming in the dog. *Anesthesiology* 1980; 52:224-230.
42. Legos JJ, Mangoni AA, Read SJ et al. Programmable microchip monitoring of post-stroke pyrexia: effects of aspirin and paracetamol on temperature and infarct size in the rat. *J Neurosci Methods* 2002; 113:159-166.
43. Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest: *N Engl J Med* 2002; 346:549-556.

44. Bernard SA, Gray TW, Buist MD et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002; 346:557-563.
45. Holzer M, Bernard SA, Hachimi-Idrissi S et al. Hypothermia for neuroprotection after cardiac arrest: systematic review and individual patient data meta-analysis. *Crit Care Med* 2005; 33:414-418.
46. Clasen RA, Pandolfi S, Stuart D et al. Hypothermia and hypotension in experimental cerebral injury. *J Neuropathol Exp Neurol* 1968; 27:127-128.
47. Laskowski EJ, Klatzo I, Baldwin M. Experimental study of the effects of hypothermia on local brain injury. *Neurology* 1960; 10:499-505.
48. Dietrich WD, Alonso O, Halley M et al. Delayed posttraumatic brain hyperthermia worsens outcome after fluid percussion brain injury: a light and electron microscopic study in rats. *Neurosurgery* 1996; 38:533-541.
49. Clifton GL, Allen S, Barrodale P et al. A phase II study of moderate hypothermia in severe brain injury. *J Neurotrauma* 1993; 10:263-271.
50. Clifton GL. Systemic hypothermia in treatment of severe brain injury: a review and update. *J Neurotrauma* 1995; 12:923-927.
51. Shiozaki T, Sugimoto H, Taneda M et al. Effect of mild hypothermia on uncontrollable intracranial hypertension after severe head injury. *J Neurosurg* 1993; 79:363-368.
52. Clifton GL. Systemic hypothermia in treatment of severe brain injury. *J Neurosurg Anesthesiol* 1995; 7:152-156.
53. Marion DW, Obrist WD, Carlier PM et al. The use of moderate therapeutic hypothermia for patients with severe head injuries: a preliminary report. *J Neurosurg* 1993; 79:354-362.
54. Marion DW, Darby JM. Hyperventilation and head injury. *J Neurosurg* 1995; 83:1113-1114.
55. Marion DW, Penrod LE, Kelsey SF et al. Treatment of traumatic brain injury with moderate hypothermia. *N Engl J Med* 1997; 336:540-546.
56. Rosomoff HL, Kochanek PM, Clark R et al. Resuscitation from severe brain trauma. *Crit Care Med* 1996; 24:S48-S56.
57. Safar P, Kochanek PM. Lack of effect of induction of hypothermia after acute brain injury. *N Engl J Med* 2001; 345:66.
58. Alderson P, Gadkary C, Signorini DF. Therapeutic hypothermia for head injury. *Cochrane Database Syst Rev* 2004; 4:CD001048.
59. Jiang JY, Xu W, Li WP et al. Effect of long-term mild hypothermia or short-term mild hypothermia on outcome of patients with severe traumatic brain injury. *J Cereb Blood Flow Metab* 2006; 26:771-776.
60. Krieger DW, De Georgia MA, bou-Chebl A et al. Cooling for acute ischemic brain damage (cool aid): an open pilot study of induced hypothermia in acute ischemic stroke. *Stroke* 2001; 32:1847-1854.
61. Steiner T, Friede T, Aschoff A et al. Effect and feasibility of controlled rewarming after moderate hypothermia in stroke patients with malignant infarction of the middle cerebral artery. *Stroke* 2001; 32:2833-2835.
62. Schwab S, Schwarz S, Spranger M et al. Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. *Stroke* 1998; 29:2461-2466.
63. De Georgia MA, Krieger DW, Bou-Chebl A et al. Cooling for Acute Ischemic Brain Damage (COOL AID): a feasibility trial of endovascular cooling. *Neurology* 2004; 63:312-317.
64. Kammersgaard LP, Rasmussen BH, Jorgensen HS et al. Feasibility and safety of inducing modest hypothermia in awake patients with acute stroke through surface cooling: A case-control study: the Copenhagen Stroke Study. *Stroke* 2000; 31:2251-2256.
65. Meijer RJ, Visser H, Koudstaal PJ et al. Lowering body temperature in acute ischemic stroke without artificial ventilation and heavy sedation: A feasibility study. *Journal of Stroke & Cerebrovascular Diseases* 2001; 10:157-160.

66. Knoll T, Wimmer ML, Gumpinger F et al. The low normothermia concept--maintaining a core body temperature between 36 and 37 degrees C in acute stroke unit patients. *J Neurosurg Anesthesiol* 2002; 14:304-308.
67. Weber UJ, Indredavik B, Norrving B et al. The Nordisc cooling stroke study. *The American Stroke Association* 2003 (abst.CTP8) [Ref 6838].
68. Leslie K, Williams D, Irwin K et al. Pethidine and skin warming to prevent shivering during endovascular cooling. *Anaesth Intensive Care* 2004; 32:362-367.
69. Mokhtarani M, Mahgoub AN, Morioka N et al. Buspirone and meperidine synergistically reduce the shivering threshold. *Anesth Analg* 2001; 93:1233-1239.
70. Doufas AG, Sessler DI. Physiology and clinical relevance of induced hypothermia. *Neurocrit Care* 2004; 1:489-498.
71. Kulkens S, Ringleb PA, Hacke W. Recommendations of the European Stroke Initiative (EUSI) for treatment of ischemic stroke--update 2003. Part 2: prevention and rehabilitation]. *Nervenarzt* 2004; 75:380-388.
72. Masferrer JL, Zweifel BS, Seibert K et al. Selective regulation of cellular cyclooxygenase by dexamethasone and endotoxin in mice. *J Clin Invest* 1990; 86:1375-1379.
73. Kargman S, Wong E, Greig GM et al. Mechanism of selective inhibition of human prostaglandin G/H synthase-1 and -2 in intact cells. *Biochem Pharmacol* 1996; 52:1113-1125.
74. Seibert K, Masferrer JL. Role of inducible cyclooxygenase (COX-2) in inflammation. *Receptor* 1994; 4:17-23.
75. Mattammal MB, Zenser TV, Brown WW et al. Mechanism of inhibition of renal prostaglandin production by acetaminophen. *J Pharmacol Exp Ther* 1979; 210:405-409.
76. Botting RM. Mechanism of action of acetaminophen: is there a cyclooxygenase 3? *Clin Infect Dis 2000*; 31:S202-S210.
77. Mitchell JA, Akarasereenont P, Thiemermann C et al. Selectivity of nonsteroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci* 1993; 90:11693-11697.
78. Koennecke HC, Leistner S. Prophylactic antipyretic treatment with acetaminophen in acute ischemic stroke: a pilot study. *Neurology* 2001; 57:2301-2303.
79. Kasner SE, Wein T, Piriyawat P et al. Acetaminophen for altering body temperature in acute stroke: a randomized clinical trial. *Stroke* 2002; 33:130-134.
80. Dippel DW, van Breda EJ, van Gemert HM et al. Effect of paracetamol (acetaminophen) on body temperature in acute ischemic stroke: a double-blind, randomized phase II clinical trial. *Stroke* 2001; 32:1607-1612.
81. Dippel DW, van Breda EJ, van der Worp HB et al. Effect of paracetamol (acetaminophen) and ibuprofen on body temperature in acute ischemic stroke PISA, a phase II double-blind, randomized, placebo-controlled trial [ISRCTN98608690]. *BMC Cardiovasc Disord* 2003; 3:2.
82. Dippel DW, van Breda EJ, van der Worp HB et al. Timing of the effect of acetaminophen on body temperature in patients with acute ischemic stroke. Neurology 2003; 61:677-679.
83. Sulter G, Elting JW, Maurits N et al. Acetylsalicylic acid and acetaminophen to combat elevated body temperature in acute ischemic stroke. *Cerebrovasc Dis* 2004; 17:118-122.
84. van Breda EJ, van der Worp HB, van Gemert HM et al. Reduction of body temperature with paracetamol in patients with acute stroke: randomised clinical trials are needed. *Cerebrovasc Dis* 2004; 18:350.
85. van Breda EJ, van der Worp HB, van Gemert HM et al. Treatment of stroke by reducing the body temperature; 'Paracetamol (acetaminophen) in stroke' (PAIS): start of a clinical trial. *Ned Tijdschr Geneeskd* 2003; 147:1976-1978.
86. Correia M, Silva M, Veloso M. *Cooling therapy for acute stroke*. Cochrane DatabaseSyst Rev 2000; 2:CD001247.

Temperature-lowering therapy for acute stroke

Heleen M Den Hertog[1], H Bart van der Worp[2], Mei-Chiun Tseng[3], Diederik WJ Dippel[1]

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Business Management, National Sun Yat-Sen University, Kaohsiung, Taiwan

Stroke 2009 Epub

Based on *Cochrane Database Syst Rev* 2009; 1:CD001247

BACKGROUND

Body temperatures over 37.5˚C have been observed in 4 to 25% of patients within the first 24 to 36 hours after stroke onset and are associated with poor long-term outcome. In the observational Copenhagen Stroke study, a 1°C increase in body temperature measured within 12 hours after stroke onset doubled the odds of poor outcome.

In animal models of focal cerebral ischemia, cooling reduces infarct volume. Hypothermia is successfully used in cardiac surgery and has been associated with a more favorable neurological outcome in patients who were resuscitated after cardiac arrest.

These observations suggest that reduction of body temperature and prevention of fever may improve functional outcome after stroke. However, the potentially beneficial effects of temperature-lowering therapy might be offset by side effects such as infections, cardiac arrhythmias, hemorrhagic transformation of infarcts, and deep venous thrombosis.

OBJECTIVES

The aim of this review was to assess the relation between interventions to reduce body or brain temperature and functional outcome or death in patients with acute stroke, and to determine whether there is any clear evidence that temperature reduction of any kind is beneficial, or whether the intervention is sufficiently promising to merit further trials.

SEARCH STRATEGY

We updated the 1999 Cochrane review "Cooling therapy for acute stroke". Relevant trials were identified in the Specialised Register of Controlled Trials (last search, December 2007). Additional searches were performed in MEDLINE and EMBASE (January 1998 to December 2007). We scanned references and contacted authors of included trials.

SELECTION CRITERIA

We considered all completed randomized or non-randomized controlled clinical trials, published or unpublished, where pharmacological and/or physical strategies to reduce body or brain temperature were applied in patients with acute ischemic stroke or intracerebral hemorrhage and the effect on clinical outcome was reported.

DATA COLLECTION AND ANALYSIS

Two reviewers independently selected trials for inclusion. Thereafter, two of three reviewers assessed the methodological quality of each identified trial and extracted the data. Outcome measures were death or dependency (modified Rankin Scale score >2) and death at the end of follow-up, and adverse effects.

MAIN RESULTS

Five pharmacological temperature reduction trials and three physical temperature reduction trials involving a total of 423 patients were included. We found no statistically significant effect of pharmacological or physical temperature-lowering therapy in reducing the risk of death or dependency (OR 0.9, 95% CI 0.6-1.4) (Figure 1) or death (OR 0.9, 95% CI 0.5-1.5). Both interventions were associated with a non-significant increase in the occurrence of infections (OR 1.5, 95% CI 0.8-2.6).

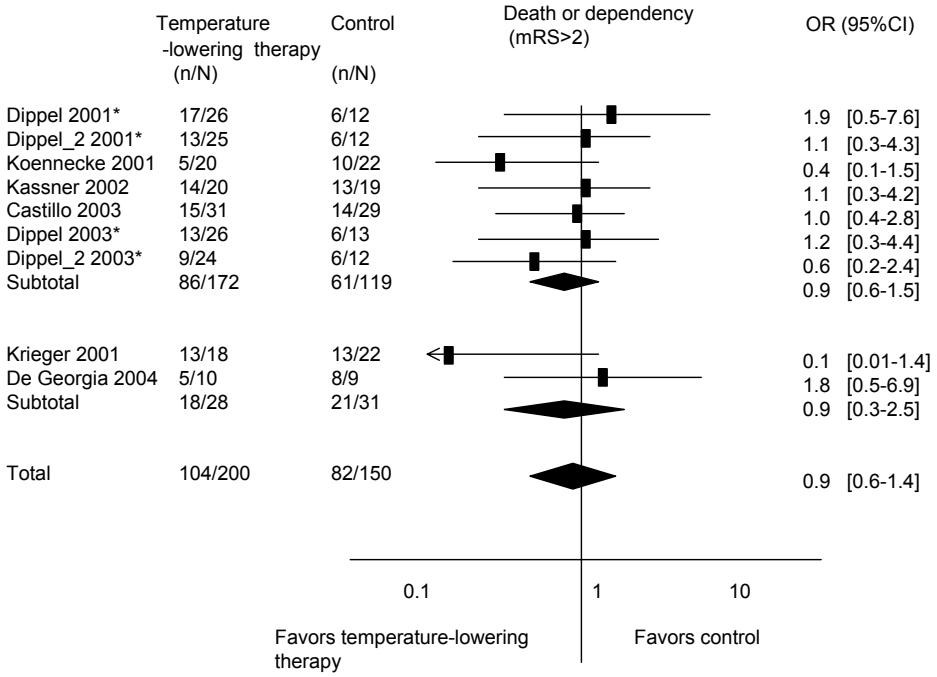

Figure 1: Effect of temperature-lowering therapy on death or dependency (score on the modified Rankin Scale >2) at final follow up.

*Two intervention groups; the number of patients with poor outcome and the total number of patients in the control group were divided by 2, in order to avoid multiple comparisons using the same subset of patients.

IMPLICATIONS FOR PRACTICE AND FUTURE RESEARCH

There is currently no evidence from randomized trials to support routine use of physical or pharmacological strategies to reduce temperature in patients with acute stroke.

Large randomized clinical trials are needed to study the safety, optimal duration and the effectiveness of both physical and pharmacological temperature reduction in patients with acute stroke. Attention should be paid to trial quality issues such as randomization, blinded outcome assessment, relevant intervention contrast and relevant outcome measures and to the occurrence of infections.

REFERENCES

The full text, data tables, analyses, results and reference list of this article are available in the Cochrane Library.[1]

1. Heleen M den Hertog, H Bart van der Worp, Mei-Chiun Tseng et al. Cooling therapy for acute stroke. *The Cochrane Database Syst Rev* 2009; 1:CD001247.

Paracetamol for temperature reduction in acute stroke: potential, but unproven benefits

Heleen M den Hertog[1], H Bart van der Worp[2], H Maarten A van Gemert[3], Diederik WJ Dippel[1]

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rupolph Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Neurology, Meander Medical Center, Amersfoort, The Netherlands

Stroke 2007; 38:e131

TO THE EDITOR:

We read the review by Hemmen and Lyden on induced hypothermia for acute ischemic stroke[1] with interest. The review focuses on physical cooling methods in acute stroke. Some points however, deserve in our opinion a more extensive discussion.

The authors state that controlling body temperature below 36.5 °C has been proven to correlate with good clinical outcome. They do not cite controlled clinical trials to support their point, but refer to two observational studies instead.

Indeed, well-designed studies have shown a consistent relationship between increased body temperature measured within 12 to 24 hours after stroke onset, and outcome, but not beyond that interval.[2] These studies suggest that reductions in body temperature of 0.5 degrees Celsius might lead to a relative risk reduction in poor outcome of 10%. Reductions in body temperature of this magnitude have been proven feasible in two pilot studies of high-dose paracetamol.[3,4]

In our view, the uncontrolled studies and small randomized trials in acute ischemic stroke that have been conducted so far have not provided sufficient evidence of safety and feasibility of physical cooling methods. We need more randomized phase II studies with several cooling devices. These studies should explore the relation between intensity of treatment, temperature reduction, and adverse events, such as arterial hypotension, infections, and cardiac arrhythmias. On the other hand, the risk of complications, the high costs of this mode of treatment, and other logistical barriers imply that there is a need for a simple medical intervention that may reduce body temperature to lesser extent, but is cheap and safe.

Although several national and international guidelines recommend the use of paracetamol in patients with fever after stroke, it is recognized that there is no evidence available of a therapeutic effect.[5] Moreover, most patients develop fever after the first 24 hours, and it appears unlikely that late treatment will have an effect on outcome.

Why not treat all patients early with high-dose paracetamol? The main reason is that this treatment strategy has not yet been proven to improve functional outcome after stroke. In addition, paracetamol can be dangerous in high doses and cause liver failure. Use of paracetamol may delay the recognition of pneumonia and urinary tract infections, and thus lead to delayed treatment and poor outcome.

We conclude that a large pragmatic placebo-controlled randomized clinical trial of paracetamol in acute ischemic stroke is needed. Exactly such a trial is underway in the Netherlands, PAIS: Paracetamol (Acetaminophen) In Stroke. More than 1200 patients have been included in the trial as of January 2007. We expect to be able to present the trial's results in 2008.[6]

REFERENCES

1. Hemmen TM, Lyden PD. Induced hypothermia for acute stroke. *Stroke* 2007; 38:794-799.
2. Reith J, Jorgensen HS, Pedersen PM et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996; 347:422-425.
3. Dippel DW, van Breda EJ, van Gemert HM et al. Effect of paracetamol (acetaminophen) on body temperature in acute ischemic stroke: a double-blind, randomized phase II clinical trial. *Stroke* 2001; 32:1607-1612.
4. Dippel DW, van Breda EJ, van Gemert HM et al. Effect of paracetamol (acetaminophen) and ibuprofen on body temperature in acute ischemic stroke PISA, a phase II double-blind, randomized, placebo-controlled trial. *BMC Cardiovasc Disord* 2003; 3:2
5. Adams H, Adams R, del Zoppo G et al. Guidelines for the Early Management of Patients With Ischemic Stroke. 2005 Guidelines Update. A Scientific Statement From the Stroke Council of the American Heart Association/American Stroke Association. *Stroke* 2005; 36;916-923.
6. Van Breda EJ, van der Worp HB, van Gemert HM et al. PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial [ISRCTN 74418480] *BMC Cardiovasc Disord* 2005; 5:24.

Chapter 3

Pharmacological temperature-lowering therapy

"Introduction of sliding dichotomy analysis in the Paracetamol (Acetaminophen) In Stroke trial: a protocol change

Heleen M den Hertog[1], H Bart van der Worp[2], H Maarten A van Gemert[3], Ale Algra[2,4], L Jaap Kappelle[2], Jan van Gijn[2], Peter J Koudstaal[1], Diederik WJ Dippel[1], on behalf of the PAIS investigators

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Neurology, Meander Medical Center, Amersfoort, The Netherlands

[4]Julius Center for Health Sciences and Primary care, University Medical Center Utrecht, Utrecht and Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

BMC Cardiovasc Disord 2008; 8:29

ABSTRACT

Background The Paracetamol (Acetaminophen) In Stroke (PAIS) study is a phase III multicenter, double blind, randomized, placebo-controlled clinical trial of high-dose paracetamol in patients with acute stroke. The trial compares treatment with a daily dose of 6 g paracetamol, started within 12 hours of symptom onset, with matched placebo. The purpose of this study is to assess whether treatment with paracetamol for 3 days will result in improved functional outcome through a modest reduction in body temperature or prevention of fever.

The previously planned statistical analysis based on a dichotomization of the scores on the modified Rankin Scale (mRS) may not make the most efficient use of the available baseline information. Therefore, the planned primary analysis of the PAIS study has been changed from fixed dichotomization of the mRS to a sliding dichotomy analysis.

Methods Instead of taking a single definition of good outcome for all patients, the definition is tailored to each individual patient's baseline prognosis on entry into the trial.

Conclusion The protocol change was initiated because of both advances in statistical approaches and to increase the efficiency of the trial by improving statistical power.

Trial registration: Current Controlled Trials [ISRCTN74418480]

abstract

INTRODUCTION

The Paracetamol (Acetaminophen) In Stroke (PAIS) Study is a phase III multicenter, double blind, randomized, placebo-controlled clinical trial of high-dose paracetamol in patients with acute stroke. The trial compares treatment with a daily dose of 6 g paracetamol, started within 12 hours after symptom onset, with matched placebo. The purpose of this study is to assess whether early treatment with paracetamol will result in improved long-term functional outcome through a modest reduction in body temperature or prevention of fever.[1]

In the original protocol, the primary outcome measure is a dichotomized score on the modified Rankin Scale (mRS)[2] assessed at 3 months from onset of symptoms, with good functional outcome defined as a score of 0-2 and poor functional outcome as a score of 3-death.

The common approach of dichotomizing the mRS, an ordinal outcome scale, has several disadvantages. First, it may not correspond with everyday clinical practice. Dichotomized outcome analyses convert ordinal scales into binary outcome measures. Most treatment strategies tested in acute stroke trials are not expected to be completely curative, but to lead to improvement. Therefore, it is also informative to show that treatment moves patients from severe to moderate disability or from moderate disability to recovery, and not only to demonstrate differences in the numbers of patients with a good or poor functional outcome. Secondly, dichotomization may limit statistical power. The sample size calculation used for the PAIS study assumes that each patient in the placebo group has a 50% probability of poor outcome at 3 months.[1] In practice, there will be substantial prognostic heterogeneity because of differences in baseline variables between patients. If this is not taken into account, the chance of finding a true treatment effect may be reduced. Thirdly, by assessing only a single health state transition, investigators may be forced to discard a substantial amount of outcome information. This may lead to under-estimation of treatment benefit or harm.

Because of these disadvantages of dichotomization, new approaches to outcome analysis have been proposed and tested in acute stroke trials.[2-7] These novel outcome analyses consider the full range of the ordinal outcome scale and lead to a single and meaningful estimate of the treatment effect. One of these new approaches is the so-called "sliding dichotomy".[5] Other approaches are shift analysis and analyses that make use of ordinal logistic regression.[3,4,7]

For the PAIS study, we decided to change the planned analysis for the primary outcome measure from a fixed dichotomy to a sliding dichotomy analysis. This protocol change was initiated because of both advances in statistical approaches and to increase the efficiency of the trial by improving statistical power.

METHODS

Instead of taking a single definition of good outcome for all patients, the definition is tailored to each individual patient's baseline prognosis on entry into the trial. The outcome of an individual patient is thus regarded as good or poor depending on what would have been expected based on the severity of stroke and other prognostic factors.

Blinded data from the PAIS study[1] were used to illustrate this procedure of the sliding dichotomy analysis (Figure 1). A prognostic index was derived from a logistic regression model for favorable outcome (defined as mRS ≤2), which was generated on the basis of blinded observations in the trial. The prognostic index included age, sex, stroke severity (according to the NIHSS), previous stroke, stroke type (ischemic stroke versus intracerebral hemorrhage), and diabetes mellitus.

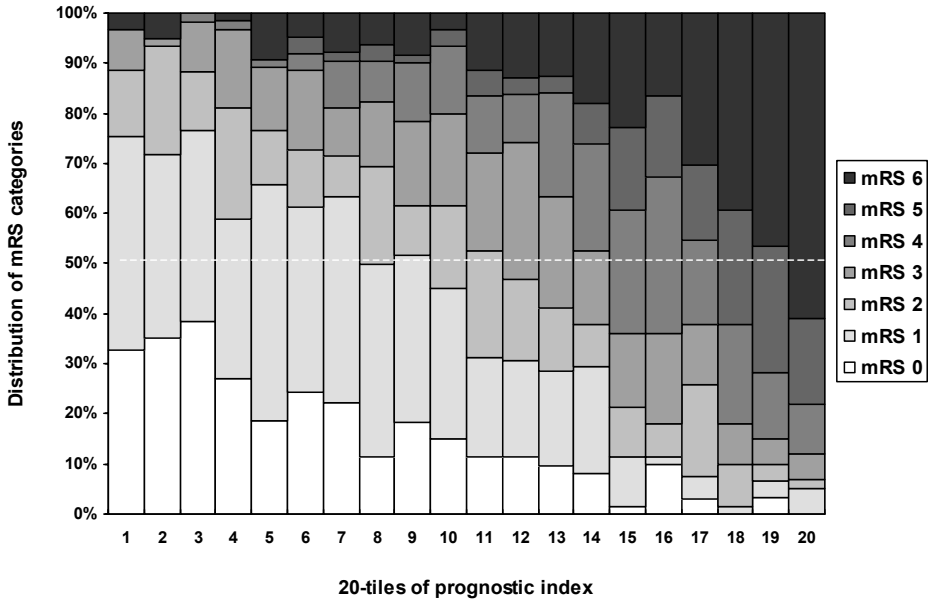

Figure 1: Distribution of predicted outcome scores on the modified Rankin Scale[2] at three months over ordered vingtiles of a prognostic index for good outcome in the PAIS trial.[1] Six bands were defined in such a way that the median of the mRS in that band was equal to the next score on the mRS (1-6). Cut-off points were taken between quantile 7 and 8, 11 and 12, 14 and 15, 17 and 18, and 19 and 20.

The study population was then divided into 20 quantiles (vingtiles) of the baseline prognostic index. For each vingtile, the distribution of outcomes on the mRS was computed (Figure 1). Thereafter, 6 bands (clusters of vingtiles) were defined in such a way that the median of the mRS in that band would be closest to one of the six grades of the mRS (1-6). Cut-off points would thus be taken between vingtiles 7 and 8, 11 and 12, 14 and 15, 17 and 18, and 19 and 20. Patients are considered to have improved beyond expectation when their score on the mRS is lower than the median grade of patients with a similar

prognostic index. The primary effect estimate will be the odds ratio for improvement. For example, for a patient of whom the prognostic risk is in the 12th vingtile, the median predicted outcome mRS is 3 and an outcome mRS <3 would be taken as favorable outcome (Figure 1). This varying bandwidth makes more efficient use of the data than an approach based on tertiles or quartiles, as originally proposed.[5] Note that the choice of cut-off point is completely independent of the treatment effect, as the data are blinded.

Although the definition of the new primary outcome is based on prognostic indicators at baseline, imbalances in these and other prognostic factors between the treatment groups may still confound the assessment of treatment effect. Adjustments will therefore be made with a multiple logistic regression model that includes time to treatment, baseline body temperature, stroke severity (as assessed with the NIHSS), stroke type (ischemic stroke versus intracerbral hemorrhage versus), ischemic stroke subtype (lacunar versus non-lacunar) and thrombolytic therapy, as proposed previously[1], as well as for age, sex, previous stroke and diabetes mellitus.

Secondary effect measures will include an estimate of the odds ratio for improvement, estimated by means of ordinal logistic regression analysis.

We will also estimate the adjusted odds ratio for the former primary outcome, i.e. favorable functional outcome, as defined as a score < 3, and alternative dichotomizations of the mRS (0 to 3 versus 4 to 6). As reported before[1], the score on the Barthel index at 3 months, body temperature at 24 hours from start of treatment, and quality of life at three months as assessed with the EuroQol-5D will also be assessed.

DISCUSSION

Several approaches to outcome analysis have recently been applied in acute stroke trials, including shift analysis[6], a rank test of original ordinal data, ordinal logistic regression[3], and the concept of a sliding dichotomy[5].

Analyzing the full range of ordinal data for functional outcome has shown to be more statistically efficient than collapsing data.[3] A recent study compared statistical approaches to the analysis of differences in functional outcome as measured on ordinal scales. The authors concluded that methods that made use of the full range of ordinal outcomes were more sensitive to treatment effects. However, in this study methods that incorporate baseline prognostic information, such as the sliding dichotomy, could not be evaluated.[3]

Shift analysis[6] is not a formal test, but a calculus to estimate the proportion of patients moving from one category on the ordinal scale to the next. This can be a useful measure to gain insight in the size of a treatment effect. A disadvantage of shift analysis is that the uncertainty concerning the effect estimate cannot be quantified, as in statistical effect estimation.

The term 'shift test' is used to designate the Mantel-Haenszel (CMH) test that compares two ordered outcome distributions after adjusting for one or more baseline variables. The test provides a p-value, but not an effect estimate. The van Elteren variant of the test was employed in the analysis of the results of the GAIN trial[8] and in a post-hoc analysis of NINDS and ECASS-2 trials[9] Ordinal logistic regression was used to estimate an effect size with its corresponding 95% confidence interval.

Classic statistical methods to compare distributions on an ordinal scale require rank tests. Translation of the results of a rank test (a p-value) to an estimate of the treatment effect is not straightforward; this may be done either by shift analysis (with the aforementioned drawbacks) or by ordinal logistic regression. This analysis does not make use of the available baseline information. Ordinal logistic regression may be used to estimate treatment effects in studies with ordered outcomes. The relative risk of a transition is estimated as an odds ratio. An assumption is that any treatment effect is similar across outcome levels, i.e. the odds of moving from mRS level 3 to 2 are similar to the odds of moving from level 5 to 4. In the Optimizing Analysis of Stroke Trials (OAST) study, a study to assess which statistical approaches are most efficient in analyzing outcomes from stroke trials, the assumption of proportional odds was not met in 8 of the 55 datasets according to the authors, but they did not specify how they tested for this assumption.[3] Furthermore, this method might be inefficient when treatment effects cluster at one transition. When this approach was tested in a large dataset of patients with severe head injury, it did not perform better than the sliding dichotomy.[5] Effect estimates should be meaningful from a clinical point of view. In ordinal logistic regression, meaningful interpretation is hampered by the point that moving one category up on the mRS may have a different clinical interpretation when it concerns low mRS scores, compared to high mRS scores.

In our view, these are important arguments for not using ordinal regression in the *primary* outcome analysis.

An advantage of the sliding dichotomy approach is that it makes the least assumptions about the type of patients who will be included in the study, the type of outcome they will experience, and the treatment effect pattern the treatment strategy under study will exert. It provides a simple outcome measure that is relatively easy to interpret, i.e. the relative risk of improvement beyond the level that could be expected from baseline prognostic information.[5] To our opinion, this approach is to be preferred in the analysis of treatment effects employing the full range of outcomes on the mRS. With the concept of sliding dichotomy, each individual patient's baseline prognosis is taken into account. This approach may be more relevant for clinical practice and may improve statistical power, as patients at the prognostic extremes have the potential to contribute to the estimation of the treatment effect.

An exact, parametric approach to sample size estimation for studies that make use of the sliding dichotomy approach for ordered categorical outcome variables is not

available. Simulation studies of randomized clinical trials in traumatic brain injury that made use of the Glasgow Outcome Scale[10], suggest that ".. substantial gains in statistical efficiency can be made". Either of these approaches along with adjustment for baseline covariates gave efficiency gains equivalent to reducing the required sample size by up to 50%.[5,11]

We realize that this approach may also have disadvantages. Patients, care givers, and clinicians may consider the results of this way of analysis more difficult to interpret than collapsing data into a binary outcome. We wonder whether this is really the case. Taken at face value, a transition across the boundary between mRS 2 and mRS 3 does not tell us much at all about the real health benefit of a treatment, and a common odds ratio based on sliding dichotomy, may be in fact more informative for those who have become familiar with this type of analysis.

CONCLUSION

In summary, because of the drawbacks of dichotomization, the primary outcome analysis of the multicenter acute stroke trial PAIS has been changed from fixed dichotomy of the mRS to the sliding dichotomy analysis. It has been approved by the PAIS steering committee on April 1, 2008, before inclusion into the trial was completed. This approach may be more relevant for clinical practice and is expected to increase the efficiency of the trial by improving statistical power.

REFERENCES

1. van Breda EJ, van der Worp HB, van Gemert HM et al. PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial [ISCRTN 74418480]. *BMC Cardiovasc Disord* 2005; 5:24.
2. van Swieten JC, Koudstaal PJ, Visser MC et al. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988; 19:604-607.
3. Bath PM, Gray LJ, Collier T et al. Can we improve the statistical analysis of stroke trials? Statistical reanalysis of functional outcomes in stroke trials. *Stroke* 2007; 38:1911-1915.
4. Joffe MM, Greenland S. Standardized estimates from categorical regression models. *Stat Med* 1995; 14:2131-2141.
5. Murray GD, Barer D, Choi S et al. Design and analysis of phase III trials with ordered outcome scales: the concept of the sliding dichotomy. *J Neurotrauma* 2005; 22:511-517.
6. Saver JL. Novel end point analytic techniques and interpreting shifts across the entire range of outcome scales in acute stroke trials. *Stroke* 2007; 38:3055-3062.
7. Whitehead J. Sample size calculations for ordered categorical data. *Stat Med* 1993; 12:2257-2271.
8. Lees KR, Zivin JA, Ashwood T et al. NXY-059 for acute ischemic stroke. *N Engl J Med* 2006; 354:588-600.
9. Savitz SI, Lew R, Bluhmki E et al. Shift analysis versus dichotomization of the modified Rankin scale outcome scores in the NINDS and ECASS-II trials. *Stroke* 2007; 38:3205-3212.
10. Jennett B, Bond M. Assessment of outcome after severe brain damage *Lancet* 1975; 1:480-484.
11. Murray GD, on behalf of the IMPACT investigators. Approaches to outcome analysis in traumatic brain injury trials. Abstract for the 8th International Neurotrauma Symposium 2006, Rotterdam, The Netherlands. *J Neurotrauma* 2006: 735.

High-dose paracetamol in acute stroke: the Paracetamol (Acetaminophen) In Stroke trial (PAIS)

Heleen M den Hertog[1], H Bart van der Worp[2], H Maarten A van Gemert[3], Ale Algra[2,4], L Jaap Kappelle[2], Jan van Gijn[2], Peter J Koudstaal[1], Diederik WJ Dippel[1], on behalf of the PAIS investigators

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Neurology, Meander Medical Center, Amersfoort, The Netherlands

[4]Julius Center for Health Sciences and Primary care, University Medical Center Utrecht, Utrecht and Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Lancet Neurol 2009; 8:434-440

ABSTRACT

Background High body temperature in the first 12 to 24 hours after stroke onset is associated with poor functional outcome. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial aimed to assess whether early treatment with paracetamol improves functional outcome in patients with acute stroke by reducing body temperature and preventing fever.
Methods In a multicenter, randomized, double-blind placebo-controlled trial, patients with ischemic stroke or intracerebral hemorrhage and body temperature between 36˚C and 39˚C were randomly assigned with paracetamol (6 g daily) or placebo within 12 hours from symptom onset. Treatment allocation was based on a computer-generated list of random numbers with varying block size. The primary outcome was the odds ratio for improvement beyond expectation on the modified Rankin scale at 3 months, according to the sliding dichotomy approach. This trial is registered, number ISRCTN74418480.
Findings Between March 2003, and May 2008, 1400 patients were randomly allocated treatment. 260 of 697 (37%) patients receiving paracetamol and 232 of 703 (33%) receiving placebo improved beyond expectation (adjusted OR 1.20; 95% CI 0.96 to 1.50). In a post-hoc analysis of patients with baseline body temperature between 37°C and 39°C, treatment with paracetamol was associated with improved outcome (adjusted OR 1.43; 95% CI 1.02 to 1.97). There were 55 serious adverse events in the paracetamol group (8%) and 70 in the placebo group (10%).
Interpretation These results do not provide routine use of high-dose paracetamol in patients with acute stroke. Paracetamol may have a beneficial effect on functional outcome in patients admitted with a body temperature between 37.0°C and 39.0°C, but this post-hoc finding needs further study.

abstract

INTRODUCTION

Many patients admitted with stroke have fever or subfebrile body temperatures, which are associated with case fatality and poor functional outcome. About a third of patients have temperatures greater than 37.5˚C within the first hours after stroke onset.[1-4] The odds of poor outcome were doubled for every degree increase in body temperature, measured within 12 hours of stroke onset.[4] This association was independent of initial stroke severity, lesion volume, age, sex, and stroke type. The relation with clinical outcome is probably limited to body temperatures measured in the first 12 to 24 hours of stroke onset.[3-5]

High body temperature may be a direct consequence of stroke or a result of accompanying infections. Studies in animals have suggested that high body temperature increases damage caused by cerebral ischemia through increased metabolic demands, an increased release of neurotransmitters, increased free-radical production, breakdown of the blood-brain barrier, and increased proteolysis.[6] Mitigation of even mild spontaneous hyperthermia conferred neuroprotection in animal models[6], and induced hypothermia reduced infarct volume and improved functional outcome.[7] Therefore, reduction of body temperature and prevention of fever may form a promising approach in treatment to improve functional outcome after stroke.

Guidelines for the treatment of patients with acute ischemic stroke[8,9] or intracerebral hemorrhage[10] recommend antipyretic drugs in patients with fever or body temperature above 37.5˚C, and most European stroke specialists view the monitoring of body temperature as an essential component of care in stroke units.[11] However, no evidence from randomized trials has shown that strategies to prevent or treat high body temperature reduce case fatality and improve functional outcome after stroke.

Paracetamol (acetaminophen) is one of the most commonly used antipyretic drugs. Potent inhibition of prostaglandin production in the central nervous system presumably confers its antipyretic properties.[12] Paracetamol is usually well tolerated by patients with acute stroke and has almost no side effects in doses up to 6 g per day.[13-16] In patients with acute ischemic stroke, paracetamol at a daily dose of 6 g reduces body temperature by about 0.3˚C within 4 hours from start of treatment.[14]

In the Paracetamol (Acetaminophen) In Stroke (PAIS) trial, we aimed to assess whether early treatment with paracetamol improves functional outcome in patients with acute stroke.

METHODS

Patients

PAIS was a multi-center, randomized, double-blind, placebo-controlled, clinical trial in which patients were enrolled between March 2003, and May 2008. The study protocol has been published previously.[17] In brief, patients were eligible for inclusion if they were 18 years or older, had a clinical diagnosis of ischemic stroke or intracerebral hemorrhage, and were able to receive the study drug within 12 hours of symptom onset. In patients who noticed symptoms after waking from sleep, the time they were last seen well was taken as the time of symptom onset. The clinical diagnosis was confirmed with computed tomography or magnetic resonance imaging. Patients were excluded if they had a body temperature below 36˚C or over 39˚C, a history of liver disease or alcohol abuse, high concentrations of liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, or gamma-glutamyl transferase increased to more than twice the upper limit of normal), hypersensitivity to paracetamol, or impairment before stroke that had led to dependency (modified Rankin scale (mRS) >2)[18], or if death appeared imminent.

The trial was approved by national and local institutional review boards, and written informed consent was obtained from all patients or from their legal representatives.

Randomization and masking

Patients were randomly allocated to paracetamol or an identical placebo given orally or rectally. Patients treated with paracetamol received a daily dose of 6 times 1 g for 3 days consecutively. The study drug was provided in white paper boxes, with ascending numbers. Treatment allocation was based on a computer-generated list of random numbers with varying block size and linked to a unique treatment number. An independent statistician, who was otherwise not involved in the study, provided the list. The pharmacist of each center received a confidential list that indicated the treatment allocation for each randomized patient. Treatment assignment was masked for all investigators, study personnel, and patients were blind to the treatment assignments throughout the trial. In the first 3 days after enrollment, concurrent treatment with open-label paracetamol was not allowed.

Procedures

The patients were assessed at enrollment and at 14 days (or at discharge, if earlier) and 3 months after enrollment. The initial assessment included cardiovascular risk factors and a quantification of stroke severity according to the National Institutes of Health Stroke

Scale (NIHSS), a 15-item scale in which scores can range from 0 to 42, with higher values indicating increasing severity.[19] Body temperature (tympanic or rectal) was measured at enrollment and 24 hours later. For each individual patient, the same method of thermometry was used at baseline and after 24 hours. After 14 days (or at discharge, if earlier), functional status was assessed by means of the Barthel Index (BI), which ranges from 0 to 20, with 20 indicating no disability and 0 indicating complete dependence.[20] At 3 months, scores on the mRS, BI, and European Quality of Life-5 Dimensions (EuroQol-5D)[21] were assessed by telephone interview. Scores on the mRS range from 0 (no symptoms at all) to 5 (severe disability)[18]; for statistical purposes, death is assigned a score of 6.

Trial conduct was monitored by a steering committee. An independent data monitoring committee (DMC) did five interim safety and effectiveness analyses after intervals of one year.

Serious adverse events were reported by local investigators to the PAIS trial office; these included any infection that led to prolonged hospital stay or was life threatening, death from any cause, liver failure, and gastro-intestinal hemorrhage that occurred during hospitalisation and within the first 14 days after randomization.

Statistical analysis

Analyses were by intention-to-treat and all patients who were randomly assigned treatment were included in the prespecified analyses. The primary outcome measure was the score on the mRS at 3 months. The original primary effect measure was the odds ratio for unfavorable outcome defined as a score on the mRS of 3 to 6. With this primary effect measure, we estimated that 2500 participants would have provided 80% power to detect a statistically significant ($p<0.05$) benefit, assuming a 6% absolute or 12% relative reduction of unfavorable outcome with paracetamol treatment. During the study, we realised that the target of 2500 patients could not be achieved within a reasonable time frame with the available financial resources and recruitment rate. As a result, the steering committee stopped inclusion on May 1, 2008 with final patient follow-up on August 1, 2008.

Early stopping of the trial at 1400 patients would lead to a reduction of power to 60%. For this reason, and because of advances in statistical methods during the course of the study[22-24], the steering committee decided to change the primary effect estimate to the odds ratio for "improvement beyond expectation", according to the sliding dichotomy approach.[23] This decision was made on April 10, 2008, before data were unmasked, and the protocol change was subsequently published.[25] Improvement beyond expectation (in short, improvement) was defined as a score on the mRS lower than the median grade of patients with a similar prognostic index.[25]

We also analyzed the effect of treatment in patients who completed at least the first 24 hours of treatment (on-treatment analysis).

Secondary effect estimates were the odds ratio for improvement of at least one category on the mRS assessed by ordinal logistic regression and furthermore the odds ratio for favorable outcome defined as a score on the mRS of 2 or less, a score on the mRS of 3 or less, and a score on the BI of 20.

Adjustments were made with a multiple logistic regression model that included the following factors: age, sex, time to treatment, baseline body temperature, stroke severity, stroke type (ischemic stoke versus intracerebral hemorrhage), ischemic stroke subtype (lacunar versus non-lacunar), treatment with rt-PA, previous stroke, atrial fibrillation, and diabetes mellitus.

Quality of life was assessed by means of the EuroQol-5D.[21] A utility score was calculated with population-based preference weights for combined health scores.[26] A utility score of 1 represents perfect health, a score of 0 represents death and negative scores represent health states considered worse than death.

The mean absolute difference in body temperature between the two treatment groups was calculated with adjustment for baseline body temperature by means of multiple linear regression. The relation between improvement on the one hand and baseline body temperature and body temperature 24 hours after start of treatment on the other was expressed as an odds ratio per 1.0˚C increase in body temperature with a corresponding 95% confidence interval, through logistic regression. An adjustment for the impact of age, sex, NIHSS score, stroke type, and ischemic stroke subtype was made by use of multiple logistic regression.

Planned subgroup analyses were based on the time from stroke onset to start of treatment (dichotomized at the median), stroke type (intracerebral hemorrhage versus ischemic stroke), and ischemic stroke subtype (non-lacunar versus lacunar infarction). Post-hoc subgroup analyses were done according to baseline body temperature, dichotomized at the median, and treatment with rt-PA.

We also updated our previous meta-analysis of pharmacological temperature-lowering therapy in acute stroke.[27]

All analyses were performed with Stata/SE 9.2 for Windows (Statacorp, College Station, Texas). This trial is registered as an International Standard Randomized Controlled trial [ISRCTN74418480].

Role of the funding source

The funding sources had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The study steering committee had full access to all data and had final responsibility for the decision to submit for publication.

RESULTS

In 29 participating centers in the Netherlands, 697 patients were randomly assigned to treatment with paracetamol and 703 to placebo. 21 patients assigned paracetamol (3%) and 34 assigned placebo (5%) discontinued treatment within the first 24 hours of treatment, and about 30% of all patients did not complete the 3-day treatment period. Early discharge (41%) or death (20%) were the most common reasons for not completing the full treatment period. One patient was lost to follow-up (Figure 1).

Baseline characteristics were well balanced (Table 1), except for atrial fibrillation, which was more common in patients allocated placebo (Table 1). In the total study population, the mean age was 69.8 years (SD 13.0), 56% were men, and 88% had ischemic stroke. Eligibility was determined by clinical diagnosis on admission; in 3 patients (0.2%), the final diagnosis turned out not to be stroke. The median score on the NIHSS was 6 (range 0-30). The mean baseline body temperature was 36.9°C (SD 0.6). Median time from stroke onset to treatment was about 6 hours.

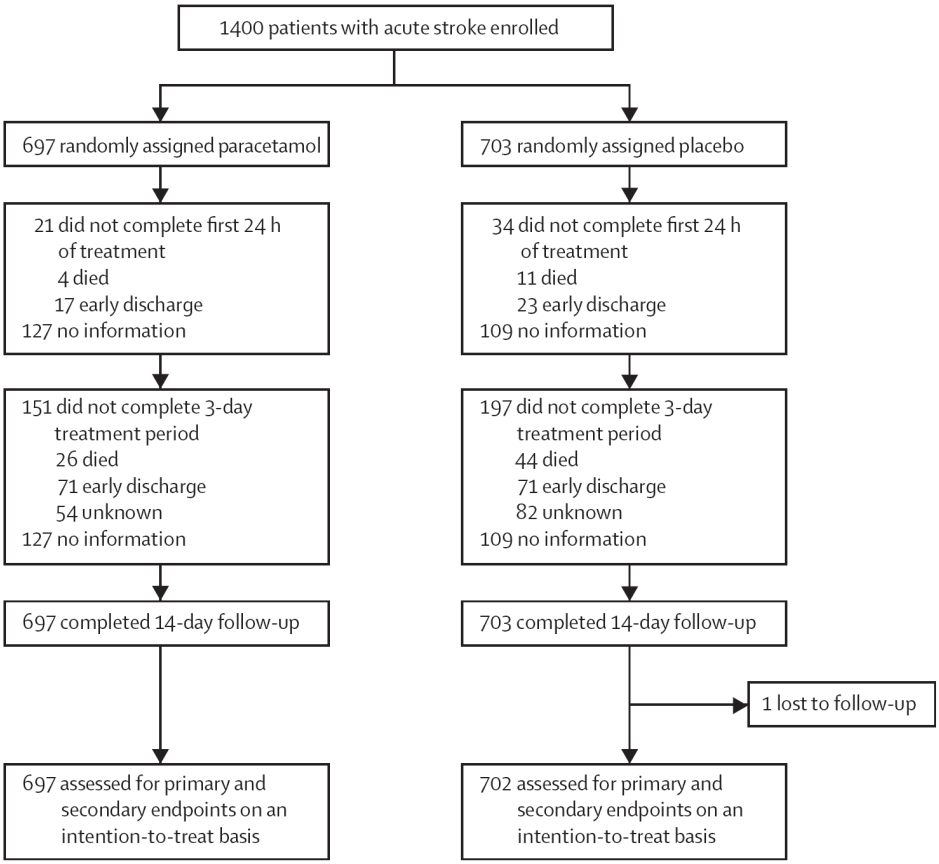

Figure 1: Enrolment and follow-up.

Table 1: Baseline characteristics of the patients.

	Paracetamol (n=697)	Placebo (n=703)
Demographics		
Mean (SD) age (years)	69.6 (13.0)	70.1 (13.0)
Men	374 (54%)	410 (58%)
Cardiovascular risk factors		
Hypertension	343 (49%)	346 (49%)
Atrial fibrillation	88 (13%)	123 (18%)
Diabetes mellitus	99 (14%)	110 (16%)
Current smoking	197 (29%)	209 (30%)
Hypercholesterolemia	169 (24%)	177 (26%)
Medical history		
Stroke	147 (21%)	133 (19%)
Myocardial infarction	77 (11%)	85 (12%)
Peripheral vascular disease	64 (9%)	68 (10%)
Stroke type		
Ischemic stroke	610 (87%)	617 (89%)
Ischemic stroke subtype*		
Large vessel disease (≥50% stenosis)	66 (11%)	84 (14%)
Cardiac source of embolism	109 (18%)	124 (20%)
Small vessel occlusion	108 (18%)	90 (15%)
Other determined etiology	49 (8%)	49 (8%)
Undetermined / negative evaluation	251 (41%)	241 (39%)
Missing information	27 (4%)	29 (5%)
Stroke severity		
Median (range) NIHSS[§] score	6 (0-28)	7 (0-30)
Physical examination		
Mean (SD) body temperature (°C)	36.9 (0.6)	36.9 (0.6)
Treatment		
Median (IQR) time from stroke onset to start of treatment (minutes)	345 (240-540)	360 (240-525)
Treated within 12 hours of onset	661 (95%)	673 (96%)
Treatment with rt-PA	150 (22%)	147 (21%)

*Based on the definitions of the Trial of ORG 10172 in Acute Stroke Therapy (TOAST) criteria.
[§]Scores on the National Institutes of Health Stroke Scale (NIHSS), range from 0 to 42, with higher values indicating more severe stroke.

More patients in the paracetamol group than in the placebo group improved beyond expectation (Figure 2), but this effect was not statistically significant (adjusted OR 1.20; 95% CI, 0.96 to 1.50) (Table 2). In the on-treatment analysis, the effect on improvement was much the same (1.20 (95% CI, 0.90 to 1.59). No analyses of secondary measures showed positive effects of treatment with paracetamol (Table 2). Quality of life according to the EuroQol-5D measurement was much the same in both groups, with a negligible difference of 0.004 (95% CI: -0.029 to 0.034).

Mean body temperature at 24 hours from the start of treatment was 0.26°C (95% CI, 0.18 to 0.31) lower with paracetamol than with placebo. After adjustment for age, sex,

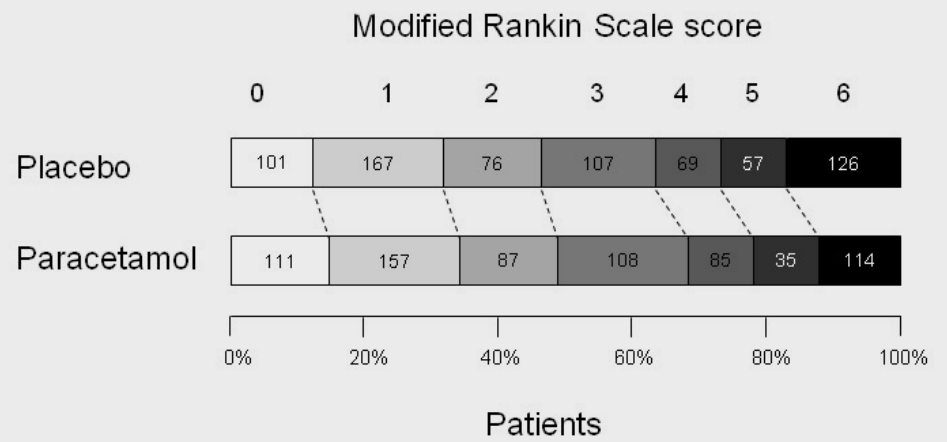

Figure 2: Distribution of grades on the modified Rankin Scale at 3 months.

Table 2: Primary and secondary outcome measures.

	Paracetamol (n=697)	Placebo (n=703)	OR (95% CI)	aOR (95% CI)*
Improvement (sliding dichotomy)	260 (37%)	232 (33%)	1.21 (0.97-1.50)	1.20 (0.96-1.50)
Improvement (ordinal regression)			1.10 (0.91-1.32)	1.03 (0.85-1.25)
Flavorable outcome (mRS ≤2)	355 (51%)	344 (50%)	1.09 (0.88-1.33)	1.02 (0.78-1.32)
Favorable outcome (mRS ≤3)	463 (66%)	451 (64%)	1.11 (0.88-1.39)	1.00 (0.76-1.32)
Favorable outcome (BI = 20)	436 (63%)	446 (63%)	0.96 (0.78-1.20)	1.09 (0.81-1.49)

*Adjusted for age, sex, NIHSS score, stroke type, ischemic stroke subtype, baseline body temperature, time to treatment, previous stroke, diabetes mellitus, treatment with rt-PA, and atrial fibrillation.

NIHSS score, stroke type, and ischemic stroke subtype, baseline body temperature was not related to improvement (adjusted OR 0.95; 95 % CI: 0.79 to 1.14). Increased body temperature at 24 hours from the start of treatment was associated with a lower likelihood of improvement (adjusted OR 0.83; 95% CI, 0.69 to 0.99).

We found no evidence of benefit of paracetamol in any of the predefined subgroups (Figure 3). In post-hoc subgroupanalysis of patients with a baseline body temperature between 37.0˚C and 39.0˚C, 131 of 326 patients (40%) in the paracetamol group improved beyond expectation compared with 106 of 335 (31%) in the placebo group (106/229) (adjusted OR 1.43; 95% CI, 1.02 to 1.97), but a stratified Mantel-Haenszel test did not formally indicate heterogeneity in the effect of paracetamol (p=0.12). The absolute risk reduction of 9% relates to a number of patients needed to treat of 11. Also, paracetamol resulted in a larger reduction in body temperature (0.30˚C; 95% CI, 0.20 to 0.40) in this subgroup than in patients with a baseline body temperature between 36.0˚C and 37.0˚C (0.19; 95% CI: 0.10 to 0.30). In a post-hoc subgroupanalysis of patients who were not treated with rt-PA, treatment with paracetamol was also associated with improvement (adjusted OR 1.29; 95% CI: 1.00 to 1.66) (Figure 3).

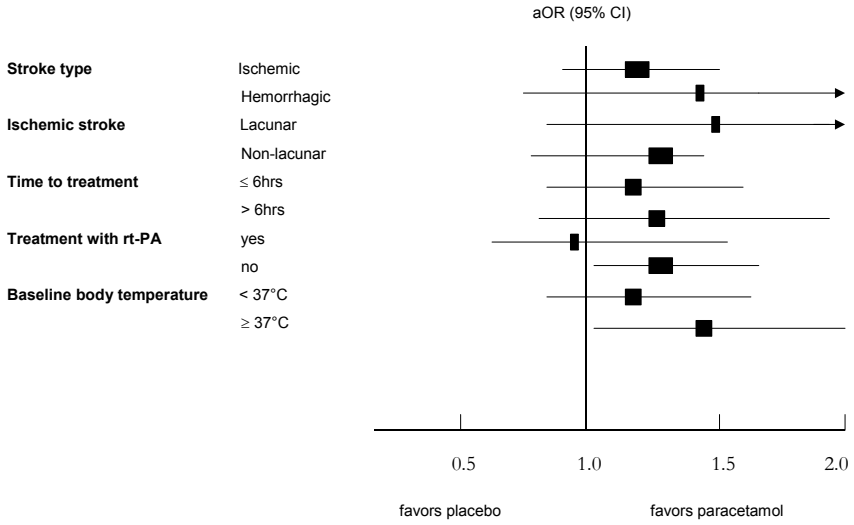

Figure 3: Subgroup analyses for the primary effect measure (improvement).

Table 3: Serious adverse events.

	Paracetamol (n=697)	Placebo (n=703)
Pneumonia	17	16
Urinary tract infection	7	3
Other infection	0	1
Sepsis	0	1
Liver function disturbance	2	0
Liver failure	0	0
Gastrointestinal hemorrhage	1	2
Death within 14 days	28	47
Total	55 (8%)	70 (9%)

The number of serious adverse events was similar in both treatment groups (Table 3). In particular, there was no increased rate of infections or liver enzyme disturbances in patients treated with paracetamol. Fewer patients treated with paracetamol died within the first 14 days than did those treated with placebo (OR 0.60; 95% CI: 0.36 to 0.90). However, no difference in case fatality was found at 3 months (OR 0.90; 95% CI: 0.68 to 1.18).

DISCUSSION

In the PAIS trial, more patients allocated to paracetamol improved beyond expectation than did patients treated with placebo, although the difference was not statistically significant. Paracetamol did not affect secondary outcome measures in predefined subgroups. However, in patients with a baseline body temperature between 37.0˚C and 39.0˚C, treatment with paracetamol was associated with improved outcome.

Two-phase II trials in patients with acute ischemic stroke showed that high-dose paracetamol lowered temperature 0.30°C, which was confirmed in the present study. In a recent Cochrane review on temperature-lowering therapy for acute stroke, 5 studies of pharmacological temperature reduction were identified.[27] These studies did not provide evidence that temperature reduction decreases the risk of death or dependency. However, the studies were small, were designed to test safety and feasibility, and long time periods were allowed between stroke onset and start of treatment. PAIS addressed the question whether temperature reduction by paracetamol improves outcome in patients with acute stroke in a large randomized phase III trial.

We updated our previous meta-analysis of temperature-lowering therapy[27] in acute stroke. The meta-analysis is now based on the data of six pharmacological temperature-lowering trials (Figure 4). A pooled analysis shows no significant difference between active treatment and control in the proportion of patients who were alive and independent (score on the mRS ≤2) at final follow-up (OR 1.1; 95% CI: 0.9 to 1.3).

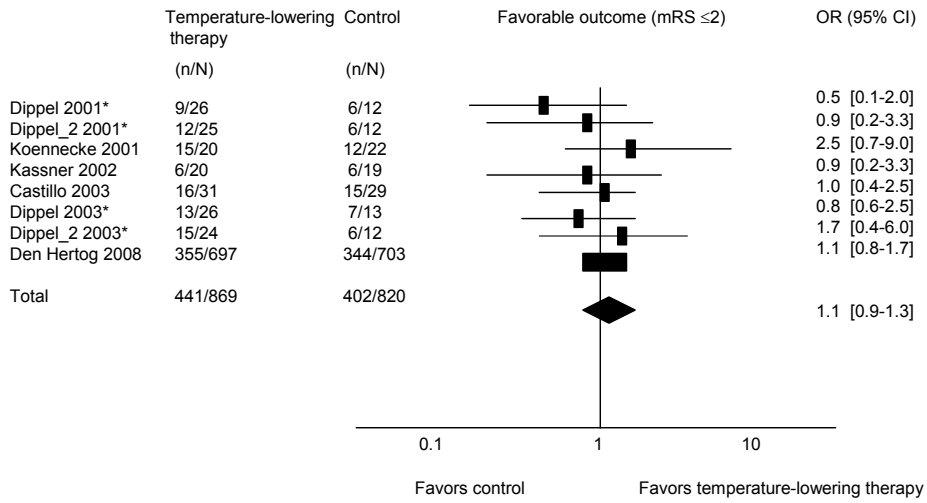

Figure 4: Update of the meta-analysis of pharmacological temperature-lowering therapy in acute stroke.[27] *Two intervention groups: the number of patients with favorable outcome and the total number of patients in the control group were divided by 2, in order to avoid multiple comparisons with the same subset of patients.

There are some controversial features in the design of our study. The analysis plan for PAIS was changed from a fixed dichotomy of the mRS to the sliding dichotomy analysis during the trial. Neither the original primary nor the sliding dichotomy showed an effect of paracetamol on functional outcome. The protocol change was prompted by advances in statistical methods[22-24] and the need to increase the efficiency of the trial. Analysis of sliding dichotomy allows each patient's baseline prognosis to be taken into account and

seems more relevant for clinical practice, because even those at the prognostic extremes contribute to the estimation of the treatment effect, which may increase statistical power.

Enrollment in the trial was stopped after inclusion of 1400 patients despite a target of 2500, because of lack of funding. This reduced the power of the study to detect a statistically significant effect for the original primary outcome measure of poor outcome defined as a score on the mRS of 3 to 6. However, the effect of adopting the sliding dichotomy approach might be almost the same as a doubling the sample size.[23]

Although 95% of patients completed the first 24 hours of treatment, only 70% completed the full treatment period of three days. Early discharge, because of a complete or almost complete recovery or because of death, were the most common reasons for not completing the 3-day treatment period. Because overall benefit in the on-treatment analysis and intention-to-treat analysis was similar, we think that the suboptimal treatment adherence will not have had a large effect on the results of the trial. Moreover, as the rise in temperature occurs mainly within the first 24 hours after stroke onset[2,28], the first 24 hours might be the most important treatment period. In addition, most observational studies showed that the relation between body temperature and clinical outcome is probably limited to the first 12 to 24 hours after stroke onset.[3-5]

Few patients with severe stroke were included in the PAIS trial, possibly because we did not specify a lower NIHSS limit in the eligibility criteria of the trial. Underrepresentation of patients with severe strokes might affect generalizability if the effect of treatment would be less in these patients. This is a point of concern for subsequent clinical trials with antipyretic drugs.

Contrary to most observational studies[1,3,4,30], we found no relation between body temperature on admission and functional outcome at 3 months. However, patients with increased body temperatures at 24 hours from the start of treatment did have a lower likelihood of improvement. This disparity suggests that an early rise in body temperature is more important in predicting clinical outcome after stroke than the body temperature itself.

Post-hoc subgroup analysis indicated a beneficial baseline of paracetamol on improvement in patients with a baseline body temperature between 37.0˚C and 39.0˚C. Treatment with high-dose paracetamol also induced a larger reduction in body temperature in this group of patients than in others, because paracetamol leads to larger temperature reductions when the initial body temperature is higher.[31]

Any possible explanations for this finding should be interpreted with caution, because this subgroup was not defined in advance. Post-hoc subgroup analysis also indicated a beneficial effect of paracetamol in patients who were not treated with rt-PA. This might be explained by a higher recovery rate in the patients treated with rt-PA than in those not treated with thrombolysis.

With organized care on a specialized stroke unit, patients are more likely to survive, regain independence, and return home than with a less well-organized stroke service.

The features of organized stroke care that confers benefits are unclear.[32] Monitoring and early treatment of physiological variables, such as glucose levels, blood pressure and body temperature, probably contribute to the advantages.[32]

Although the PAIS trial does not provide sufficient evidence to support the routine use of high-dose paracetamol in patients with acute stroke, its results are promising. In the subgroup of patients with a baseline body temperature between 37.0˚C and 39.0˚C, treatment with paracetamol resulted in a 9% absolute increase in the number of patients who improved beyond expectation, corresponding with a number needed to treat of only 11. This observation requires confirmation in an independent study. If such an effect can be confirmed, a simple, safe, and cheap treatment with a long time window for start of therapy will be available for patients with acute ischemic stroke or intracerebral hemorrhage.

Data-monitoring committee
PAG Sandercock, M Vermeulen, M Correia, EW Steyerberg

Executive committee
HM den Hertog, HB van der Worp, HMA van Gemert and DWJ Dippel

Steering committee
HM den Hertog (trial-coordinator), DWJ Dippel (co-principal investigator), HB van der Worp (co-principal investigator), HMA van Gemert, A Algra, J van Gijn, LJ Kappelle, PJ Koudstaal

Central trial office
N El Ghannouti, ES van der Heijden

Participating centers (with numbers of patients randomized and names of investigators)
Meander MC Amersfoort (297; HMA van Gemert), Erasmus MC Rotterdam (246; DWJ Dippel), UMC Utrecht (185; HB van der Worp), Sint Fransicus Gasthuis Rotterdam (93; FH Vermeij), Slotervaart Ziekenhuis Amsterdam (79; VIH Kwa), Gelre ziekenhuizen Apeldoorn (71; HP Bienfait), TweeSteden ziekenhuis Tilburg (44; BPW Jansen), Sint Elisabeth Ziekenhuis Tilburg (42; PLM de Kort), Westfriesgasthuis Hoorn (39; TC van der Ree), Maasstad ziekenhuis Rotterdam (34; R Saxena), Ziekenhuis Bethesda Hoogeveen (34; PG Oomes), Dirksland Ziekenhuis Dirksland (34; UW Huisman), Sint Lucas Andreas Ziekenhuis Amsterdam (32; EJ Wouda), Martini Ziekenhuis Groningen (29; C Bouwsma), Catharina Ziekenhuis Eindhoven (25; K Keizer) Albert Schweitzer ziekenhuis Dordrecht (24; RP Kleyweg/H Kerkhoff), Amphia Ziekenhuis Breda (18; HBC Verbiest/SLM Bakker), Rijnland Ziekenhuis Leiderdorp (16; ELLM De Schryver), Diakonessenhuis Utrecht (10; RCJM Donders), Slingeland Ziekenhuis Doetinchem (10; RA van der Kruijk), IJsselland

Ziekenhuis Capelle aan den Ijssel (8; J Heerema), Jeroen Bosch Ziekenhuis Den Bosch (7; RAJAM Bernsen), Ziekenhuis Hilversum Hilversum (6; D Herderschêe), Diaconessenhuis Meppel (5; EJW Keuter), Vlietlandziekenhuis Schiedam (4; WC Baart), Spaarne Ziekenhuis Hoofddorp (3; RJ Meijer), Beatrixziekenhuis Gorinchem (3; RB Alting van Geusau), Streekziekenhuis Koningin Beatrix Winterswijk (1; JP de Ruiter), Wilhelmina Ziekenhuis Assen (1; JN Wessel/AE Bollen).

ACKNOWLEDGEMENT

The PAIS trial was sponsored by the Netherlands Heart Foundation. Grant number 2002B148.
EJ van Breda coordinated the study from March 2003 to June 2005.
We would like to thank HF Lingsma who supported the analyses.
We are grateful to all patients, nurses, secretaries, neurologists and residents who contributed to the PAIS trial.

REFERENCES

1. Azzimondi G, Bassein L, Nonino F et al. Fever in acute stroke worsens prognosis. A prospective study. *Stroke* 1995; 26:2040-2043.
2. Boysen G, Christensen H. Stroke severity determines body temperature in acute stroke. *Stroke* 2001; 32:413-417.
3. Castillo J, Davalos A, Marrugat J et al. Timing for fever-related brain damage in acute ischemic stroke. *Stroke* 1998; 29:2455-2460.
4. Reith J, Jorgensen HS, Pedersen PM et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996; 347:422-425.
5. Jorgensen HS, Reith J, Pedersen PM et al. Body temperature and outcome in stroke patients. *Lancet* 1996; 348:193.
6. Ginsberg MD, Busto R. Combating hyperthermia in acute stroke: a significant clinical concern. *Stroke* 1998; 29:529-534.
7. van der Worp HB, Sena ES, Donnan GA et al. Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. *Brain* 2007; 130:3063-3074.
8. Adams HP, del Zoppo G, Alberts MJ et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. *Circulation* 2007; 115:e478-e534.
9. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. *Cerebrovasc Dis* 2008; 25:457-507.
10. Broderick J, Connolly S, Feldmann E et al. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update: a guideline from the American Heart Association/American Stroke Association Stroke Council, High Blood Pressure Research Council, and the Quality of Care and Outcomes in Research Interdisciplinary Working Group. Circulation 2007;116: e391-e413.
11. Leys D, Ringelstein EB, Kaste M et al. The main components of stroke unit care: results of a European expert survey. *Cerebrovasc Dis* 2007; 23:344-352.
12. Clissold SP. Paracetamol and phenacetin. Drugs 1986; 32:46-59.
13. Dippel DW, van Breda EJ, van Gemert HM et al. Effect of paracetamol (acetaminophen) on body temperature in acute ischemic stroke: a double-blind, randomized phase II clinical trial. *Stroke* 2001; 32:1607-1612.
14. Dippel DW, van Breda EJ, van der Worp HB et al. Timing of the effect of acetaminophen on body temperature in patients with acute ischemic stroke. *Neurology* 2003; 61:677-679.
15. Koennecke HC, Leistner S. Prophylactic antipyretic treatment with acetaminophen in acute ischemic stroke: a pilot study. *Neurology* 2001; 57:2301-2303.
16. Kasner SE, Wein T, Piriyawat P et al. Acetaminophen for altering body temperature in acute stroke: a randomized clinical trial. *Stroke* 2002; 33:130-134.
17. van Breda EJ, van der Worp HB, van Gemert HM et al. PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial [ISCRTN 74418480]. *BMC Cardiovasc Disord* 2005; 5:24.
18. van Swieten JC, Koudstaal PJ, Visser MC et al. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988; 19:604-647.

19. Brott T, Adams HP, Olinger CP et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989; 20:864-870.
20. Mahoney FI, Barthel DW. Functional evaluation: The Barthel Index. *Md State Med J* 1965; 14:61-65.
21. The EuroQol Group. EuroQol -a new facility for the measurement of health-related quality of life. *Health Policy* 1990; 16:199-208.
22. Bath PM, Gray LJ, Collier T et al. Can we improve the statistical analysis of stroke trials? Statistical reanalysis of functional outcomes in stroke trials. *Stroke* 2007; 38:1911-1915.
23. Murray GD, Barer D, Choi S et al. Design and analysis of phase III trials with ordered outcome scales: the concept of the sliding dichotomy. *J Neurotrauma* 2005; 22:511-517.
24. Saver JL, Yafeh B. Confirmation of tPA treatment effect by baseline severity-adjusted end point reanalysis of the NINDS-tPA stroke trials. *Stroke* 2007; 38:414-416.
25. den Hertog HM, van der Worp HB, van Gemert HM et al. Correction: PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial. [ISRCTN 74418480]. *BMC Cardiovasc Disord* 2008; 8:29.
26. Lamers LM, Stalmeier PF, McDonnell J et al. Measuring the quality of life in economic evaluations: the Dutch EQ-5D tariff. *Ned Tijdschr Geneeskd* 2005;149:1574-1578.
27. den Hertog HM, van der Worp HB, Tseng MC et al. Cooling therapy for acute stroke. *Cochrane Database Syst Rev* 2009; 1:CD001247.
28. Wong AA, Davis JP, Schluter PJ et al. The time course and determinants of temperature within the first 48 h after ischaemic stroke. *Cerebrovasc Dis* 2007; 24:104-110.
29. Schwarz S, Hafner K, Aschoff A et al. Incidence and prognostic significance of fever following intracerebral hemorrhage. Neurology 2000; 54:354-361.
30. Wang Y, Lim LL, Levi C et al. Influence of admission body temperature on stroke mortality. *Stroke* 2000; 31:404-409.
31. Van Esch A, Van Steensel-Moll HA, Steyerberg EW et al. Antipyretic efficacy of ibuprofen and acetaminophen in children with febrile seizures. *Arch Pediatr Adolesc Med 1995*; 149:632-637.
32. Govan L, Weir CJ, Langhorne P et al. Organised inpatient (stroke unit) care for stroke. *Cochrane Database Syst Rev* 2007; 4:CD000197.

An early rise in body temperature is related to unfavorable outcome after stroke

Heleen M den Hertog[1], H Bart van der Worp[2], H Maarten A van Gemert[3] , Ale Algra[2,4], L Jaap Kappelle[2], Jan van Gijn[2], Peter J Koudstaal[1], Diederik WJ Dippel[1], on behalf of the PAIS investigators

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Neurology, Meander Medical Center, Amersfoort, The Netherlands

[4]Julius Center for Health Sciences and Primary care, University Medical Center Utrecht, Utrecht, and Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Submitted

ABSTRACT

Background and purpose Subfebrile temperature or fever is present in about a third of patients on the first day after stroke onset and is associated with poor outcome. However, the temporal profile of this association is not well established. We aimed to assess the relationship between body temperature, on admission as well as the change in body temperature from admission to 24 hours thereafter and functional outcome and death.
Methods We analyzed data of 1332 patients admitted within 12 hours of stroke onset. The relation between body temperature on admission or the change in body temperature from admission to 24 hours thereafter (adjusted for body temperature on admission) on the one hand and poor outcome (death, or a modified Rankin Scale score>2) at 3 months on the other were expressed as odds ratio per 1.0˚C increase in body temperature. Adjustments for potential confounders were made with a multiple logistic regression model.
Results No relation was found between admission body temperature and poor outcome (aOR 1.06; 95% CI 0.85 to 1.33) and death (aOR 1.26; 95% CI 0.97-1.64). In contrast, increased body temperature in the first 24 hours after stroke onset was associated with poor outcome (aOR 1.29; 95% CI 1.03 to 1.60) and death (aOR 1.48; 95% CI 1.13 to 1.93).
Conclusion An early rise in body temperature rather than elevated body temperature on admission is a risk factor for poor functional outcome in patients with acute stroke.

abstract

BACKGROUND

Subfebrile temperature or fever is common in patients with acute stroke. Body temperatures over 37.5°C have been reported in up to a quarter of patients within the first six hours[1], and in about a third at 24 hours after stroke onset.[2] This elevation of body temperature may be a direct consequence of brain damage inflicted by stroke, or a result of an associated infection.[3]

Increased body temperature markedly exacerbates neuronal injury in experimental models of cerebral ischemia.[4] An association between increased body temperature and poor outcome has also been shown in patients with acute stroke.[1, 2, 5-12] However, the temporal profile of this relation is not well established. Several prospective studies found that body temperature on admission was associated with poor outcome.[1, 2, 5, 9, 11] Others found that an increased body temperature in the first 24 hours after admission was a prognostic factor for clinical outcome.[6, 7, 10]

The aim of our study was to asses the relationship between body temperature within the first 12 hours after symptom onset, and the change in body temperature from admission to 24 hours thereafter on the one hand, and functional outcome and death on the other in a large sample of stroke patients.

METHODS

All patients participated in the Paracetamol (Acetaminophen) In Stroke (PAIS) trial. The PAIS trial was a multicenter, randomized, placebo-controlled clinical trial of high-dose paracetamol in patients with acute stroke.[13] In short, patients with ischemic stroke or intracerebral hemorrhage with no history of liver disease or significant pre-stroke impairment (grade on the modified Rankin Scale (mRS) ≥ 2)[14] were included within 12 hours of symptom onset and treated with high-dose paracetamol (6g daily) or placebo for the next three days.

The PAIS trial was approved by national and institutional review boards, and written informed consent was obtained from all patients or from their legal representatives.

Clinical characteristics

Baseline clinical information included stroke severity assessed by means of the NIH Stroke Scale (NIHSS), a 15-item scale with scores that range from 0 to 42 and higher values indicating greater severity[15], ischemic stroke subtype according to the TOAST classification[16], and cardiovascular risk factors. In addition, any infection that led to prolonged hospital stay or was life threatening was recorded. The diagnosis of infection was left with the treating physician.

Body temperature

Body temperature was measured with a tympanic or rectal thermometer within 12 hours from stroke onset and 24 hours later. In each patient, a similar method of thermometry was used at these two points in time.

Outcome measures

Poor outcome was defined as a grade of more than 2 on the mRS at 3 months from stroke onset. Scores on the mRS range from 0 (no symptoms at all) to 5 (severe disability); for statistical purposes, death has a score of 6.

A secondary outcome measure was death at 3 months. Outcome assessment was blinded for body temperature.

Statistical analysis

Statistical analyses were performed with Stata/SE 9.2 for Windows (Statacorp, College Station, Texas). Changes in body temperature from baseline to 24 hours thereafter and corresponding 95% confidence intervals (CIs) were calculated with linear regression analysis. The relation between body temperature within 12 hours from stroke onset and the change in body temperature from admission to 24 hours thereafter (adjusted for admission body temperature) on the one hand and poor outcome or death on the other was expressed as an odds ratio per 1.0˚C increase in body temperature with a corresponding 95% confidence interval (CI), as estimated with logistic regression.

Adjustments were made with a multiple logistic regression model that included the following factors: age, sex, baseline NIHSS score, stroke type (ischemic stroke versus intracerebral hemorrhage), ischemic stroke subtype (lacunar versus non-lacunar infarction), and the occurrence of infections. We performed additional analyses to account for the potential modifying effect of antipyretic treatment by stratifying for treatment with paracetamol, which was the experimental drug treatment in the PAIS trial.

Furthermore, we assessed the relation between several clinical variables including stroke type, ischemic stroke subtype, and stroke severity (expressed in quartiles of the baseline NIHSS score) and the change in body temperature from baseline to 24 hours thereafter, adjusted for baseline body temperature, with multiple linear regression.

RESULTS

Fourteen hundred patients with a clinical diagnosis of ischemic stroke or intracerebral hemorrhage were included in the PAIS trial. Of these patients, 68 were excluded from the

present study either because the final diagnosis was not a stroke (n=3) or because body temperature at 24 hours was not recorded (n=65).

In the remaining 1332 patients, mean age was 70 years (SD 13), 56% of the patients were male, and 88% had an ischemic stroke (Table 1). The infarction was of the lacunar type in 193 patients (17%). The median score on the NIHSS scale was 6 (range 0-30). Median time from stroke onset to measurement of the admission body temperature was 360 minutes (IQR 240-540). A total of 660 patients (50%) was treated with paracetamol.

Table 1: Baseline clinical characteristics of the patients (n=1332).

Demographics	
Mean (SD) age (years)	70 (13)
Sex (male)	749 (56%)
Cardiovascular risk factors	
Hypertension	652 (49%)
Atrial fibrillation	198 (15%)
Diabetes mellitus	195 (15%)
Current cigarette smoking	390 (29%)
Hypercholesterolemia	332 (25%)
Medical history	
Stroke	267 (20%)
Myocardial infarction	155 (12%)
Peripheral vascular disease	128 (10%)
Stroke type	
Ischemic stroke	1169 (88%)
Ischemic stroke subtype*	
Large vessel disease (≥50% stenosis)	146 (12%)
Cardiac source of embolism	216 (18%)
Small vessel occlusion	193 (17%)
Other determined etiology	90 (8%)
Undetermined	477 (41%)
Missing information	47 (4%)
Stroke severity	
Median (range) NIHSS score§	6 (0-30)
Physical examination	
Mean (SD) body temperature (°C)	36.9 (0.6)
Median (IQR) time from stroke onset to measurement of baseline body temperature (minutes)	360 (240-540)
Treatment	
Paracetamol	660 (50%)
Intravenous alteplase	281 (21%)

*Based on the Trial of ORG 10172 in Acute Stroke Therapy (TOAST) criteria.[16]
§Scores on the National Institutes of Health Stroke Scale (NIHSS).[15]

The mean body temperature was 36.9°C (SD 0.6) on admission, and 37.1°C (SD 0.7) 24 hours later. Body temperature over 37.5˚C was observed in 160 patients (12%) on admission, and in 298 (22%) 24 hours later. The increase in body temperature from baseline to 24 hours thereafter was larger in patients with body temperatures of 37.0˚C or less than in those with body temperatures over 37.0˚C (Figure 1). After 24 hours, 96 of 660 patients (15%) treated with paracetamol and 202 of 672 patients (30%) on placebo had a body temperature over 37.5˚C (difference 15%; 95% CI 11% to 19%). Treatment with paracetamol was associated with a 0.26°C (95% CI, 0.18 to 0.31) reduction in mean body temperature measured 24 hours after admission.

Four of the 160 patients (3%) with baseline body temperatures over 37.5°C and 24 of 298 patients (8%) with body temperatures over 37.5°C 24 hours later developed an infection during hospitalization.

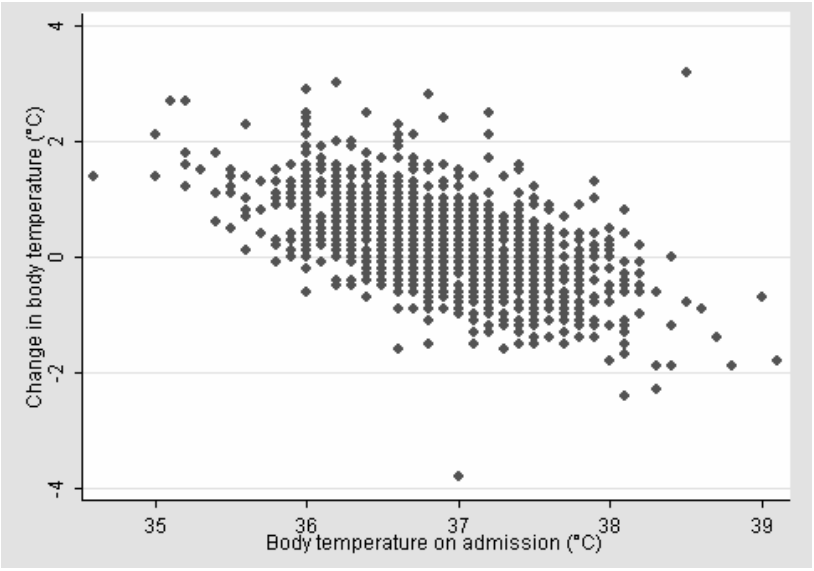

Figure 1: The change in body temperature in the first 24 hours from admission to 24 hours thereafter (ºC) as a function of body temperature on admission (ºC).

The proportion of patients with admission body temperatures over 37.5˚C was similar in patients with ischemic stroke (145/1169 (12%)) and in those with intracerebral hemorrhage (16/163 (10%)) and also in patients with non-lacunar infarction (136/1139 (10%) and lacunar infarction (24/193 (12%)). However, the increase in body temperature from admission to 24 hours thereafter was 0.24°C larger (95% CI, 0.14 to 0.33) in patients with non-lacunar infarction than in those with lacunar infarction and also 0.33°C (95% CI, 0.23 to 0.44) larger in patients with intracerebral hemorrhage than in patients with ischemic stroke.

We did not observe a proportional increase in the association between stroke severity and body temperature on admission. In the highest quartile of the NIHSS score (NIHSS

score ³ 13), baseline body temperature was 0.20°C (95% CI, 0.12 to 0.27) lower than body temperature in the lower quartiles of the NIHSS score (NIHSS<13) (Figure 2). Body temperature 24 hours later was associated with baseline stroke severity. For every quartile of the NIHSS score, body temperature at 24 hours rose on average with 0.16°C (95% CI, 0.13 to 0.19) (Figure 2).

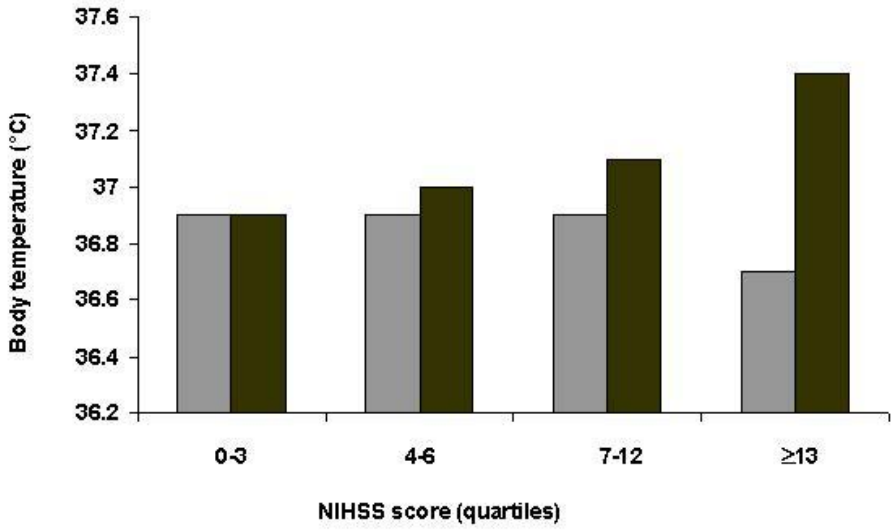

Figure 2: Stroke severity (quartiles) and body temperature within 12 hours from stroke onset, (grey bars) and 24 hours later (black bars).

Body temperature on admission was not related to poor outcome or death (Table 2). An increased body temperature in the first 24 hours after admission, however, was associated with poor outcome or death at 3 months (Table 2). Adjustment for potential confounders slightly attenuated this association (Table 2). An additional stratified analysis to account for the potentially modifying effect of treatment with paracetamol did not affect the association (Table 2).

Table 2: Association of body temperature with poor outcome or death at 3 months.

	Poor outcome	Overall OR (95% CI)*	Overall aOR (95% CI)*$	Paracetamol aOR (95% CI)*$	Placebo aOR (95% CI)*$
Within 12 hrs from stroke onset	mRS>2 (n=663)	0.82 (0.68-0.98)	1.06 (0.85-1.33)	0.90 (0.65 to 1.24)	1.22 (0.89 to 1.68)
	Death (n=218)	0.87 (0.68-1.12)	1.26 (0.97-1.64)	1.15 (0.77 to 1.72)	1.39 (0.96 to 2.02)
Change from admission to 24 hours#	mRS>2 (n=663)	1.98 (1.65-2.39)	1.28 (1.03-1.60)	1.22 (0.86 to 1.72)	1.31 (0.97 to 1.77)
	Death (n=218)	2.36 (1.86-2.99)	1.49 (1.13-1.94)	1.50 (0.96 to 2.32)	1.57 (1.10 to 2.26)

*Per 1.0 degree Celsius increase of body temperature

$Adjusted for age, sex, NIHSS score, stroke type, ischemic stroke subtype, and occurrence of infections

#Adjusted for body temperature on admission

After exclusion of patients who developed an infection during the first two weeks after stroke onset, the adjusted odds ratio for poor outcome was 1.27 (95% CI 1.02 to 1.59), and for death 1.47 (95% CI 1.10 to 1.96).

Figure 3 illustrates the strength of the association between the change in body temperature from admission to 24 hours thereafter and poor outcome. The absolute difference in the risk of poor outcome between patients in the lowest and patients in the highest body temperature category is more than 20%.

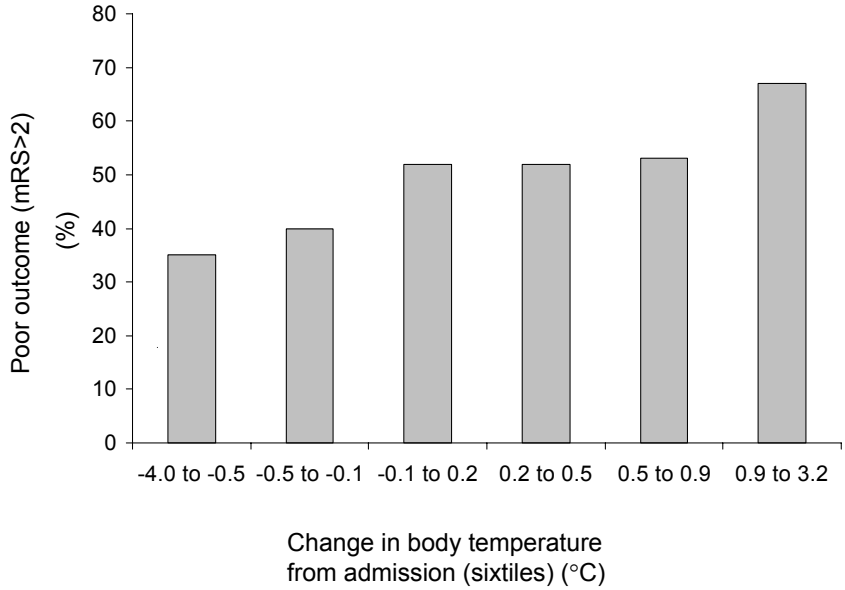

Figure 3: Probability of poor outcome (mRS>2) at 3 months in relation to change in body temperature from baseline to 24 hours thereafter (sixtiles) (°C).

DISCUSSION

Our results do not confirm the previously reported association between body temperature on admission and poor outcome in patients with acute stroke. However, patients with increased body temperatures in the first 24 hours after admission did have an increased risk of poor functional outcome or death at 3 months.

Before these results can be interpreted, some methodological issues have to be discussed. First, one of the strengths of our study is its large sample size. Other strengths include robust outcome measures and detailed information on confounders. Second, PAIS was a randomized double-blind placebo controlled trial that addressed the question whether temperature reduction by paracetamol would improve outcome in patients with acute stroke.[13] Half of the study population was treated with paracetamol in a daily dose of 6 g for 3 consecutive days. As a result, body temperature at 24 hours after admission was lowered and fewer patients may have experienced a poor outcome,

although the overall effect of paracetamol on outcome was not statistically significant. We did separate regression analyses in the patients treated with paracetamol and those allocated to placebo. The estimates of the association between the change in body temperature from admission to 24 hours thereafter and outcome were similar in the two groups, suggesting that the study population could be analyzed as one group.

A second issue is that we only recorded body temperature at two points in time, i.e. within 12 hours from symptom onset and 24 hours later and did not repeated measurements of body temperature. As a consequence, it might be more difficult to compare our results with the results of previous studies.

Finally, relatively few patients with very severe stroke were included, possibly because we did not specify a lower NIHSS score limit in the eligibility criteria of the PAIS trial. However, our results showed that patients with more severe strokes had higher body temperatures at 24 hours after admission, and thereby underrepresentation of patients with severe strokes potentially weakens rather than exaggerates the observed relation between body temperature at 24 hours after admission and poor outcome.

Previous studies have found that increased body temperature within 6 hours up to at least 24 hours after stroke onset is an independent prognostic factor for poor outcome.[1, 2, 5-12] However, the temporal profile of this association is still under debate. Several studies showed that high body temperature on admission was associated with poor outcome. In three of these, no adjustments were made for baseline stroke severity assessed with validated stroke scales.[2, 5, 8] A retrospective study of 509 patients with ischemic stroke or intracerebral hemorrhage found an association between elevated admission body temperature and poor outcome only in patients with ischemic stroke.[11] In a prospective study of 390 patients with either ischemic stroke or intracerebral hemorrhage admitted within 6 hours of symptom onset, the odds of poor outcome doubled for every degree of Celsius increase in baseline body temperature.[1]

A prospective study of 725 patients admitted within 6 hours of symptom onset did find a relation between increased body temperature at 10 to 12 hours after stroke onset and poor outcome, but no relationship between body temperature on admission and poor outcome was found.[6] Although these results seem contradictory to our findings, our results indeed suggest that increased body temperature in the first 24 hours after admission rather than body temperature on admission is an important prognostic factor for clinical outcome after stroke. We found that patients with severe stroke had relatively low body temperatures at baseline, possibly because of a faster loss of body temperature before hospital admission as a result of less muscle activity. This finding might explain in part that body temperature on admission was not associated with clinical outcome.

In line with our results, two studies showed that increased body temperature relative to baseline in the first 24 to 72 hours after stroke onset was associated with unfavorable outcome.[7, 10] These studies included only patients with intracerebral hemorrhage[10] or patients with ischemic stroke treated with intravenous thrombolysis.[7]

Increased body temperature may be attributed to a systemic inflammatory response following ischemic stroke or to accompanying infections. In addition, it has been suggested that increased body temperature indicates hypothalamic damage following stroke.[17] The mechanisms of the association between an early rise in body temperature and poor outcome remain speculative. Secondary infections are common in the first week of stroke and are associated with poor outcome, but they usually occur more than 12 hours after stroke onset.[18] In our study, only three percent of patients with baseline body temperatures over 37.5°C and eight percent of patients with body temperatures over 37.5°C, 24 hours later developed an infection during hospitalization. Besides, the relationship between body temperature at 24 hours after admission and poor outcome was independent of the occurrence of infections. Furthermore, exclusion of patients developing an early infection during hospitalization did not substantially affect the relation between body temperature and poor functional outcome or death. Therefore, infections cannot account for the observed relation between temperature and outcome.

The change in body temperature from admission was associated with baseline stroke severity. One may argue that increased body temperature does not accelerate the ischemic cascade, but merely reflects extensive cerebral damage and, thereby, of poor outcome. However, the relation between increased body temperature and poor outcome was independent of baseline stroke severity.

Our results provide a rationale for the hypothesis that prevention of an early rise in body temperature could reduce death and improve functional outcome. Guidelines for the treatment of patients with acute ischemic stroke[19] or intracerebral hemorrhage[20] recommend the administration of antipyretic medication in case of fever or a temperature above 37.5˚C. This might be too late, as the rise in body temperature has already occurred, and the time period in which the brain lesion is most vulnerable has subsided. On the other hand, there is still no convincing evidence from randomized trials that strategies to prevent or treat elevated body temperature reduce case fatality and improve functional outcome after stroke.[13, 21]

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The PAIS trial was sponsored by the Netherlands Heart Foundation, grant number 2002B148. We are grateful to all patients, nurses, secretaries, and neurologists who contributed to this study.

Participating centers with names of investigators

Meander MC Amersfoort (HMA van Gemert), Erasmus MC Rotterdam (DWJ Dippel), UMC Utrecht (HB van der Worp), Sint Fransicus Gasthuis Rotterdam (FH Vermeij), Slotervaart

Ziekenhuis Amsterdam (VIH Kwa), Gelre ziekenhuizen Apeldoorn (HP Bienfait), TweeSteden ziekenhuis Tilburg (BPW Jansen), Sint Elisabeth Ziekenhuis Tilburg (PLM de Kort), Westfriesgasthuis Hoorn (TC van der Ree), Maasstad ziekenhuis Rotterdam (R Saxena), Ziekenhuis Bethesda Hoogeveen (PG Oomes), Dirksland Ziekenhuis Dirksland (UW Huisman), Sint Lucas Andreas Ziekenhuis Amsterdam (EJ Wouda), Martini Ziekenhuis Groningen (C Bouwsma), Catharina Ziekenhuis Eindhoven (K Keizer) Albert Schweitzer ziekenhuis Dordrecht (RP Kleyweg/H Kerkhoff), Amphia Ziekenhuis Breda (HBC Verbiest/SLM Bakker), Rijnland Ziekenhuis Leiderdorp (ELLM De Schryver), Diakonessenhuis Utrecht (RCJM Donders), Slingeland Ziekenhuis Doetinchem (RA van der Kruijk), IJsselland Ziekenhuis Capelle aan den Ijssel (J Heerema), Jeroen Bosch Ziekenhuis Den Bosch (RAJAM Bernsen), Ziekenhuis Hilversum Hilversum (D Herderschêe), Diaconessenhuis Meppel (EJW Keuter), Vlietlandziekenhuis Schiedam (WC Baart), Spaarne Ziekenhuis Hoofddorp (RJ Meijer), Beatrixziekenhuis Gorinchem (RB Alting van Geusau), Streekziekenhuis Koningin Beatrix Winterswijk (JP de Ruiter), Wilhelmina Ziekenhuis Assen (JN Wessel/AE Bollen).

REFERENCES

1. Reith J, Jorgensen HS, Pedersen PM et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. Lancet 1996; 347:422-425.
2. Castillo J, Davalos A, Marrugat J et al. Timing for fever-related brain damage in acute ischemic stroke. Stroke 1998; 29:2455-2460.
3. den Hertog H, van der Worp B, van Gemert M et al. Therapeutic hypothermia in acute ischemic stroke. Expert Rev Neurother 2007; 7:155-164.
4. Ginsberg MD, Busto R. Combating hyperthermia in acute stroke: a significant clinical concern. Stroke 1998; 29:529-34.
5. Azzimondi G, Bassein L, Nonino F et al. Fever in acute stroke worsens prognosis. A prospective study. Stroke 1995; 26:2040-2043.
6. Boysen G, Christensen H. Stroke Severity Determines Body Temperature in Acute Stroke. Stroke 2001; 32:413-417.
7. Ernon L, Schrooten M, Thijs V. Body temperature and outcome after stroke thrombolysis. Acta Neurol Scand 2006; 114:23-28.
8. Hindfelt B. The prognostic significance of subfebrility and fever in ischaemic cerebral infarction. Acta Neurol Scand 1976; 53:72-9.
9. Jorgensen HS, Reith J, Pedersen PM et al. Body temperature and outcome in stroke patients. Lancet 1996; 348:193.
10. Schwarz S, Hafner K, Aschoff A et al. Incidence and prognostic significance of fever following intracerebral hemorrhage. Neurology 2000; 54:354-361.
11. Wang Y, Lim LL, Levi C, et al. Influence of admission body temperature on stroke mortality. Stroke 2000; 31:404-409.
12. Hajat C, Hajat S, Sharma P. Effects of poststroke pyrexia on stroke outcome : a meta-analysis of studies in patients. Stroke 2000; 31:410-414.
13. den Hertog HM, van der Worp HB, van Gemert HM et al. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial: a multicentre, randomised, placebo-controlled, phase III trial. Lancet Neurol 2009; 8:434-440
14. van Swieten JC, Koudstaal PJ, Visser MC et al. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988; 19:604-607.
15. Brott T, Adams HP, Olinger CP et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke.* 1989; 20:864-870.
16. Adams HPJ, Bendixen BH, Kappelle LJ et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993; 24:35-41.
17. Przelomski MM, Roth RM, Gleckman RA et al. Fever in the wake of a stroke. Neurology 1986; 36:427-429.
18. Emsley HC, Hopkins SJ. Acute ischaemic stroke and infection: recent and emerging concepts. Lancet Neurol 2008; 7:341-53.
19. Adams HP, del Zoppo G, Alberts MJ et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. Circulation 2007; 115:e478-e534.

20. Broderick JP, Adams HP, Barsan W et al. Guidelines for the management of spontaneous intracerebral hemorrhage: A statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Stroke 1999; 30:905-915.
21. den Hertog HM, van der Worp HB, Tseng MC et al. Cooling therapy for acute stroke. Cochrane Database Syst Rev 2009; 1:CD001247.

High-dose paracetamol reduces systolic blood pressure in acute stroke

Heleen M den Hertog[1], H Bart van der Worp[2], H Maarten A van Gemert[3], Jan van Gijn[2], Peter J Koudstaal[1], Diederik WJ Dippel[1]

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Neurology, Meander Medical Center, Amersfoort, The Netherlands

Submitted

ABSTRACT

Background Early administration of paracetamol may improve outcome of patients with acute stroke and a baseline body temperature of 37°C or above by lowering body temperature and preventing fever. Besides its antipyretic effects, paracetamol may also reduce blood pressure through cyclooxygenase-2 inhibition. We therefore aimed to assess the effect of high-dose paracetamol on blood pressure in patients with acute stroke.
Methods We analyzed data of 540 patients admitted within 24 hours of stroke onset who were randomized to treatment with either paracetamol (6 g daily) or placebo. Blood pressures were measured at 12, 24, and 48 hours from the start of treatment. Changes in blood pressure from baseline in the two treatment groups and corresponding 95% confidence intervals (CI) were calculated with linear regression analysis. Adjustments for potential confounders were made with a multiple linear regression model.
Results Treatment with high-dose paracetamol was associated with a significant reduction in systolic blood pressure of 4.7 mm Hg (95% CI 0.7 to 8.6) at 12 hours from the start of treatment. This effect was no longer present after 24 and 48 hours.
Conclusion High-dose paracetamol reduces not only body temperature, but also blood pressure in patients with acute stroke. Both effects may improve functional outcome after stroke, but this needs further study.

abstract

BACKGROUND

Guidelines for the treatment of acute ischemic stroke or intracerebral hemorrhage recommend administration of antipyretic medication in patients with subfebrile temperatures or fever, on the assumption that this will improve functional outcome.[1, 2] We have recently reported that in a large randomized trial, treatment with high-dose paracetamol improved functional outcome in patients with acute stroke and a baseline body temperature of 37°C or above, although this was a post-hoc finding that requires confirmation.[3]

Despite its widespread use, the mechanism of action of paracetamol has not been fully clarified. Paracetamol is a potent inhibitor of cyclooxygenase (COX)-1 and COX-2-mediated prostaglandin production in the central nervous system.[4, 5] This presumably accounts for its antipyretic and analgesic properties. A different effect of paracetamol may be a COX-2-mediated change in blood pressure. Two small observational studies suggested that in patients admitted to an intensive care unit, administration of paracetamol reduces systolic arterial pressure and mean arterial pressure.[6, 7] On the other hand, long-term use of some COX-2 inhibitors may be associated with an increase in blood pressure by inhibiting the synthesis of vasodilatory prostaglandins, sodium retention, or increased endothelin 1 production.[8]

Most patients with acute stroke initially have an elevated blood pressure, which spontaneously normalizes over the first few days.[9, 10] High blood pressure in the acute phase of stroke is associated with increased case fatality and dependency, and moderate lowering of blood pressure may therefore improve outcome.[11, 12]

We aimed to assess the effect of high-dose paracetamol on blood pressure in patients with acute stroke.

METHODS

Study design

The study population was derived from the Paracetamol (Acetaminophen) in Patients with Acute Stroke (PAPAS) trial[13], the Paracetamol (Acetaminophen) and Ibuprofen in Acute Stroke (PISA) trial[14], and the Paracetamol (Acetaminophen) In Stroke (PAIS) trial.[3]

In the first study (PAPAS)[13], 76 patients with ischemic stroke admitted within 24 hours of symptom onset were treated with either 3 g or 6 g paracetamol or placebo for 5 days. Study medication was provided as suppositories. The second study (PISA)[14] compared high-dose paracetamol (6 g daily) and ibuprofen (2.4 g daily) with placebo in 75 patients with ischemic stroke, admitted within 24 hours of symptom onset. The first dose of the study medication was provided as a suppository and the remainder as tablets. In the

third study (PAIS)[3], patients with ischemic stroke or intracerebral hemorrhage admitted within 12 hours of symptom onset were treated with paracetamol in a daily dose of 6 g for 3 consecutive days. The study medication was administered orally, except for the first four gifts that could be administered as a suppository. In all three studies, patients were eligible for inclusion if they had a body temperature between 36°C and 39°C and had no history of liver failure or alcohol abuse. All three trials were randomized and double-blind; detailed protocols have been published.[13-16]

For the purpose of the present study, we included patients from PAPAS and PISA who were treated with either 6 g paracetamol or placebo and patients from PAIS who were enrolled in the two largest recruiting centers (Erasmus Medical Center, Rotterdam, and Meander Medical Center, Amersfoort, the Netherlands). Patients were excluded in case of missing data on blood pressure at 12 hours of stroke onset.

National and institutional review boards approved all trials, and written informed consent was obtained from all patients or from their legal representatives.

Baseline data

Baseline clinical information included stroke severity assessed by means of the NIH Stroke Scale (NIHSS)[17], ischemic stroke subtype according to the TOAST classification[18], and cardiovascular risk factors. Additionally, the use of antihypertensive drugs and COX-1 and COX-2 inhibitors was recorded.

Blood pressure

According to local protocols, systolic blood pressure, diastolic blood pressure and heart rate were measured on admission, every 2 hours within the first 24 hours, and every 4 hours thereafter, up to 48 hours after hospitalization. We collected data on systolic and diastolic blood pressure levels and heart rate on admission and at 12, 24, and 48 hours from the start of treatment.

Outcome measures

The primary outcome measures of the present study were the systolic and diastolic blood pressure at 12 hours from the start of treatment. Secondary outcome measures were systolic and diastolic blood pressure at 24 and 48 hours from the start of treatment. Outcome assessment was blinded for treatment with the study drug.

Statistical analyses were performed with Stata/SE 9.2 for Windows (Statacorp, College Station, Texas).

The differences between the baseline blood pressure and the blood pressures on follow-up were compared between patients allocated to paracetamol and those on placebo. Linear regression analysis was performed to estimate differences between the changes in blood pressure in the two treatment groups and corresponding 95% confidence intervals (CI). Adjustments were made with a multiple linear regression model that included the following factors: age, sex, stroke severity, time to treatment, stroke type (ischemic stroke versus intracerebral hemorrhage), ischemic stroke subtype (lacunar versus non-lacunar), diabetes mellitus, hypertension, use of other COX inhibitors, and use of antihypertensive drugs. Between-study effects and between-hospital effects were explored. We also analyzed the effect of treatment in patients who completed at least the first 48 hours of treatment (on-treatment analysis), to evaluate the full pharmacological effect of paracetamol on blood pressure.

RESULTS

Of the 151 patients included in the PAPAS en PISA trial, 52 were treated with high-dose paracetamol and 50 with placebo. Of the 543 patients in the PAIS trial who were enrolled at the Erasmus Medical Center or the Meander Medical Center, 105 patients were excluded from the present study, because of insufficient data on blood pressure 12 hours after the start of treatment. No significant differences in baseline characteristics or case fatality were found between included patients (n=540) and excluded patients (n=105) (data not shown).

Blood pressure levels were available for 502 of the 540 patients at 24 hours from the start of treatment, and for 423 patients at 48 hours. Early discharge (n = 87) and death (n = 13) were the most common reasons for missing blood pressure levels at these two points in time.

Of the 540 included patients, 60% were male, mean age was 68 years (SD 14), and 86% percent had an ischemic stroke (Table 1). The infarction was of the lacunar type in 56 patients (12%). Forty-seven percent of patients had a history of hypertension and about half of the patients received one or more antihypertensive drugs. The median score on the NIHSS scale was 6 (range, 0-40). Median time from stroke onset to start of treatment was 6.5 hours (IQR, 4.6 to 10.0).

The mean systolic/diastolic blood pressure on admission was 157/86 mm Hg in the paracetamol group and 160/81 mm Hg in patients on placebo.

Table 1: Baseline characteristics of the patients (n=540).

	Paracetamol (N=281)	Placebo (N=259)
Demographics		
Mean (SD) age (years)	69 (14)	68 (14)
Sex (male)	158 (56%)	163 (63%)
Cardiovascular risk factors		
Hypertension	132 (47%)	122 (47%)
Atrial fibrillation	36 (13%)	36 (14%)
Diabetes mellitus	37 (13%)	35 (14%)
Current cigarette smoking	90 (32%)	91 (35%)
Hypercholesterolemia	58 (21%)	59 (23%)
Medical history		
Previous stroke	56 (20%)	55 (21%)
Previous myocardial infarction	34 (12%)	27 (10%)
Peripheral vascular disease	18 (6%)	20 (8%)
Stroke type		
Ischemic stroke	247 (88%)	218 (84%)
Ischemic stroke subtype*		
Large vessel disease (≥50% stenosis)	32 (13%)	37 (17%)
Cardiac source of embolism	44 (18%)	39 (18%)
Small vessel occlusion	32 (13%)	24 (11%)
Other determined etiology	20 (8%)	13 (6%)
Undetermined	109 (44%)	94 (43%)
Missing information	10 (4%)	11 (5%)
Stroke severity		
Median (range) NIHSS score[$]	6 (0-42)	7 (0-40)
Physical examination		
Mean (SD) systolic blood pressure (mm Hg)	157 (29)	160 (29)
Mean (SD) diagnostic blood pressure (mm Hg)	78 (19)	81 (18)
Mean (SD) heart rate (beats per minute)	82 (20)	81 (22)
Treatment		
Median (IQR) time from stroke onset to start of treatment (hours)	6.5 (4.6 to 9.3)	7.0 (4.6 to 10.6)
Antihypertensive drugs	134 (48%)	112 (43%)
Other Cox-1 and 2 inhibitors	51 (18%)	35 (14%)
Intravenous alteplase	57 (20%)	49 (19%)

*Based on the Trial of ORG 10172 in Acute Stroke Therapy (TOAST) criteria.[18]
[$]Scores on the National Institutes of Health Stroke Scale (NIHSS).[17]

At 12 hours from the start of treatment, the mean systolic blood pressure had dropped 15.7 mm Hg (95% CI, 12.7 to 18.6) in the paracetamol group and 12.2 mm Hg (95% CI, 8.6 to 15.6) in patients on placebo (difference 3.5 mm Hg; 95% CI, 0.5 to 8.4) (Table 2 and Figure 1). The mean diastolic blood pressure had dropped 5.8 mm Hg (95% CI, 3.7 to 8.2) in patients on paracetamol, and 4.4 mm Hg (95% CI, 1.7 to 8.6) in patients on placebo (difference 1.4; 95% CI -1.5 to 4.3) (Table 2 and Figure1). Adjustment for confounding factors increased the effect of paracetamol on systolic blood pressure after 12 hours to 4.7 mm

Table 2: Mean difference in decrease in blood pressure (mm Hg) between patients treated with paracetamol and those who received placebo during the treatment period of 48hrs.

Time from the start of treatment		Difference* (95% CI)	Adjusted difference*# (95% CI)	Adjusted difference for on-treatment analysis*# (95% CI)
12 hours	SBP	3.5 (0.5 to 8.4)	4.7 (0.7 to 8.6)	5.3 (1.1 to 9.5)
	DBP	1.4 (-1.5 to 4.3)	1.2 (-1.7 to 4.1)	1.6 (-1.4 to 4.7)
24 hours	SBP	-1.4 (-5.4 to 2.5)	-0.9 (-4.8 to 3.1)	-0.7 (-4.5 to 3.5)
	DBP	-1.0 (-3.6 to 1.7)	-0.5 (-3.2 to 2.3)	-1.1 (-3.8 to 1.7)
48 hours	SBP	-1.8 (-6.2 to 2.7)	-1.1 (-5.5 to 3.4)	-0.8 (-5.4 to 3.8)
	DBP	-0.1 (-3.1 to 3.1)	-0.1 (-3.1 to 3.1)	-0.1 (–3.2 to 3.2)

SBP, systolic blood pressure; DBP, diastolic blood pressure

*Adjusted for baseline systolic or diastolic blood pressure

#Adjusted for age, sex, NIHSS score, time to treatment, hypertension, diabetes mellitus, stroke type, ischemic stroke subtype, use of antihypertensive drugs, and use of other COX inhibitors.

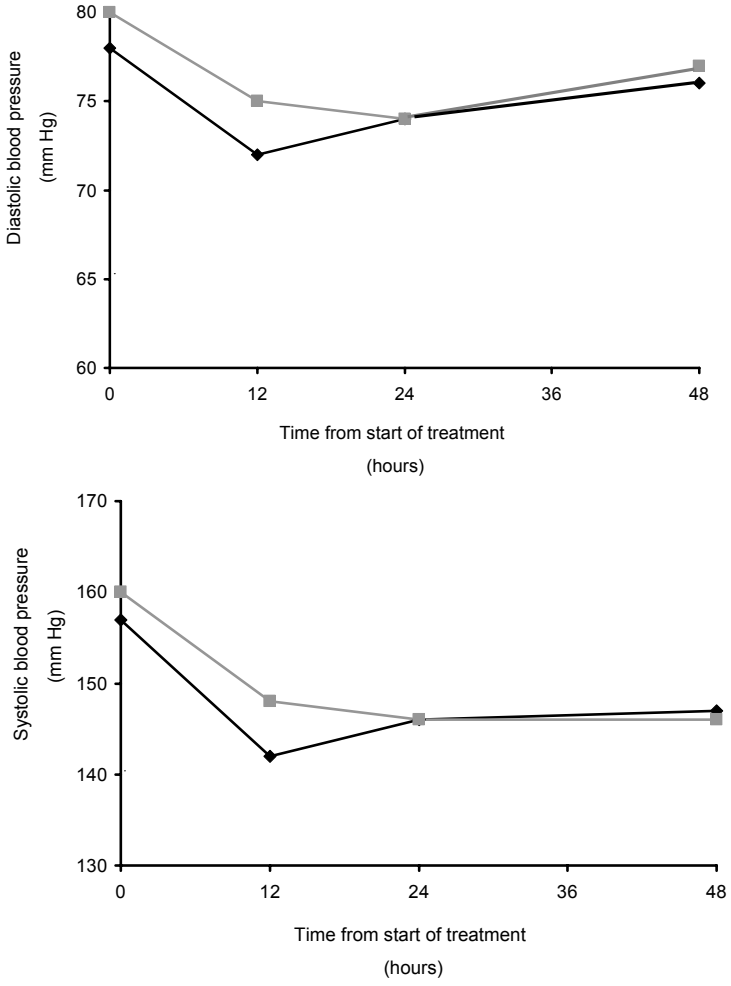

Figure 1: Systolic and diastolic blood pressures in patients on paracetamol (black dots) and in patients on placebo (grey dots).

Hg (95% CI 0.7 to 8.6) (Table 2). Furthermore, paracetamol was associated with a decrease in heart rate of 4.2 beats per minute (95% CI, 0.5 to 8.5) as compared with placebo.

At 24 and 48 hours from the start of treatment, no effect of paracetamol was observed on either systolic or diastolic blood pressure (Table 2 and Figure1), or on heart rate.

There was no hospital effect. The direction of the effect of paracetamol was consistent across the trials. However, the decrease in systolic blood pressure by paracetamol at 12 hours from the start of treatment was larger in PAPAS (11.4 mm Hg; 95% CI, -6.8 to 29.6) than in PISA (2.7 mm Hg; 95% CI -9.2 to 14.6) than in PAIS (3.8 mm Hg; 95% CI, -0.5 to 8.1).

In the on-treatment analysis, the effect of paracetamol on systolic blood pressure 12 hours from start of treatment increased to 5.3 mm Hg (95% CI, 1.1 to 9.5) (Table 2).

DISCUSSION

We showed that treatment with high-dose paracetamol, started within 24 hours after stroke onset, was associated with a significant reduction in systolic blood pressure of 4.7 mm Hg at 12 hours from the start of treatment. This effect was no longer present after 24 and 48 hours.

Several methodological issues need to be discussed. First, this study was not designed to evaluate the effect of high-dose paracetamol on blood pressure in acute stroke. As a consequence, blood pressure was not measured in a standardized way. However, this would more likely result in a random error than in a systematic error. Second, we did not compare the effect of different doses of paracetamol on blood pressure. However, only in the PAPAS trial two doses of paracetamol were compared, and the number of patients included in this trial (76) was far too small for reliable analyses. Third, about a sixth of the patients enrolled in the three trials had to be excluded because of missing data on blood pressure at 12 hours. On the other hand, comparison of the excluded patients with the study population showed no significant differences with regard to baseline characteristics or case fatality. Fourth, the study population included patients from three different randomized trials. For this reason, we performed separate regression analyses in the patients included in either PAPAS or PISA and in those included in the PAIS trial. The absence of heterogeneity in the effect of paracetamol across the three studies suggests that the study population could indeed be analyzed as a single group. Finally, strengths of our study are its large sample size and assessment of blood pressure that was blinded for treatment allocation.

To our knowledge, our study is the first that assessed the effect of high-dose paracetamol on blood pressure in patients with acute stroke. This effect of paracetamol has been previously assessed in two small studies, both in patients admitted to an intensive care unit. The first study found a reduction in mean arterial pressure of approximately 10 percent within 15 minutes after oral administration of 1 g paracetamol, persisting for 90 minutes.[6]

The second study reported a reduction in mean arterial pressure of 6 mm Hg at 2 hours after administration of an unspecified dose of paracetamol.[7]

On the first day after stroke, blood pressure is elevated in up to 75% of the patients, but returns to pre-stroke values over a period of a few days.[9, 10] We propose two explanations for the short-term increase in blood pressure after stroke and for the effect of paracetamol.

Elevated blood pressure following acute stroke has been associated with increased levels of both urinary catecholamines and salivary cortisol, possibly reflecting a neuroendocrine stress response.[21] About a fifth of patients with ischemic stroke and more than half of patients with intracerebral hemorrhage experience headache.[22, 23] Headache can lead to an increase in blood pressure by increased sympathetic activity. It may therefore be hypothesized that paracetamol decreases blood pressure by pain relief. This might also explain why paracetamol affected heart rate and why the effect of paracetamol on systolic blood pressure in our study seemed to be limited to the first 36 hours after start of treatment. Unfortunately we did not record the presence and severity of headache, nor did we measure levels of cortisol or catecholamines.

A more speculative hypothesis is that inflammation might lead to increased blood pressure after stroke. High blood pressure has been attributed to elevated levels of C-reactive protein.[24,25] COX-2 may be upregulated as a consequence of the inflammatory processes following ischemic stroke.[25]Paracetamol is a weak COX-1 and COX-2 inhibitor [4, 5], but at higher doses it may exhibit stronger and nonselective COX inhibitor activity, thereby affecting blood pressure.[20] This suggests that paracetamol may decrease blood pressure by inhibition of COX-2- mediated inflammation. Unfortunately, we did not measure inflammatory markers in all patients in this cohort.

The best possible management of blood pressure in patients with acute stroke is uncertain, as has been pointed out in various guidelines for the management of acute ischemic stroke or intracerebral hemorrhage, and in a recent Cochrane meta-analysis.[2, 26, 27] However, longitudinal studies have indicated that stroke patients with systolic blood pressures of 10 mm Hg above 150 mm Hg may have a 4% increased risk of recurrent stroke and a 4% increased risk of death or dependency.[11, 12] Also, preliminary studies suggest that antihypertensive treatment lowers blood pressure by 10-15 mm Hg after stroke.[26, 28-30] The effect of paracetamol on systolic blood pressure, which amounted to approximately 5 mm Hg in the present study, may therefore be large enough to be of clinical significance.

In conclusion, early administration of high-dose paracetamol in patients with acute stroke does not only reduce body temperature, but systolic blood pressure as well. This finding provides an additional argument for further studies of the effect of prophylactic high-dose paracetamol on functional outcome in acute stroke.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The PAIS trial was sponsored by the Netherlands Heart Foundation, grant number 2002B148. We would like to thank professor C Patrono for his useful comments on our manuscript. We are grateful to all patients, nurses, secretaries, and neurologists who contributed to this study.

Participating centers (with numbers of patients included in the present study and names of local principal investigators)

Meander MC Amersfoort (276; HMA van Gemert), Erasmus MC Rotterdam (259; DWJ Dippel), UMC Utrecht (5; HB van der Worp).

REFERENCES

1. Adams HP, del Zoppo G, Alberts MJ et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. *Circulation* 2007; 115:e478-e534.
2. Broderick JP, Broderick J, Connolly S. American Heart Association; American Stroke Association Stroke Council; High Blood Pressure Research Council; Quality of Care and Outcomes in Research interdisciplinary Working Group. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update: a guideline from the American Heart Association/American Stroke Association Stroke Council, High Blood Pressure Research Council, and the Quality of Care and Outcomes in Research Interdisciplinary Working Group.*Stroke* 2007; 38:2001-23.
3. den Hertog HM, van der Worp HB, van Gemert HM et al. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial: a multicentre, randomised, placebo-controlled, phase III trial. *Lancet Neurol* 2009; 8:434-40.
4. Clissold SP. Paracetamol and phenacetin. *Drugs.* 1986; 32 Suppl 4:46-59.
5. Flower RJ, Vane JR. Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). *Nature* 1972; 240:410-411.
6. Boyle M, Hundy S, Torda TA. Paracetamol administration is associated with hypotension in the critically ill. *Aust Crit Care* 1997; 10:120-122.
7. Mackenzie I, Forrest K, Thompson F et al. Effects of acetaminophen administration to patients in intensive care. *Intensive Care Med* 2000; 26:1408.
8. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. *JAMA* 2006; 296:1633-1644.
9. Britton M, Carlsson A, de Faire U. Blood pressure course in patients with acute stroke and matched controls. *Stroke* 1986; 17:861-864.
10. Saxena R, Wijnhoud AD, Koudstaal PJ et al. Induced elevation of blood pressure in the acute phase of ischemic stroke in humans. *Stroke* 2000; 31:546-548.
11. Leonardi-Bee J, Bath PM, Phillips SJ et al. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke* 2002; 33:1315-1320.
12. Willmot M, Leonardi-Bee J, Bath PM. High blood pressure in acute stroke and subsequent outcome: a systematic review. *Hypertension* 2004; 43:18-24.
13. Dippel DWJ, van Breda EJ, Van Gemert HMA et al. Effect of paracetamol (acetaminophen) on body temperature in acute ischemic stroke: a double-blind, randomized phase II clinical trial. *Stroke* 2001; 32:1607-1612.
14. Dippel DWJ, van Breda EJ, van der Worp HB et al. Effect of paracetamol (acetaminophen) and ibuprofen on body temperature in acute ischemic stroke PISA, a phase II double-blind, randomized, placebo-controlled trial [ISRCTN98608690]. *BMC Cardiovascular Disorders* 2003; 6;3:2.
15. den Hertog HM, van der Worp HB, van Gemert HM et al. Correction: PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial. [ISRCTN74418480]. *BMC Cardiovasc Disord* 2008; 8:29.
16. van Breda EJ, van der Worp HB, van Gemert HM et al. PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial [ISRCTN 74418480]. *BMC Cardiovasc Disord* 2005; 5:24.

17. Brott T, Adams HP, Olinger CP et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; 20:864-870.
18. Adams HP, Bendixen BH, Kappelle LJ et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993; 24:35-41.
19. Curhan GC, Willett WC, Rosner B et al. Frequency of analgesic use and risk of hypertension in younger women. *Arch Intern Med* 2002; 162:2204-2208.
20. Hinz B, Cheremina O, Brune K. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. *FASEB J* 2008; 22:383-390.
21. Ahmed N, de la Torre B, Wahlgren NG. Salivary cortisol, a biological marker of stress, is positively associated with 24-hour systolic blood pressure in patients with acute ischaemic stroke. *Cerebrovasc Dis* 2004; 18:206-213.
22. Koudstaal PJ, van Gijn J, Kappelle LJ. Headache in transient or permanent cerebral ischemia. Dutch TIA Study Group. *Stroke* 1991; 22:754-759.
23. Melo TP, Pinto AN, Ferro JM. Headache in intracerebral hematomas. *Neurology* 1996; 47:494-500.
24. Di Napoli M, Papa F. Association between blood pressure and C-reactive protein levels in acute ischemic stroke. *Hypertension* 2003; 42:1117-1123.
25. Vongpatanasin W, Thomas GD, Schwartz R et al. C-reactive protein causes downregulation of vascular angiotensin subtype 2 receptors and systolic hypertension in mice. *Circulation* 2007; 115:1020-1028.
26. Geeganage CM, Bath PM. Interventions for Deliberately Altering Blood Pressure in Acute Stroke. *Stroke* 2009 Epub.
27. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. *Cerebrovasc Dis* 2008; 25:457-507.
28. Anderson CS, Huang Y, Wang JG et al. Intensive blood pressure reduction in acute cerebral haemorrhage trial (INTERACT): a randomised pilot trial. *Lancet Neurol* 2008; 7:391-399.
29. Sare GM, Gray LJ, Wardlaw J et al. Is lowering blood pressure hazardous in patients with significant ipsilateral carotid stenosis and acute ischaemic stroke? Interim assessment in the 'Efficacy of Nitric Oxide in Stroke' trial. *Blood Press Monit* 2009; 14:20-25.
30. Potter J, Mistri A, Brodie F et al. Controlling hypertension and hypotension immediately post stroke (CHHIPS) a randomised controlled trial. *Health Technol Assess* 2009; 13:48-73.

Chapter 4

Inflammatory responses, prognostic markers

C-reactive protein in the very early phase of acute ischemic stroke: association with poor outcome and death

HM den Hertog[1], JA van Rossum[1], HB van der Worp[2], HMA van Gemert[3], R de Jonge[4] , PJ Koudstaal[1], DWJ Dippel,[1] and the PAIS investigators

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Neurology, Meander Medical Center, Amersfoort, The Netherlands

[4]Department of Clinical Chemistry, Erasmus MC University Medical Center, Rotterdam, The Netherlands

J Neurol 2009 Epub

ABSTRACT

Objective Acute ischemic stroke may trigger an inflammatory response that leads to increased levels of C-reactive protein (CRP). High levels of CRP may be associated with poor outcome either because they reflect an inflammatory reaction or tissue damage. We evaluated the prognostic value of CRP within 12 hours of onset of ischemic stroke.

Methods Levels of CRP were routinely obtained within 12 hours of symptom onset in 561 patients with ischemic stroke. CRP values were dichotomized as <7 mg/L or ≥7 mg/L. The full range of CRP values was used to detect a possible level-risk relationship. We studied the relation between CRP values and poor outcome (modified Rankin Scale score >2) or death at 3 months. A multiple logistic regression model was applied to adjust for age, sex, NIHSS score, current cigarette smoking, diabetes mellitus, hypertension, statin use, and stroke subtype.

Results After adjustment for potential confounders, patients with CRP levels ≥7 mg/L had a significantly increased risk of poor outcome (adjusted OR 1.6, 95% CI 1.1-2.4) or death (adjusted OR 1.7, 95% CI 1.0-2.9) at 3 months. In addition, the risk of poor outcome or death at 3 months increased with higher levels of CRP.

Conclusion CRP within 12 hours of ischemic stroke is an independent prognostic factor of poor outcome at 3 months.

abstract

BACKGROUND

Elevated serum levels of C-reactive protein (CRP) are found in up to three quarters of patients with ischemic stroke.[1, 2] Increases in CRP may reflect a systemic inflammatory response following ischemic stroke, the extent of tissue injury, or concurrent infections. Moreover, in animal models of focal cerebral ischemia, CRP increased secondary brain damage through activation of the complement system.[3, 4]

Several studies have assessed the value of CRP in the very early phase of stroke as a prognostic factor of functional outcome. These studies were either small, included a selected group of patients, or only tested the relation between CRP and mortality instead of functional outcome. The findings were inconclusive as some found a positive association[5-7], but others not.[8, 9]

Verification of the role of CRP as an early prognostic factor of functional outcome after ischemic stroke may be of clinical importance, because it is an easy to measure and readily available inflammatory marker. The aim of our study was therefore to determine the prognostic value of CRP measured in the very early phase of ischemic stroke for poor functional outcome and death in large sample of patients with acute ischemic stroke.

METHODS

Study design

All patients included in the present study participated in the Paracetamol (Acetaminophen) In Stroke (PAIS) trial, a multicenter, randomized, placebo-controlled clinical trial of high-dose paracetamol in patients with acute stroke. The study protocol has been published earlier.[10, 11] In short, patients with ischemic stroke or intracerebral hemorrhage within 12 hours of symptom onset with no history of liver disease or pre-stroke impairment (modified Rankin Scale (mRS) score <2)[12] were included in this study. Patients with ischemic stroke included in the PAIS trial between March 2003 and March 2007 in centers where CRP was measured as a part of routine laboratory assessment on admission were included in the present study if venous blood sampling for CRP was accomplished within 12 hours of symptom onset.

Baseline variables

Baseline clinical information was extracted from the trial records. This included body temperature on admission and 24 hrs later, stroke subtype according to the TOAST classification[13], stroke severity as assessed with the NIH Stroke Scale (NIHSS)[14], and cardio-

vascular risk factors. In addition, the occurrence of infections during hospitalization was assessed. Body temperature was measured with either tympanic or rectal thermometers.

Blood samples and CRP assay

Blood samples for assessment of CRP and white blood cell count were taken on admission. The time of CRP measurement relative to stroke onset was recorded in all patients.

Levels of CRP were determined with a clinically validated assay. Participating centers used different analyzers (Dade Behring, Beckman LX-20, Beckman Synchron, Beckman Coulter DXC, Olympus 640, Ortho Vitros, Roche Cobas 6000, Roche Cobas Integra, Roche Hitachi 917, Roche Modular) to establish CRP levels. Intra- and inter-assay variation of all participating centers was evaluated by means of the external quality control scheme of the Dutch Foundation for Quality Assessment in Clinical Laboratories (SKML). If the intra-assay and/or inter-assay coefficient of variation was higher than 7,5%, patients from these centers were excluded from further analysis. All centers were subjected to quality review by the Dutch Foundation for Quality Assessment in Clinical Laboratories (SKML (www.skml.nl).

Some centers did not report exact values of CRP below a certain cut-off point, as values below these cut-off points were considered "normal" values. These cut-off points varied between 1 mg/L and 7 mg/L.

As extremely high levels of CRP likely reflect an infection at the time of blood sampling, patients in whom the level of CRP was higher than 2 SD above the mean, were excluded from further analysis.

Outcome measures

Poor outcome was defined as a score of more than 2 on the mRS, including death, at 3 months from stroke onset. A secondary outcome was death at 3 months. Outcome was assessed without knowledge of baseline CRP levels.

Statistical analysis

Statistical analyses were performed with Stata/SE 8.2 for Windows (Statacorp, College Station, Texas). The relation between outcome and dichotomized CRP levels was expressed as an odds ratio (OR) with a corresponding 95% confidence interval (CI), through logistic regression.

CRP values were dichotomized at 7 mg/L. This cut-off point was selected, as this was the highest cut-off point below which values were considered normal, and not reported as exact values by some centers. In order to study a possible level-risk relationship, all CRP values were recoded with 7 mg/L as the lower bound. Odds ratios were expressed per unit increase in logarithmically transformed CRP levels. Adjustment for the impact of

age, sex, baseline NIHSS score, cigarette smoking status, diabetes mellitus, hypertension, statin use, and stroke subtype (cardioembolic stroke versus non-cardioembolic stroke) was made with multiple logistic regression. We performed additional analyses to account for the potential confounding effect of antipyretic treatment by stratifying for treatment with acetaminophen.

RESULTS

Between March 1, 2003 and March 1, 2007, 1187 patients were included in the PAIS trial. Sixteen of the 29 participating centers routinely performed CRP measurements on admission for acute stroke. In these 16 centers, 897 patients where included in the PAIS trial in the period under study. Of these patients, 336 were excluded from the present study because of hemorrhagic stroke (n=117), CRP measurement not accomplished within 12 hours of stroke onset or time of assessment unknown (n=154), CRP measurements with analyzers with a variation coefficient >7.5% (n=56), or CRP levels over 110 mg/L (2 SD; n= 9).

The median CRP level was 5 mg/L (IQR 2-8) and 33% of patients had CRP levels of 7 mg/L or above.

Table 1 shows the baseline characteristics of the patients with CRP levels <7 mg/L or ≥7 mg/L. Median time from onset of symptoms to CRP measurement was 137 minutes (range, 0-696). Patients with CRP ≥7 mg/L were older, had higher scores on the NIHSS, had a slightly higher body temperature on admission, were more often smokers, more frequently had hypertension, diabetes mellitus, or atrial fibrillation, and less often used statins than patients with low CRP levels. Cardioembolic strokes were observed more often in patients with CRP ≥7 mg/L.

Sixteen patients with CRP ≥7 mg/L (9%) and 15 patients (4%) with CRP <7 mg/L developed an infection during hospitalization. Two patients with CRP ≥7 mg/L had an infection (both pneumonia) within 24 hours of stroke onset. No infections within 24 hours were reported in patients with CRP <7 mg/L.

No association between CRP levels and the body temperature at 24 hours after study enrollment was found.

Patients with CRP levels^3 7 mg/L more often had a poor outcome (57% versus 42%; p=0.006) or died (23% versus 13%; p=0.0007) than patients with lower CRP levels (Figure 1). Adjustment for potential confounders did not change these results (Table 2).

An additional stratified analysis to account for the potential confounding effect of treatment with paracetamol did not affect the association.

A level-risk relationship was observed between CRP and poor outcome or death at 3 months. The relation between CRP and poor outcome was attenuated after adjustment for potential confounders (Table 3).

Table 1: Baseline characteristics of the study population.

	CRP<7 mg/L	CRP ≥7 mg/L
Demographics		
N	377	184
Age (years), mean (SD)	69.1 (13.4)	70.9 (13.8)
Male	226 (60%)	110 (60%)
Stroke severity[§]		
NIHSS, mean (SD)	7.5 (6.0)	8.9 (6.6)
Risk factors		
Arterial hypertension	183 (49%)	107 (58%)
Atrial fibrillation	47 (12%)	47 (26%)
Diabetes mellitus	50 (13%)	37 (20%)
Current cigarette smoking	108 (29%)	70 (38%)
Hypercholesterolemia	104 (28%)	42 (23%)
History		
Stroke	84 (22%)	40 (22%)
Myocardial infarction	47 (12%)	25 (14%)
Peripheral vascular disease	25 (7%)	21 (11%)
Physical examination		
Systolic blood pressure, mean (SD)	169 (31)	171 (34)
Diastolic blood pressure, mean (SD)	89 (18)	89 (21)
Body temperature, mean (SD)	36.9 (0.6)	37.0 (0.6)
Laboratory assessments		
Time until CRP measurement (minutes), median (range)	140 (2-696)	129 (0-681)
Leukocytes 10^9/L, mean (SD)	8.2 (2.6)	9.4 (3)
Stroke type (TOAST)*		
Undetermined	189 (50%)	81 (44%)
Large vessel disease (≥50% stenosis)	38 (10%)	28 (15%)
Cardiac source of embolism	60 (16%)	44 (24%)
Small vessel occlusion	49 (13%)	24 (13%)
Other determined etiology	41 (11%)	7 (4%)
Treatment		
RtPA	95 (25%)	50 (27%)
Ace inhibitor	65 (17%)	38 (21%)
Statin	101 (27%)	36 (20%)

[§]Scores on the National Institutes of Health Stroke Scale (NIHSS) range from 0 to 42, with higher values indicating more severe stroke.[5]

*Based on the definitions of the Trial of ORG 10172 in Acute Stroke Therapy (TOAST) criteria.[1]

After exclusion of patients who developed an infection during the first two weeks after stroke onset, the adjusted odds ratio for poor outcome was 1.5 (95% CI 1.0-2.3; p=0.07), and for death 1.9 (95% CI 1.1-3.4).

DISCUSSION

In this study, patients with CRP levels ≥7 mg/L within 12 hours of ischemic stroke onset had a significantly increased risk of poor functional outcome or death at 3 months, even

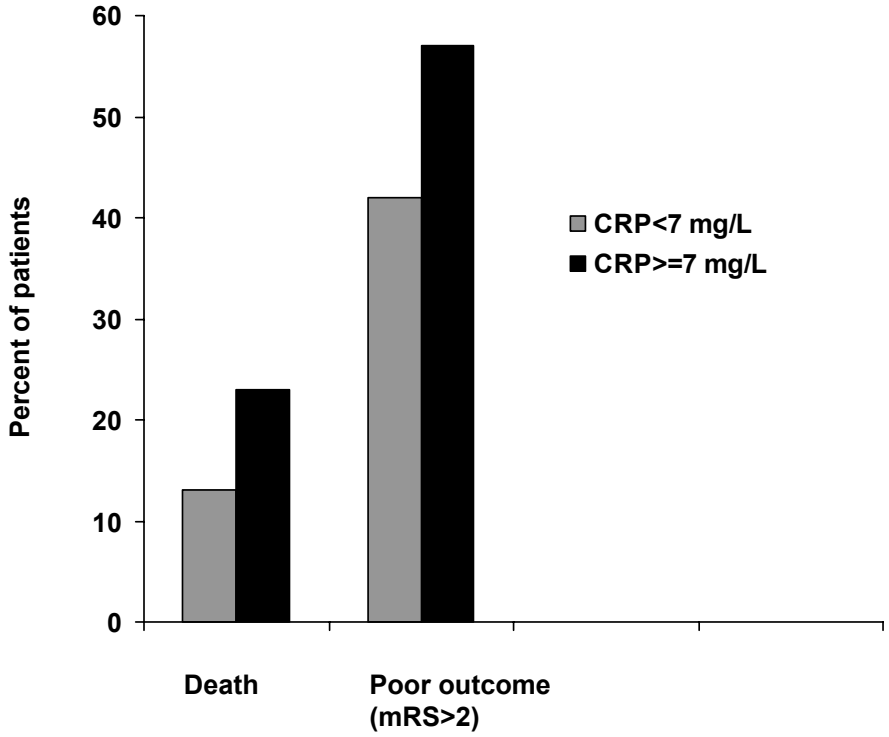

Figure 1: Association of CRP levels on admission with poor outcome or death at 3 months.

Table 2: Association between increased CRP (≥7 mg/L) at baseline and outcome after ischemic stroke.

	OR (95% CI)	Adjusted OR (95% CI)*
Poor outcome (mRS>2)	1.9 (1.3-2.7)	1.6 (1.1-2.4)
Death	2.0 (1.3-3.2)	1.7 (1.0-2.9)

*Adjusted for age, sex, NIHSS score, cigarette smoking, diabetes mellitus, hypertension, statin use, and stroke subtype.

Table 3: Level-risk relationship CRP and outcome.

	OR[$] (95% CI)	Adjusted OR*[$] (95% CI)
Poor outcome (mRS>2)	1.6 (1.2-2.2)	1.3 (0.9-1.9)
Death	2.1 (1.5-3.0)	1.9 (1.2-2.8)

[$]ORs were expressed per 1 unit increase in logarithmically transformed CRP levels.
*Adjusted for age, sex, NIHSS score, cigarette smoking, diabetes mellitus, hypertension, statin use, and stroke subtype.

after adjustment for potential confounders. In addition, a level-risk relationship was found between CRP and poor outcome and death.

Several studies have found an association between increased CRP levels and clinical outcome in the time window between 12 and 72 hours after ischemic stroke.[1, 2, 5-9, 15-17] The results of previous studies that have aimed to assess the prognostic value of CRP in the

very early phase of stroke are ambiguous. Two prospective studies did not observe a relation between CRP levels obtained within 6 or 12 hours after symptom onset and death or dependency at follow-up.[8, 9] Both studies were rather small (127 and 111 patients) and may therefore have lacked the power to detect an association. In addition, one of these studies included only patients treated with rt-PA.[8]

In line with our results, three other studies found an association between CRP and outcome. In one of these studies, CRP was measured within 24 hours of stroke onset, and 25% of the patients had a TIA instead of a stroke, which may have influenced the observed association.[5] In addition, only the relation between CRP and mortality, and not disability, was studied. Other studies included only patients aged 75 years or older [6], or patients who had a middle cerebral artery occlusion and received rt-PA.[7] In the latter, an association with mortality was found, but functional outcome was not assessed.[7]

The strengths of the present study are its large sample size, robust outcome measures and early CRP measurement, which increase the relevance of our findings. Furthermore, detailed information on confounders was available.

Some methodological limitations should be discussed. First, this study was part of a larger clinical trial, and not designed to evaluate the prognostic value of CRP with regard to clinical outcome in acute ischemic stroke. As a consequence, a detailed history of inflammatory conditions was not recorded.

Levels were determined with different analyzers, which may have resulted in a systematic error. For this reason, measurements performed with analyzers with an inter-assay coefficient of variation ≥ 7,5% or that demonstrated bias relative to the other participating centers were excluded from further analysis. Secondly, the lowest detection limit for CRP varied from 1 mg/L to 7 mg/L among the centers. Therefore, CRP levels had to be dichotomized. This makes it more difficult to compare our results with previous studies. However, in three of the five studies that assessed the relation between CRP levels in the very early phase of acute stroke and clinical outcome, a similar cut-off point level was selected.[20, 27, 30] Furthermore, all CRP values were recoded with 7 as the lower bound, in order to study a possible level-risk relationship.

A fourth issue might be that rather few patients with very severe stroke were included, which may affect the generalisability of the results.

In patients with ischemic stroke, increased levels of CRP may reflect the pre-existing degree of atherosclerosis or the presence of vascular risk factors. We found that patients with higher levels of CRP were more often smokers and more frequently had hypertension, diabetes mellitus, or atrial fibrillation. Furthermore, cardioembolic strokes were observed more often in patients with higher levels of CRP. Moreover, patients with CRP ≥7 mg/L used statins less often than patients with low CRP levels, suggesting that drugs in this class reduce levels of CRP. These findings are supported by previous studies.[6, 18-22]

Why could CRP concentration be a prognostic factor for poor outcome and death? Early after onset of ischemic stroke, increased CRP levels may reflect an accompanying inflam-

matory reaction. Inflammatory processes play an important role in the pathophysiology of ischemic stroke.[23] Cerebral ischemia triggers an inflammatory response characterized by activation and release of acute phase proteins such as C-reactive protein (CRP) and cytokines.[23-25] The inflammatory processes may start within 2 hours after stroke onset and sustain for days [26], and may contribute to ischemic brain damage even in that early stage.[27]

Elevated CRP levels may be a reflection of the extent of brain injury. In our study, patients with CRP levels ≥7 mg/L had higher NIHSS scores on admission. Previous studies have shown that patients with increased CRP levels have larger infarctions.[26] Although patients with CRP levels ≥7 mg/L taken as a group had worse outcomes at 3 months as assessed with the mRS, multiple logistic regression modeling indicated that the adverse outcome in this group remained even after adjustment for initial stroke severity by NIHSS score and multiple other risk factors for poor outcome. This suggests that CRP not only reflects the amount of tissue damage, but may also indicates a state of enhanced risk due to increased inflammation or cytokine excess. Interestingly, recent experimental studies have shown that CRP itself may contribute to secondary brain damage after focal cerebral ischemia, possibly via a complement-mediated exacerbation of tissue injury.[3, 4] In rats, treatment with human CRP after middle cerebral artery occlusion resulted in larger infarcts.[3] Similar results have been observed in experimental models of myocardial infarction.[28]

It is therefore conceivable that increased levels of CRP following stroke are not only a consequence of brain infarction, but contribute to ischemic damage as well.

Increased CRP levels following ischemic stroke may also reflect concurrent infections. Secondary infections are common in the first week of stroke and are associated with poor outcome[27, 29, 30], but they usually occur more than 12 hours after stroke onset. In our study, only two clinically overt infections were reported within 24 hours after stroke onset. In addition, we excluded patients in whom the CRP level exceeded 2 SD above the mean, who may have had an infection before stroke onset. Furthermore, exclusion of patients developing an early infection during hospitalization did not substantially affect the relation between CRP levels and poor functional outcome or death.

The use of biomarkers as predictors of stroke lesion evolution and prognosis is becoming increasingly important, as they may be valuable tools in the search for an optimal management of stroke patients. The present study confirms results from previous studies that have advocated CRP as a powerful prognostic marker in patients with ischemic stroke.[1, 5-7, 9, 15-17, 26] It may provide important prognostic information beyond conventional clinical parameters. Further studies are needed to assess if CRP is also a predictor of outcome after stroke.

Moreover, the fact that animal studies have found that CRP might exacerbate brain tissue damage following ischemic stroke, might stimulate research into the underlying pathogenetic mechanisms and development of new, more targeted, medical treatments for acute ischemic stroke.

In conclusion, elevated CRP levels in the very early phase of acute ischemic stroke are independent prognostic factors for poor outcome at 3 months.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement and ethics

The PAIS trial was sponsored by the Netherlands Heart Foundation, grant number 2002B148. We are grateful to all patients, secretaries, neurologists who contributed to this study.

All patients participated in the PAIS trial; written informed consent was obtained from all patients or from their legal representatives for inclusion in the trial and for follow-up. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Participating centers with names of investigators

Meander MC Amersfoort (HMA van Gemert), Erasmus MC Rotterdam (DWJ Dippel), UMC Utrecht (HB van der Worp), Sint Fransicus Gasthuis Rotterdam (FH Vermeij), Slotervaart Ziekenhuis Amsterdam (VIH Kwa), Gelre ziekenhuizen Apeldoorn (HP Bienfait), TweeSteden ziekenhuis Tilburg (BPW Jansen), Sint Elisabeth Ziekenhuis Tilburg (PLM de Kort), Westfriesgasthuis Hoorn (TC van der Ree), Maasstad ziekenhuis Rotterdam (R Saxena), Ziekenhuis Bethesda Hoogeveen (PG Oomes), Dirksland Ziekenhuis Dirksland (UW Huisman), Sint Lucas Andreas Ziekenhuis Amsterdam (EJ Wouda), Martini Ziekenhuis Groningen (C Bouwsma), Catharina Ziekenhuis Eindhoven (K Keizer) Albert Schweitzer ziekenhuis Dordrecht (RP Kleyweg/H Kerkhoff), Amphia Ziekenhuis Breda (HBC Verbiest/SLM Bakker), Rijnland Ziekenhuis Leiderdorp (ELLM De Schryver), Diakonessenhuis Utrecht (RCJM Donders), Slingeland Ziekenhuis Doetinchem (RA van der Kruijk), IJsselland Ziekenhuis Capelle aan den Ijssel (J Heerema), Jeroen Bosch Ziekenhuis Den Bosch (RAJAM Bernsen), Ziekenhuis Hilversum Hilversum (D Herderschêe), Diaconessenhuis Meppel (EJW Keuter), Vlietlandziekenhuis Schiedam (WC Baart), Spaarne Ziekenhuis Hoofddorp (RJ Meijer), Beatrixziekenhuis Gorinchem (RB Alting van Geusau), Streekziekenhuis Koningin Beatrix Winterswijk (JP de Ruiter), Wilhelmina Ziekenhuis Assen (JN Wessel/AE Bollen).

REFERENCES

1. Di Napoli M, Papa F, Bocola V. C-reactive protein in ischemic stroke: an independent prognostic factor. *Stroke* 2001; 32:917-924.
2. Smith CJ, Emsley HC, Vail A et al. Variability of the systemic acute phase response after ischemic stroke. *J Neurol Sci* 2006; 251:77-81.
3. Gill R, Kemp JA, Sabin C et al. Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats. *J Cereb Blood Flow Metab* 2004; 24:1214-1218.
4. Pepys MB, Hirschfield GM, Tennent GA et al. Targeting C-reactive protein for the treatment of cardiovascular disease. *Nature* 2006 27; 440:1217-1221.
5. Christensen H, Boysen G. C-reactive protein and white blood cell count increases in the first 24 hours after acute stroke. *Cerebrovasc Dis* 2004; 18:214-219.
6. Masotti L, Ceccarelli E, Forconi S et al. Prognostic role of C-reactive protein in very old patients with acute ischemic stroke. *J Intern Med* 2005; 258:145-152.
7. Montaner J, Fernandez-Cadenas I, Molina CA et al. Poststroke C-reactive protein is a powerful prognostic tool among candidates for thrombolysis. *Stroke* 2006; 37:1205-1210.
8. Topakian R, Strasak AM, Nussbaumer K et al. Prognostic value of admission C-reactive protein in stroke patients undergoing iv thrombolysis. *J Neurol* 2008; 255:1190-1196.
9. Winbeck K, Poppert H, Etgen T et al. Prognostic relevance of early serial C-reactive protein measurements after first ischemic stroke. *Stroke* 2002; 33:2459-2464.
10. den Hertog HM, van der Worp HB, van Gemert HM et al. Correction: PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial. [ISCRTN74418480]. *BMC Cardiovasc Disord* 2008; 8:29.
11. van Breda EJ, van der Worp HB, van Gemert HM et al. PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial [ISCRTN 74418480]. *BMC Cardiovasc Disord* 2005; 5:24.
12. van Swieten JC, Koudstaal PJ, Visser MC et al. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988 ;19:604-607.
13. Adams HP, Bendixen BH, Kappelle LJ et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993 ; 24:35-41.
14. Brott T, Adams HP, Olinger CP et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; 20:864-870.
15. Di Napoli M, Papa F, Bocola V. Prognostic influence of increased C-reactive protein and fibrinogen levels in ischemic stroke. *Stroke* 2001; 32:133-138.
16. Kocer A, Canbulat C, Gozke E et al. C-reactive protein is an indicator for fatal outcomes in first-time stroke patients. *Med Sci Monit* 2005 ; 11:CR540-CR544.
17. Muir KW, Weir CJ, Alwan W et al. C-reactive protein and outcome after ischemic stroke. *Stroke* 1999; 30:981-985.
18. Albert MA, Danielson E, Rifai N et al. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA* 2001; 286:64-70.
19. Di Napoli M, Schwaninger M, Cappelli R et al. Evaluation of C-reactive protein measurement for assessing the risk and prognosis in ischemic stroke: a statement for health care professionals from the CRP Pooling Project members. *Stroke* 2005; 36:1316-1329.
20. Ladenvall C, Jood K, Blomstrand C et al. Serum C-reactive protein concentration and genotype in relation to ischemic stroke subtype. *Stroke* 2006; 37:2018-2023.

21. Ridker PM, Rifai N, Pfeffer MA et al. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation* 1999; 100:230-235.
22. Ridker PM, Danielson E, Fonseca FA et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008; 359:2195-2207.
23. McColl BW, Allan SM, Rothwell NJ. Systemic inflammation and stroke: aetiology, pathology and targets for therapy. *Biochem Soc Trans* 2007; 35:1163-1165.
24. Barone FC, Feuerstein GZ. Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* 1999; 19:819-834.
25. Kushner I, Agrawal A. CRP can play both pro-inflammatory and anti-inflammatory roles. *Mol Immunol* 2007; 44:670-671.
26. Smith CJ, Emsley HC, Gavin CM et al. Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. *BMC Neurol* 2004; 4:2.
27. Emsley HC, Hopkins SJ. Acute ischemic stroke and infection: recent and emerging concepts. *Lancet Neurol* 2008; 7:341-353.
28. Griselli M, Herbert J, Hutchinson WL et al. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med* 1999; 190:1733-1740.
29. Aslanyan S, Weir CJ, Diener HC et al. Pneumonia and urinary tract infection after acute ischemic stroke: a tertiary analysis of the GAIN International trial. *Eur J Neurol* 2004; 11:49-53.
30. Emsley HC, Smith CJ, Gavin CM et al. An early and sustained peripheral inflammatory response in acute ischemic stroke: relationships with infection and atherosclerosis. *J Neuroimmunol* 2003; 139:93-101.

Variation in the C-reactive protein (CRP) gene is associated with serum levels of CRP in patients with acute ischemic stroke

Heleen M den Hertog[1$], Evita G van den Herik[1], Diederik WJ Dippel[1], Peter J Koudstaal[1], Moniek PM de Maat[2]

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2] Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

Under Revision

ABSTRACT

Background and purpose Elevated levels of C-reactive protein (CRP) are found in up to three quarters of patients with acute ischemic stroke and are associated with poor outcome. We investigated whether haplotypes representing common variations in the CRP gene are associated with levels of CRP in patients with acute ischemic stroke.

Methods We included 185 patients with ischemic stroke in whom CRP was measured within 24 hours of symptom onset. Common haplotypes within the CRP gene were determined by 3 genotype-tagging single-nucleotide polymorphisms (SNPs).

Results Four haplotypes with frequencies >5% covered 99.2% of the genetic variation. Haplotype 4 (CCG, frequency 8.3%) was associated with a 20.6 mg/L (95% CI, 9.8 to 30.4) stronger increase in CRP level as compared with haplotype 1 (CTC, frequency 33.7%).

Conclusion Variation in the CRP gene is associated with levels of CRP in acute ischemic stroke.

abstract

BACKGROUND

C-reactive protein (CRP) is a strong acute phase reactant. Elevated levels of serum CRP are found in up to three quarters of patients with acute ischemic stroke and are associated with poor outcome.[1-4]This leads to interest in the use of CRP as a biomarker of prognosis of ischemic stroke. Moreover, CRP may contribute to secondary brain damage after focal cerebral ischemia, possibly via a complement-mediated exacerbation of tissue injury.[5]

The CRP concentrations are under genetic influence. Family and twin studies found that additive genetic factors account for 27–40% of the variance in CRP**,** indicating a role of DNA sequence variation in determining serum protein levels.[6] Furthermore, there is accumulating evidence for an association between single nucleotide polymorphisms (SNPs) in the CRP-gene and levels of CRP.[7]

Only limited and inconsistent data are available on the association between SNPs in the CRP gene and CRP concentrations in patients with acute ischemic stroke.[3, 8]

In the present study, we investigated whether haplotypes representing common variations in the CRP gene are associated with levels of CRP in patients with acute ischemic stroke.

METHODS

Study population

We evaluated 185 consecutive patients with acute ischemic stroke admitted between 2005 and 2008 in whom venous blood was collected within 24 hours of symptom onset from the Rotterdam Stroke Biobank, a project aimed at collecting clinical information, blood samples and DNA of all patients with neurovascular diseases admitted to Erasmus University Medical Center. Written informed consent was obtained from all patients, signed by the participants or a first degree relative, as approved by the Institutional Ethics Committee.

Baseline characteristics

Baseline clinical information was extracted from the trial records. This included a quantification of stroke severity according to the National Institutes of Health Stroke Scale (NIHSS)[9], ischemic stroke subtype according to the TOAST classification[10], and cardiovascular risk factors.

Measurement of CRP

CRP levels were determined by means of the Roche Modular assay. The range of measurement is 1–285 mg/L with a variation coefficient of 4.6%.

CRP polymorphism genotyping

In the Seattle SNPs program for Genomic applications, 31 SNPs were identified in the CRP gene on chromosome 1q21 (**http://www.pga.gs.washington.edu/data/crp**, "visual haplotype" option).

In 23 unrelated individuals of European descent, these SNPs formed 4 well-defined haplotypes with frequencies of more than 5%. By selecting 3 tagging SNPs, the total common variation in the CRP gene is described. We selected the following tagging SNPs: 1184 C>T (rs1130864), 2042 C>T (rs1205) and 2911C>G (rs3093068).

DNA was extracted according to standard procedures and stored at -20°C. Genotypes were determined in genomic DNA of 2 ng with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, California) and end-point readings of fluorescence were performed on Taqman Prism 7900HT (Applied Biosystems, Foster City, California)

Statistical analysis

SNPs were tested for Hardy Weinberg equilibrium with a Chi square test. The association between CRP haplotypes and levels of CRP were assessed with the non-parametric haplo.score function of Haplo Stats (http://cran-r.project.org/scr/contrib/Descriptions/haplostats). We adjusted for age, sex and stroke severity on admission by means of multiple linear regression.

RESULTS

In the total study population, the mean age was 64 years (SD 15), 53% were male and the median NIHSS score was 4 (range 0-22) (Table 1). Mean CRP level was 10 mg/L (SD 27). Median time from onset of symptoms to CRP measurement was approximately 7 hours.

All SNPs were in Hardy Weinberg equilibrium. The 1184T-allele was present in 25.9%, the 2042T-allele was present in 34.5% and the 2911G-allele in 8.6% of 370 chromosomes.

Four common haplotypes were identified (Table 2). The remaining haplotypes (CTG, TCG, TTC) were present in less than 5%.

Haplotype 4 (CCG) was associated with a significant stronger increase in CRP level (20.6 mg/L; 95% CI, 9.8 to 30.4) relative to the reference haplotype 1 (CTC) (Table 2 and Figure 1). Adjustment for age, sex and NIHSS score did not attenuate this association (Figure 1).

Table 1: Clinical characteristics of the patients (n=185).

Demographics	
Mean (SD) age (years)	64 (15)
Sex (male)	98 (53%)
Cardiovascular risk factors	
Hypertension	98 (53%)
Atrial fibrillation	22 (12%)
Diabetes mellitus	29 (16%)
Current cigarette smoking	35 (19%)
Hypercholesterolemia	85 (46%)
Medical history	
Previous stroke	50 (27%)
Previous myocardial infarction	31 (17%)
Peripheral vascular disease	13 (7%)
Stroke subtype*	
Large vessel disease (≥50% stenosis)	28 (15%)
Cardiac source of embolism	30 (16%)
Small vessel occlusion	27 (15%)
Other determined etiology	21 (11%)
Undetermined / negative evaluation	79 (43%)
Stroke severity	
Median (range) NIHSS score[$]	4 (0-22)
Physical examination	
Mean (SD) systolic blood pressure on admission (mm Hg)	167 (34)
Mean (SD) diastolic blood pressure on admission (mm Hg)	86 (20)
Laboratory assessments	
Median (IQR) time from stroke onset to measurement of CRP (hrs)	6.6 (3 to 13.9)
Treatment	
Treatment with rt-PA	36 (19%)

*Based on the Trial of ORG 10172 in Acute Stroke Therapy (TOAST) criteria.[10]
[$]Scores on the National Institutes of Health Stroke Scale (NIHSS).[9]

Table 2: Frequency of haplotypes and association between CRP haplotypes and levels of CRP.

Haplotype	Haplotype frequency	Haplo-score	P-value*
1 (CTC)	33.7%	-0.75	0.45
2 (CCC)	31.7%	-0.01	0.98
3 (TCC)	25.5%	0.41	0.68
4 (CCG)	8.3%	4.49	<0.01

*P-values were obtained using haplo.score, after 2377 simulations

DISCUSSION

In this study, we found that SNPs in the CRP gene were associated with levels of CRP within 24 hours of ischemic stroke onset. Haplotype 4 (CCG) was associated with significant higher levels of CRP than haplotype 1 (CTC).

Our results provide further proof that, apart from environmental factors, genetic variations may influence levels of CRP. Several studies found an association between

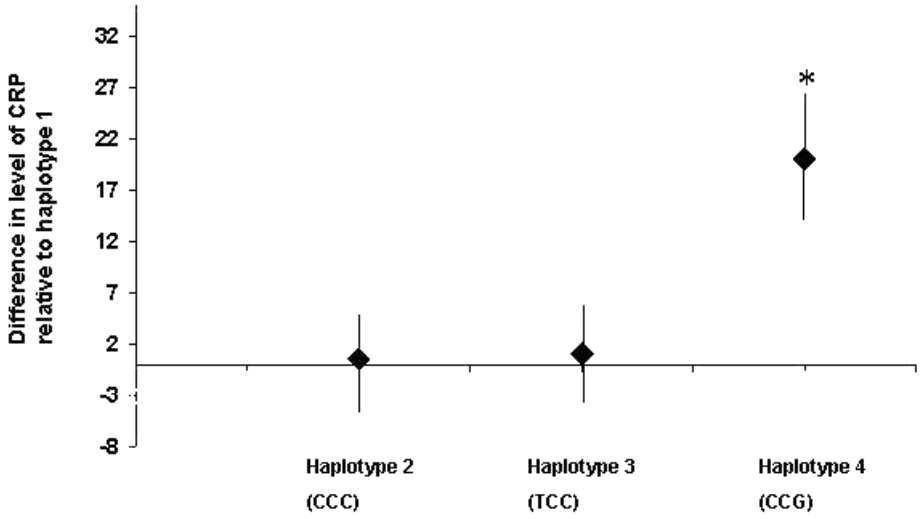

Figure 1: Relative effects of CRP gene haplotypes on levels of CRP (mg/L) *P-value<0.01. Regression coefficients were adjusted for age, sex and NIHSS score on admission. Coefficients reflect the mean difference in levels of CRP relative to haplotype 1 (CTC).

CRP haplotypes and levels of CRP in diseases in which inflammation is involved.[7, 11, 12] The direction of the haplotype-CRP-level associations are also supported by previous studies that have used the same polymorphisms to reconstruct the haplotypes.[7, 11, 12] Two previous studies that investigated the association between SNPs in the CRP gene and levels of CRP in patients with acute ischemic stroke cannot be compared directly with ours, because only single SNPs were studied.[3, 8]

The present study has some limitations. The study compromised a small number of patients. Furthermore, our results did not include information about ethnicity. Allele frequencies have been reported to differ between ethnic groups. However, the haplotype structures are similar and therefore the direction and size of changes in CRP levels associated with most SNPs might be similar regardless of ethnic group.[12]

Increases in CRP levels following ischemic stroke may reflect a systemic inflammatory response, the extent of tissue injury, or concurrent infections. Interestingly, in animal models of focal cerebral ischemia, CRP increased secondary brain damage through activation of the complement system.[5, 13] These findings suggest that understanding genotype–phenotype associations of *CRP* polymorphisms may provide a genetic basis for the future development of tailored therapeutic strategies in ischemic stroke.

In conclusion, variation in the CRP gene is associated with levels of CRP in acute ischemic stroke. Further studies are needed to investigate the relation between variation in the CRP gene, levels of CRP and clinical outcome after acute ischemic stroke.

REFERENCES

1. Di Napoli M, Schwaninger M, Cappelli R et al. Evaluation of C-reactive protein measurement for assessing the risk and prognosis in ischemic stroke: a statement for health care professionals from the CRP Pooling Project members. *Stroke* 2005; 36:1316-1329.
2. Masotti L, Ceccarelli E, Forconi S et al. Prognostic role of C-reactive protein in very old patients with acute ischaemic stroke. *J Intern Med* 2005; 258:145-152.
3. Montaner J, Fernandez-Cadenas I, Molina CA et al. Poststroke C-reactive protein is a powerful prognostic tool among candidates for thrombolysis. *Stroke* 2006; 37:1205-1210.
4. den Hertog HM, van Rossum J, van der Worp HB et al. C-reactive protein in the very early phase of acute ischemic stroke: association with poor outcome and death. *J of Neurology* 2009 Epub.
5. Gill R, Kemp JA, Sabin C et al. Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats. *J Cereb Blood Flow Metab* 2004; 24:1214-1218.
6. Pankow JS, Folsom AR, Cushman M et al. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. *Atherosclerosis* 2001; 154:681-689.
7. Kardys I, de Maat MP, Uitterlinden AG et al. C-reactive protein gene haplotypes and risk of coronary heart disease: the Rotterdam Study. *Eur Heart J* 2006; 27:1331-1337.
8. Ben-Assayag E, Shenhar-Tsarfaty S, Bova I et al. Triggered C-reactive protein (CRP) concentrations and the CRP gene -717A>G polymorphism in acute stroke or transient ischemic attack. *Eur J Neurol* 2007; 14:315-320.
9. Brott T, Adams HP, Olinger CP et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; 20:864-870.
10. Adams HP, Bendixen BH, Kappelle LJ et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993 ; 24:35-41.
11. Miller DT, Zee RY, Suk DJ et al. Association of common CRP gene variants with CRP levels and cardiovascular events. *Ann Hum Genet* 2005; 69:623-638.
12. Teng MS, Hsu LA, Wu S et al. Association between C-reactive protein gene haplotypes and C-reactive protein levels in Taiwanese: Interaction with obesity. *Atherosclerosis* 2008; 204:e64-e69 .
13. Griselli M, Herbert J, Hutchinson WL et al. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med* 1999; 190:1733-1740.

Chapter 5

Future research, a first step

Introduction to animal models of ischemic stroke

Heleen M den Hertog[1],
Peter J Koudstaal[1]

[1]Department of Neurology, Erasmus MC
University Medical Center, Rotterdam,
The Netherlands

BACKGROUND

The notion that ischemic stroke is not only preventable but also an attractive target for acute therapeutic interventions has provided a major impetus to stroke research. At present, aspirin, surgical decompression and rt-PA are available proven therapeutic options for acute stroke.[1-4] Animal experiments help to learn us about the pathogenesis of ischemic stroke and are therefore an integral part of research on cerebral ischemia.

Ideally, an animal model of ischemic stroke meets the following criteria:

1. It should be relevant to human ischemic stroke
2. The ischemic lesion should be reproducible
3. The technique applied to induce cerebral ischemia should be relatively easy to perform and minimally invasive
4. Relevant outcome measures

First, I will give a short overview of the different models of ischemic stroke that have been used to date, the advantages and disadvantages of these models and the role of animals models in relation to stroke research. Next, a first step into further research in acute stroke aimed at integrating basic and clinical science will be presented.

ANIMAL SELECTION

Experimental models of ischemic stroke have been developed in many species, including larger mammals such as cats, rabbits and nonhuman primates, as well as small rodents such as rats and mice. Rats and mice are most commonly used, since they are relatively inexpensive and their physiology and cerebrovascular anatomy closely resemble that of humans. The commercially available inbred rats are genetically relatively homogenous[5], allowing good reproducibility of infarctions. Mice have been of increased interest, because of availability of transgenic technology, which offers new insights in the molecular processes involved in acute ischemic stroke.

Animal studies typically use young, healthy populations of animals that are homogeneous for sex and age, whereas the typical stroke patient is elderly with numerous risk factors. Therefore, the influence of age, sex and comorbidities on experimental ischemic stroke should also be taken into account.

TECHNIQUES FOR INDUCING FOCAL CEREBRAL ISCHEMIA

Focal cerebral ischemia models usually involve occlusion of the middle cerebral artery and can be divided into transient and permanent ischemia models.

The intraluminal monofilament model[6, 7]

This is the most commonly used model. Briefly, a nylon monofilament is introduced into the internal carotid artery and forwarded until the tip occludes the origin of the middle cerebral artery . The monofilament can be coated with silicone, a harder or it can be used without coating. Both permanent and transient ischemia can be achieved by respectively maintaining or withdrawing the monofilament. Advantages of this approach are that it is relatively easy to perform, less invasive than other models and is reproducible. A disadvantage is that this model is not appropriate for evaluation of treatment with rt-PA.

Transcranial model[8]

The middle cerebral artery is surgically dissected, and subsequently occluded by a clip or ligation. This technique produces reproducible infarctions. Furthermore, it results into smaller infarctions, and hence is appropriate for experiments with long-term survival. One of the disadvantages of this approach is that it is difficult and requires more investigator experience. Furthermore, it is invasive, and is associated with an increased risk of subarchnoid hemorrhage.

The thrombo-embolic model[9]

This model is of great interest because it resembles human ischemic stroke and because of its role in evaluating treatment with rt-PA. The middle cerebral artery is occluded by fibrin-rich clots, prepared either by adding thrombin to freshly drawn arterial blood or by spontaneous coagulation. The clots are injected via a catheter in the internal carotid artery. Disadvantages of this model are that it is less reproducible and that it is associated with an increased risk of hemorrhagic stroke.

Photo-thrombosis model[10]

In this model, ischemic stroke is induced by vascular injection of a photo-active dye in combination of irradiation with a light beam. A reaction between the dye and the light beam resulted in platelet aggregation and thrombosis. The location and the extent of the lesion may be well controlled with this technique. However, it do not mimic human stroke very closely. Furthermore, this technique is associated with an increased risk of hemorrhagic stroke and is not appropriate for evaluating treatment with rt-PA.

CHOICE OF ANIMAL MODEL

Since ischemic stroke is a heterogeneous disease, no single animal model can represent human stroke. We have opted to set up a "intraluminal monofilament" model, because this is one of the most reproducible models and is widely applied.

STAIR CRITERIA

Many animal studies of ischemic stroke have been poorly designed, conducted and analyzed. Experimental studies have identified numerous targets for therapy. However, the translation of these results from bench to bedside has been disappointing. Lessons from translational failures led to the Stroke Therapy Academic Industry Roundtable (STAIR) recommendations for pre-clinical and clinical studies (see appendix).[11, 12] The STAIR criteria aim to improve the methodological quality of preclinical stroke research.

EVALUATION

The STAIR citeria recommend that at least two outcome measures should be reported, namely infarct volume and functional recovery.[11, 12]

Physiological variables

Rectal temperature, blood pressure and blood gases can be measured during the intervention. These parameters are important for the interpretation of outcome of animals.

Histology: measurement of infarct area

The infarct volume is usually assessed after 24 hours of reperfusion in transient ischemia models and after 6 hours of reperfusion in permanent ischemia models. The brain is removed, and subsequently 2-mm-thick coronal sections are obtained. Brain infarction is determined by a TTC (2,3,5 triphenyltetrazolium chloride) vital staining method. TTC is reduced by mitochondrial enzymes to a red compound. As a consequence, the non-infarcted regions are stained red, whereas the infarcted regions are not stained. Alternatively, the brains can be snap-frozen in isopentane for cryostat sectioning and stained with hematoxylin and eosin (HE), a nuclear/cytoplasmatic staining. TTC staining is easier, and less expensive than HE staining. However, after 72 hours of reperfusion, TTC staining may underestimate infarct volume, because various cells infiltrate into the infarcted

region, and mitochondria in these cells may react with TTC, resulting in obscuration of the borders of the infarct.

Infarct areas are quantitated by use of sigma 4.0 software, and infarct volumes are calculated by summing the volumes of each section directly or indirectly with the following formula: contralateral hemisphere (mm^3) – undamaged ipsilateral hemisphere (mm^3).

A number of other staining methods, including TUNEL staining and Caspase-3 staining to detect apoptotic cells can be applied.

Imaging: MRI

In the past, and to a large extent still today, evaluation of the animal experiments of cerebral ischemia have been based on invasive techniques, thus permitting only information on single time points in the evolution of the ischemic pathology. With the advent of noninvasive imaging methods, longitudinal studies on animals have become available, thus providing access to information on disease evolution. The non-invasiveness of MRI provides the potential to monitor the evolution of physiological changes within the ischemic brain and to predict therapeutic effects of new treatments. So far the application of imaging with different MR techniques in animal models is just beginning, but will rapidly expand in the near future.

Neurological evaluation

Functional impairment can be assessed by the following 4-point scale[13]:
1. Spontaneous activity: moving and exploring
0 = moving and exploring, 1 = moving without exploring, 2 = no moving or only when pulled by tail
2. Circling to the left/right
0 = none, 1 = when elevated by the tail and pushed or pulled, 2 = spontaneously, 3 = circling without displacement
3. Parachute reflex
0= symmetrical, 1 = asymmetrical, 2 = contralateral forelimb retracted
4. Resistance to left forepaw stretching
0 = stretching not allowed, 1 = stretching allowed, 2 = no resistance
The total score ranges from 0 (no neurological deficits) to 9 (severe neurological deficits). Other tests for assessment of functional impairment are the Morris water maze test[14], the paw-placement test[15] and the cylinder test.[15]

"BEDSIDE TO BENCH AND BACK APPROACH"

Animal studies may help to unravel the biology of stroke and the response to various drugs. The contribution of animal studies of acute stroke to clinical practice, however, has been poor thus far. This has led to serious doubts about the utility of animal models of acute stroke for development of therapy for human stroke. There is clearly a need to increase the quality of the design of animal studies and to develop models that more closely approximate the clinical situation.

On the other hand, it becomes clear that a strictly linear bench-to-bedside approach may not be optimal for translating findings in animal models of acute stroke into clinically effective stroke therapies. A clinical study often raises new questions and information should be obtained from the positive or negative results of each clinical trial. Animal studies might be valuable in helping to elucidate these issues. Furthermore, close communication and collaboration between the clinician and the basic stroke scientist is of high importance.

We expect that this `bench to bedside and back" approach will greatly enhance our knowledge on ischemic stroke as well as on the response of ischemic stroke to therapies and may support our clinical studies.

REFERENCES

1. Hacke W, Kaste M, Bluhmki E et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; 359:1317-1329.
2. Hofmeijer J, Kappelle LJ, Algra A et al. Surgical decompression for space-occupying cerebral infarction (the Hemicraniectomy After Middle Cerebral Artery infarction with Life-threatening Edema Trial [HAMLET]): a multicentre, open, randomised trial. *Lancet Neurol* 2009; 8:326-333.
3. Sandercock PA, Counsell C, Gubitz GJ et al. Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev* 2008; 3:CD000029.
4. Wardlaw JM, Zoppo G, Yamaguchi T et al. Thrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev* 2003; 3:CD000213.
5. Oliff HS, Weber E, Eilon G et al. The role of strain/vendor differences on the outcome of focal ischemia induced by intraluminal middle cerebral artery occlusion in the rat. *Brain Res* 1995; 675:20-26.
6. Endres M, Laufs U, Huang Z et al. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc Natl Acad Sci* 1998; 95:8880-8885.
7. Justicia C, Planas AM. Transforming growth factor-alpha acting at the epidermal growth factor receptor reduces infarct volume after permanent middle cerebral artery occlusion in rats. *J Cereb Blood Flow Metab* 1999; 19:128-132.
8. Buchan AM, Xue D, Slivka A. A new model of temporary focal neocortical ischemia in the rat. *Stroke* 1992; 23:273-279.
9. Niessen F, Hilger T, Hoehn M et al. Differences in clot preparation determine outcome of recombinant tissue plasminogen activator treatment in experimental thromboembolic stroke. *Stroke* 2003; 34:2019-2224.
10. Watson BD, Dietrich WD, Busto R et al. Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol* 1985; 17:497-504.
11. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke* 1999; 30:2752-2758.
12. Fisher M. Recommendations for advancing development of acute stroke therapies: Stroke Therapy Academic Industry Roundtable 3. *Stroke* 2003; 34:1539-1546.
13. Garcia JH, Wagner S, Liu KF et al. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. *Stroke* 1995; 26:627-634.
14. Giuliani D, Ottani A, Minutoli L et al. Functional recovery after delayed treatment of ischemic stroke with melanocortins is associated with overexpression of the activity-dependent gene Zif268. *Brain Behav Immun* 2009.
15. MacLellan CL, Davies LM, Fingas MS et al. The influence of hypothermia on outcome after intracerebral hemorrhage in rats. *Stroke* 2006; 37:1266-1270.

No effectiveness of synthetic anti-inflammatory tetrapeptides in a mouse model of ischemic stroke: a pilot study

Heleen M den Hertog[1], Willem A Dik[2], Dirk J Duncker[3], Nisar A Khan[2], Robbert Benner[2] and Peter J Koudstaal[1]

[1]Department of Neurology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[2]Department of Immunology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

[3]Department of Cardiology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

In preparation

ABSTRACT

Background and purpose Inflammation plays an important role in the pathophysiology of ischemic stroke and may contribute to secondary damage following ischemic stroke. Recently, it has been shown that synthetic oligopeptides related to human chorionic gonadotropin (hCG) and various other synthetic oligopeptides may have immunomodulatory effects.

We aimed to investigate the effects of two promising synthetic anti-inflammatory tetrapeptides, namely the hCG-related peptide AQGV and the p53-related peptide EPPE on infarct volume and on inflammatory gene expression in an animal model of focal cerebral ischemia.

Methods Mice received two injections of either AQGV (30 mg/kg bodyweight) or EPPE (30mg/kg bodyweight) or phosphate buffered saline. The first dose was administered 15 minutes before middle cerebral artery occlusion, and the second dose 45 minutes after reperfusion. Infarct volume and gene expression levels of tumor necrosis factor-α, interleukin-6, E-selectin and intercellular adhesion molecule-I (ICAM-1) were measured 24 hours after reperfusion.

Results Neither AQGV nor EPPE showed an effect on infarct volume or on transcription levels of adhesion molecules and pro-inflammatory cytokines.

Conclusion AQGV and EPPE did not show any beneficial effects in this mouse model of acute ischemic stroke. Further studies are needed to investigate the nature and dynamics of the immunomodulatory effects of synthetic oligopeptides in acute ischemic stroke.

abstract

BACKGROUND

Inflammation plays a central role in the pathophysiology of ischemic stroke. In the early phase of ischemia and reperfusion, an inflammatory cascade is set off in the ischemic core and in the penumbra, where perfusion is compromised but tissue is still viable.[1] Numerous pro-inflammatory genes are upregulated, including those for transcription factors, heat shock proteins, cytokines, chemokines and adhesion molecules. The classical pro-inflammatory cytokines interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), produced by microglia, astrocytes, endothelial cells, and neurons, mediate the inflammatory processes.[2] They activate leucocytes, stimulate the release of chemokines, and increase adhesion molecule expression on cerebral microvessels and circulating leukocytes, including intercellular adhesion molecule (ICAM-1) and E-selectin.[2] The early inflammatory phase is followed by a tissue-remodelling phase during which tissue-remodelling factors are expressed and are associated with healing of the infarcted tissue. The inflammatory processes start within 2 hours after stroke onset and sustain for several days.[3, 4] Besides its beneficial effect, inflammation may contribute to the secondary progression of ischemic brain injury. The acute inflammatory phase is believed to be detrimental, while the chronic phase inflammation may be essential for repair and regeneration.[1, 3, 4] This is a concept that holds promise for new therapeutic interventions.

The adaptations of the immune system during pregnancy are striking. The maternal immune system is under tight control to prevent rejection of the foetal allograft. This suggests that a specific hormonal environment is responsible for modulating the immune system during pregnancy. Human chorionic gonadotropin (hCG) is secreted by placental syncytiotrophoblasts, but is also produced by the pituitary gland and leucocytes in non-pregnant females and males.[5-7] HCG consists of an α- and a β-chain. In human pregnancy urine, hCG occurs in a variety of forms, including oligopeptidic breakdown products of the αβ-chain.[5] HCG preparations have been recognized to exert immunomodulatory activities that are not due to the native molecule nor to it's α- and β-chain, but resided in specific peptide fractions.[5] Based on known preferential cleavage sites, several oligopeptides from β-hCG residues have been synthesized. These oligopeptides including AQGV have shown promise in the treatment of ischemia-reperfusion injury such as septic shock[8], hemorrhagic shock[9] and renal ischemia-reperfusion injury[10]. Several regulatory oligopeptides based on the primary sequence of other proteins have also been generated and proven biologically active in different models tested. One of these regulatory oligopeptides is the p53-related tetrapeptide EPPE.

In this study, as a first step in assessing potential beneficial effects of synthetic anti-inflammatory tetrapeptides in acute ischemic stroke, we investigated the effects of the β-hCG related synthetic tetrapeptide AQGV and the p53-related synthetic tetrapeptide EPPE on infarct volume and on inflammatory gene expression in an animal model of focal cerebral ischemia.

MATERIALS AND METHODS

Animals

Experiments were performed in male C57BL/6 mice, weighing 20-30g, in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and with approval of the local Animal Care Committee.

Synthetic anti-inflammatory tetrapeptides

The β-hCG-related tetrapeptide AQGV and the p53-related tetrapeptide EPPE were synthesized (Ansynth Service BV Roosendaal, the Netherlands) using the fluorenylmethoxycarbonyl (Fmoc)/tert-butyl-based methodology with a 2-chlorotritylchloride resin as the solid support and dissolved in 0.9% sodium chloride at a concentration of 30 mg/ml.

Model of focal cerebral ischemia

Anesthesia was induced by 1.5% halothane and maintained with 1% halothane in 70% N_2O and 30% O_2. Middle cerebral artery occlusion for one hour was induced with a silicone-coated 8.0 nylon monofilament, as described previously.[11] A midline neck incision was made and the soft tissues were pushed apart. The left common carotid artery (LCCA) was dissected free from the surrounding nerves and a node was made with a 6.0 string. The left external carotid artery (LECA) was separated and a second node was made. Next, the left internal carotid artery (LICA) was isolated and was tied with a 6.0 string. After obtaining a good view of the LICA and the left pterygo palatine artery, both arteries were clipped. A small hole was cut in the LCCA before it bifurcates to the LECA and the LICA. An 8.0 nylon monofilament coated with a mixture of silicone resin and a harder was left in the artery. The clipped artery was reopened and the filament was introduced into LICA up to the origin of the left middle cerebral artery. The third node on the LICA will be closed with the filament inside. After 60 minutes, the third node was opened and the filament was withdrawn. The remaining string was cut and the skin was sutured with a 4.0 string. The core temperature of the mice during the procedure was maintained at 36.5°C using a feedback temperature control unit.

Administration of synthetic anti-inflammatory tetrapeptides

Three groups of mice (10 mice per group) received, in a randomized single- blinded fashion, either AQGV (30 mg/kg bodyweight) or EPPE (30mg/kg bodyweight) or 1 ml phosphate buffered saline (PBS), which was administered intravenously in two slow injections (1ml over 10 min). Administration of the first dose was started 15 minutes before

middle cerebral artery occlusion (MCAO). The second dose was administered 45 minutes after reperfusion.

Measurement of infarct size

The animals were sacrificed 24 hours after reperfusion. The brains were snap-frozen in isopentane for cryostat sectioning. Infarct areas were quantitated by use of sigma 4.0 software (sigmaScan Pro 4.0, Jandel scientific) on 20-µm hematoxylin-and eosin–stained cryostat sections without knowledge of treatment. Infarct volumes were calculated by summing the volumes of each section directly or indirectly with the following formula: contralateral hemisphere (mm^3) – undamaged ipsilateral hemisphere (mm^3).

Evaluation of mRNA levels by real-time quantitative polymerase chain reaction

RNA was isolated from 20-µm cryostat sections with a Qiagen RNeasy kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, and reverse transcribed into cDNA. We determined gene expression levels of TNF-α, IL-6, E-selectin and ICAM-I Applied Biosystems 7700 PCR machine (Foster City, California), as described previously.[12] The expression levels of these genes were quantified by normalization against the mRNA levels of the household gene ABL.

Data analysis and presentation

Statistical analyses were performed using SPSS version 11 software (SPSS, inc., Chicago Ill)

Data are presented as mean values +/- SD of the ten mice per group. Comparisons were made by one-way of variance (ANOVA), followed by Duncan's multiple range test.

RESULTS

Of the total 30 mice entered in the study, two mice in the EPPE group and one mouse in the AQGV group died, because of technical failure during surgery.

AQGV and EPPE do not alter infarct volume

The infarct volume was not significantly different between AQGV-treated mice and mice treated with PBS (114.9 +/- 23.9 mm^3 versus 108.7 +/- 12.1 mm^3 versus; P=0.7) and between EPPE-treated and mice treated with PBS (131.8 +/-12.8 mm^3 versus 114.9 +/- 23.9 mm^3; P=0.1) (Figure1).

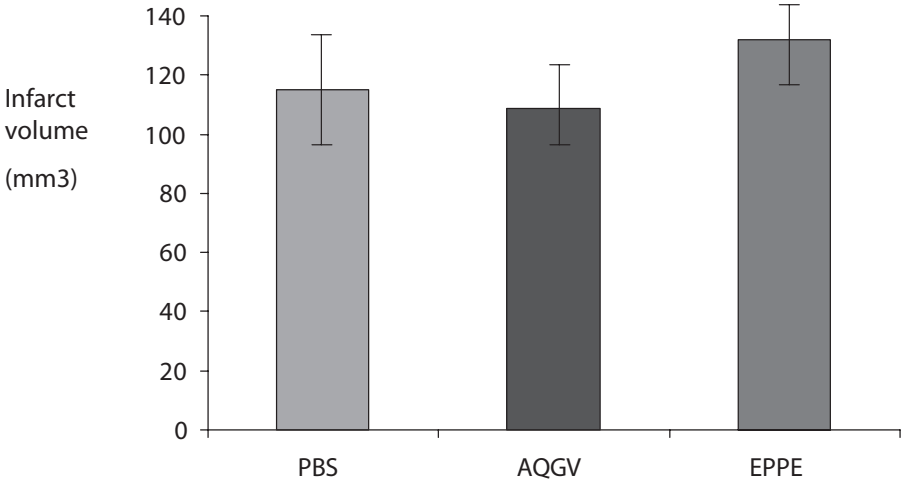

Figure 1: Effects of treatment with AQGV and EPPE versus PBS on mean infarct volume (mm3) +/- SD.

AQGV and EPPE do not influence mRNA transcription levels of TNF-α, IL-6, ICAM-1 and E-selectin

All three experimental groups displayed comparable mRNA expression levels of TNF-α, IL-6, ICAM-1 and E-selectin at 24 hours after the ischemic insult (Figure 2).

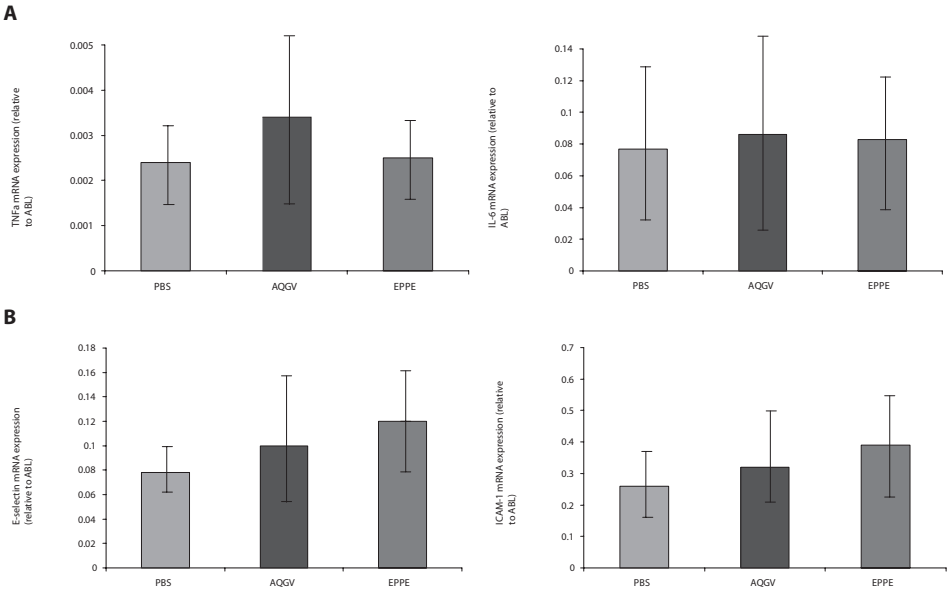

Figure 2: Relative expression of mRNA transcription levels of pro-inflammatory cytokines TNF-α and IL-6 (A) and adhesion molecules E-selectin and ICAM-1 (B) in the brain 24 hours after reperfusion. Transcription levels are normalized to the expression level of the household gene ABL. Data are presented as the mean of per group +/-SD. AQGV nor EPPE caused significant differences in the transcription levels of TNF-α and IL-6 as compared with PBS.

DISCUSSION

In this study, we found that in an established mouse model of ischemic stroke, treatment with two synthetic anti-inflammatory tetrapeptides had no effect on infarct volume or on mRNA expression levels of adhesion molecules and pro-inflammatory cytokines.

Our results deviate from the beneficial effects of treatment with synthetic anti-inflammatory oligopeptides in various other *in vivo* models of ischemia-reperfusion injury.[8-10] Treatment with such synthetic anti-inflammatory oligopeptides has been shown to inhibit renal ischemia-reperfusion injury and to increase survival rates by reducing systemic inflammation and adhesion molecule expression in the kidney[10] These peptides also attenuated inflammation and liver damage after hemorrhagic shock[9] and decreased morbidity and mortality associated with lipopolysaccharide injection[8].

There are several explanations why no such beneficial effects of treatment with synthetic anti-inflammatory oligopeptides were found in our model of acute ischemic stroke. First, the role of inflammation in the acute phase of stroke is ambivalent. The inflammatory reaction following ischemic stroke triggers the removal of noxious agents and supports tissue cleaning and repair. Contrary to these beneficial effects, it may augment secondary damage by disruption of the blood-brain barrier, release of cytotoxic agents, edema resulting from endothelial cell injury and leucocyte-mediated injury, increased body temperature and microvascular thrombosis. Since the underlying mechanisms by which the synthetic anti-inflammatory oligopeptides exert their effects are unclear, it cannot be excluded that they might also nergate the positive effects of the inflammatory reaction. On the other hand, synthetic anti-inflammatory oligopeptides did show beneficial effects in renal ischemia-reperfusion injury and there are many similarities in the pathophysiology of acute cerebral ischemia-reperfusion injury and acute renal ischemia-reperfusion injury.[10]

Furthermore, we did not compare the mRNA expression levels of adhesion molecules and pro-inflammatory cytokines with those in brains of sham-operated animals. Therefore, we do not know whether these pro-inflammatory genes were upregulated in our model of ischemic stroke. However, there is substantial evidence from previous studies that these genes are upregulated witin the first hours of stroke onset.[1-3]

It should also be noted that different synthetic anti-inflammatory oligopeptides might have different modes of action. This is supported by previous studies that have identified different synthetic anti-inflammatory oligopeptides as the most effective mediator.[8-10]

Another explanation of the negative results of our study is that it was not designed to investigate different doses of AQGV or EPPE. Therefore, we cannot rule out that there is a dose-dependent effect, as was observed in previous studies.[8, 10] Also, the two time-points of peptide administration chosen for the current study may not have been optimal. Neither can we exclude that repeated peptide administrations during the study period could have been effective.

We have studied only the effects of AQGV and EPPE on infarct volume and mRNA transcription levels at 24 hours after reperfusion. As the inflammatory reaction is initiated within a few hours after stroke onset and lasts for several days, it may be worthwhile to monitor the early evolution of brain injury at different points in time with MRI scanning.[13] The major advantage of MRI over histologic examinations is its potential to non-invasively assess changes in the brain in vivo. Finally, since no measurements of brain concentrations of the administered tetrapeptides were performed we do not know whether the oligopeptides underwent presystemic transformation and actually crossed the blood-brain barrier. However, their small size as well as the disruption of the blood–brain barrier following ischemic stroke makes it likely that tetrapeptides did cross the blood-brain barrier.

HCG is not the only protein source for regulatory oligopeptides. Sequences of other proteins such as C-reactive protein Ig heaving chain, phospholipase A2, hemoglobin, b-defensins, lactoferrin and granulysin possess anti-inflammatory activities with the function being expressed upon the degradation of the parent protein.[14-20] So far about 500 human genes encoding proteases/peptidases have been identified. This might be related to regulation of biological processes by peptidases and might imply that virtually every protein within the body can serve as a source of regulatory oligopeptides.

Our results do not negate the possibility that anti-inflammatory oligopeptides might be useful for acute stroke treatment. Further studies are needed to investigate the nature and dynamics of the immunomodulatory effects of synthetic anti-inflammatory oligopeptides in acute ischemic stroke.

REFERENCES

1. Del Zoppo GJ, Becker KJ, Hallenbeck JM. Inflammation after stroke: is it harmful? *Arch Neurol* 2001; 58(4):669-672.
2. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. *J Neuroimmunol* 2007; 184:53-68.
3. Barone FC, Feuerstein GZ. Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* 1999; 19(8):819-834.
4. Bowen KK, Naylor M, Vemuganti R. Prevention of inflammation is a mechanism of preconditioning-induced neuroprotection against focal cerebral ischemia. *Neurochem Int* 2006; 49:127-135.
5. Benner R, Khan NA. Dissection of systems, cell populations and molecules. *Scand J Immunol* 2005; 62 Suppl 1:62-66.
6. Birken S, Maydelman Y, Gawinowicz MA et al. Isolation and characterization of human pituitary chorionic gonadotropin. *Endocrinology* 1996; 137:1402-1411.
7. Yoshimoto Y, Wolfsen AR, Hirose F et al. Human chorionic gonadotropin--like material: presence in normal human tissues. *Am J Obstet Gynecol* 1979; 134:729-733.
8. Khan NA, Khan A, Savelkoul HF et al. Inhibition of septic shock in mice by an oligopeptide from the beta-chain of human chorionic gonadotrophin hormone. *Hum Immunol* 2002; 63:1-7.
9. van den Berg HR, Khan NA, van der Zee M et al. Synthetic oligopeptides related to the [beta]-subunit of human chorionic gonadotropin attenuate inflammation and liver damage after (trauma) hemorrhagic shock and resuscitation. *Shock* 2009; 31:285-291.
10. Khan NA, Susa D, van der Berg JW et al. Amelioration of renal ischemia reperfusion injury by synthetic oligopeptides related to human chorionic gonadotropin. [Nephrology Dialysis Transplantation, in press]. 2009.
11. Gertz K, Laufs U, Lindauer U, Nickenig G et al. Withdrawal of statin treatment abrogates stroke protection in mice. *Stroke* 2003; 34:551-557.
12. Dik WA, Nadel B, Przybylski GK et al. Different chromosomal breakpoints impact the level of LMO2 expression in T-ALL. *Blood* 2007; 110: 388-392.
13. Hoehn M, Nicolay K, Franke C et al. Application of magnetic resonance to animal models of cerebral ischemia. *J Magn Reson Imaging* 2001; 14:491-509.
14. King AE, Fleming DC, Critchley HO et al. Regulation of natural antibiotic expression by inflammatory mediators and mimics of infection in human endometrial epithelial cells. *Mol Hum Reprod* 2002; 8:341-349.
15. King AE, Critchley HO, Sallenave JM et al. Elafin in human endometrium: an antiprotease and antimicrobial molecule expressed during menstruation. *J Clin Endocrinol Metab* 2003; 88:4426-4431.
16. King AE, Kelly RW, Sallenave JM et al. Innate immune defences in the human uterus during pregnancy. *Placenta* 2007; 28:1099-1106.
17. Miele L, Cordella-Miele E, Facchiano A et al. Novel anti-inflammatory peptides from the region of highest similarity between uteroglobin and lipocortin I. *Nature* 1988; 335:726-730.
18. Parish CA, Jiang H, Tokiwa Y et al. Broad-spectrum antimicrobial activity of hemoglobin. *Bioorg Med Chem* 2001; 9:377-382.
19. Rawlings ND, Morton FR, Kok CY et al. MEROPS: the peptidase database. *Nucleic Acids Res* 2008; 36:D320-D325.
20. Robey FA, Ohura K, Futaki S et al. Proteolysis of human C-reactive protein produces peptides with potent immunomodulating activity. *J Biol Chem* 1987; 262:7053-7057.

Complement activation following acute ischemic stroke

Heleen M den Hertog[1], Bart C Jacobs[1,2], Peter J Koudstaal[1], Diederik WJ Dippel[1]

[1]Department of Neurology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

[2]Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

ABSTRACT

Background and purpose There is substantial evidence that inflammation plays an important role in the pathophysiology of ischemic stroke and may contribute to the secondary damage following ischemic stroke. Complement activation may be one of the key pathways in the onset and reinforcement of this inflammation process.

We aimed to assess whether the complement system is activated in acute ischemic stroke by determining the concentration of complement activation products and the remaining function of the three pathways of complement activation in peripheral blood of patients with acute ischemic stroke.

Materials and methods Blood samples from 20 patients with ischemic stroke will be collected on admission, and on day 1, 2, 3 and 5 and at 6 weeks. We will analyze plasma for components of all three pathways of complement activation and for levels of C1q, CH50, C3a, C5a levels by an enzyme-linked immunosorbent assay. Complement levels of each time point will be compared with complement levels at 6 weeks. Regression analysis will be applied to study the association between complement levels on the one hand and stroke severity and functional outcome on the other.

Clinical implications Studying the role of the complement system in the acute ischemic stroke might be very interesting as it may be an important novel therapeutic target.

abstract

BACKGROUND

Stroke is the third leading cause of death and the first cause of disability in developed countries. It is to be expected that with an increasing age of the population, the number of stroke patients will increase as well.

Since the advent of Intravenous thrombolysis with recombinant tissue-plasminogen activator, important progress in the treatment of acute ischemic stroke has been made.[1] However, only a limited number of patients will benefit from this treatment because of the short therapeutic time window and various contra-indications for treatment. There is a great need for other therapies in acute stroke.[1, 2]

There is growing evidence that inflammation plays an important role in the pathophysiology of ischemic stroke. The inflammatory reaction is a defence mechanism against invading toxic organism and noxious agents and provides for tissue cleaning and repair. Besides its beneficial effect, it may contribute to the secondary progression of ischemic brain injury. In the early phase of ischemia and reperfusion, inflammation processes ensue in the ischemic core and in the penumbra, where perfusion is compromised, but tissue is still viable.[3, 4] Numerous pro-inflammatory mediatiors are upregulated, including those for transcription factors, heat shock proteins, cytokines, chemokines and adhesion molecules.[5] The classic pro-inflammatory cytokines interleukin-b, interleukin-6, and tumor necrosis factor, produced by microglia, astrocytes, endothelial cells, and neurons, mediate these inflammation processes.[6, 7] The inflammation processes start within 2 hours and sustain for days.[5, 6] Complement activation may be one of the key pathways in the onset and reinforcement of this inflammation process.

Besides its important role in innate host defence, complement activation contributes to inflammatory and immunological responses in a number of pathological conditions. Numerous studies have demonstrated that complement contributes to secondary injury after ischemia in other organs such as myocardial tissue[8, 9], skeletal muscles[10] and kidney[11].

The role of the complement system in acute phase of ischemic stroke is unclear. Evidence for involvement of complement in neural damaging after ischemia comes from studies in animal models and patients. Recently, animal studies suggest a crucial role for complement in neurological damage after ischemic stroke and show that inhibition of complement activation can reduce infarct size.[12-14] Complement activation can occur via one of the three pathways, the classical, alternative and lectin pathway. Complement activation may trigger the inflammatory reaction after ischemia that may aggravate the damaging of neuronal cells via attraction of immune competent cells by chemotactic activated complement factors including C3a and C5a and by opsonisation of neuronal cells by C2b and C5b. Moreover, complement activation may also lead tot the production of the membrane attack complex (MAC), which can directly damage opsonized neuronal cell membranes. Additionally, this inappropriate inflammatory reaction may lead to

restenosis and recurrence of stroke. An activator of the complement cascade might be C-reactive protein (CRP).[15]

Only two small clinical studies on the role of complement in human ischemic stroke are available.[16, 17] One study with 17 ischemic stroke patients showed that levels of C3b and C3a plasma were increased in patients with acute stroke compared to controls, indicating that shortly after a stroke there is a strong complement activation which can be measured in peripheral blood.[16]The second study with 11 patients with acute ischemic stroke showed that MAC plasma levels were increased.[17] This increase in MAC was correlated with concentrations of CRP suggesting a possible role for CRP in complement activation. Neither study investigated all three pathways of complement activation.

Studying the role of the complement system in the acute phase of ischemic stroke might be very interesting as it may be an important novel therapeutic target. Questions for future research may include:

- Is activation of the complement system associated with infarct size and functionale outcome in patients with acute ischemic stroke?
- Is activation of the complement system associated with restenosis after treatment with rtPA in patients with acute ischemic stroke?
- Is activation of the complement system associated with the occurrence of infections?
- Is complement activation also found in patients with hemorrhagic stroke?

HYPOTHESIS

Against this background, our hypothesis will be that brain ischemia induces a strong complement activation that is reflected in the peripheral blood and that may contribute to the extent of the brain damaging.

AIM

To test this hypothesis by measuring the concentration of complement activation products and the remaining function of the three pathways of complement activation in peripheral blood from patients with an acute ischemic stroke.

METHODS

Study population

A cohort of 20 patients with ischemic stroke will be prospectively studied.

Inclusion criteria will be a clinical diagnosis of ischemic stroke, the possibility to confirm the diagnosis with CT or MRI within 24 hours after inclusion in the study and admission within 24 hours of symptom onset.

Patients with symptoms of an infection, malignant or other underlying inflammatory disease and/or currently receiving anti-inflammatory or immunosuppressive medication will be excluded.

In addition, blood samples of ten stroke-free controls will be derived from the Rotterdam Stroke Biobank (RSB), a project aimed at collecting clinical information, blood samples and DNA of all patients with neurovascular diseases admitted to Erasmus University Medical Center and stroke free controls. Controls will be selected on basis of age and sex.

Baseline data

A detailed history of vascular risk factors and time of symptom onset will be collected. Neurological examination will be performed on admission and on discharge. Stroke severity, neurological improvement or worsening will be assessed by means of the NIH Stroke Scale (NIHSS).[18]

Blood sampling and laboratory assessment

Blood samples (25ml) will be collected on admission, and on day 1, 2, 3 and 5 and at 6 weeks. EDTA tubes will be used to collect blood samples and plasma will be obtained by standard methods. After centrifugation, plasma will be stored at -80ºC until analysis. Plasma will be analyzed for components of all three pathways of complement activation by means of an ELISA.

Plasma will also be analyzed for levels of C1q, CH50, C3a, C5a by means of ELISA. In addition, CRP levels will be measured, using a Roche Modular assay. The range of measurement is 1–285 mg/L with a variation coefficient of 4.6%.

Statistical methods

The analysis will be carried out with STATA 10.0 statistical package (Statacorp, College Station, Texas). All data will be presented as mean +/- standard deviation. Concentrations of each time point will be compared with concentrations at 6 weeks by Student's t-test. In addition, mean concentrations of patients with ischemic stroke will be compared with concentrations of the matched control group. Logarithmic transformation will be made for data that are not normally distributed. In addition, we will use linear regression to assess the relation between complement levels on the one hand and neurological stroke severity (baseline NIHSS score) and functional outcome (NIHSS score on discharge ad-

justed for baseline NIHSS score) on the other. A multiple regression model will be applied to adjust for age and sex.

Sample size

We expect a difference in complement levels of 300 ng/ml between ischemic stroke patients and controls. Assuming a standard deviation of 200 ng/ml, a group of 20 patients and 20 controls will have a power of 80% to detect a significant (α=0.05) difference in complement levels.

Ethical considerations

The study will be conducted according to the principles of the Declaration of Helsinki (version, date, see for the most recent version: www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and acts.

REFERENCES

1. Wardlaw JM, Zoppo G, Yamaguchi T et al. Thrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev* 2003; 3:CD000213.
2. Hacke W, Kaste M, Bluhmki E et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; 359:1317-1329.
3. Baron JC. How healthy is the acutely reperfused ischemic penumbra? *Cerebrovasc Dis* 2005; 20 Suppl 2:25-31.
4. Memezawa H, Smith ML, Siesjo BK. Penumbral tissues salvaged by reperfusion following middle cerebral artery occlusion in rats. *Stroke* 1992; 23:552-559.
5. Barone FC, Feuerstein GZ. Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* 1999; 19:819-834.
6. Emsley HC, Smith CJ, Gavin CM et al. An early and sustained peripheral inflammatory response in acute ischaemic stroke: relationships with infection and atherosclerosis. *J Neuroimmunol* 2003; 139:93-101.
7. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. *J Neuroimmunol* 2007; 184:53-68.
8. Kilgore KS, Friedrichs GS, Homeister JW et al. The complement system in myocardial ischaemia/reperfusion injury. *Cardiovasc Res* 1994; 28:437-444.
9. Yasuda M, Takeuchi K, Hiruma M et al. The complement system in ischemic heart disease. *Circulation* 1990; 81:156-163.
10. Weiser MR, Williams JP, Moore FD et al. Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J Exp Med* 1996; 183:2343-2348.
11. Bonventre JV. Complement and renal ischemia-reperfusion injury. *Am J Kidney Dis* 2001; 38(2):430-436.
12. Akita N, Nakase H, Kaido T et al. Protective effect of C1 esterase inhibitor on reperfusion injury in the rat middle cerebral artery occlusion model. *Neurosurgery* 2003; 52:395-400.
13. De Simoni MG, Rossi E, Storini C et al. The powerful neuroprotective action of C1-inhibitor on brain ischemia-reperfusion injury does not require C1q. *Am J Pathol* 2004; 164:1857-1863.
14. Huang J, Kim LJ, Mealey R et al. Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. *Science* 1999; 285:595-599.
15. Gill R, Kemp JA, Sabin C et al. Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats. *J Cereb Blood Flow Metab* 2004; 24:1214-1218.
16. Mocco J, Wilson DA, Komotar RJ et al. Alterations in plasma complement levels after human ischemic stroke. *Neurosurgery* 2006; 59:28-33.
17. Pedersen ED, Waje-Andreassen U, Vedeler CA et al. Systemic complement activation following human acute ischaemic stroke. *Clin Exp Immunol* 2004;137:117-122.
18. Brott T, Adams HP, Olinger CP et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; 20:864-870.

Chapter 6

General discussion

In my thesis, I have focused on body temperature and temperature-lowering therapy in acute stroke. Furthermore, I have explored the role of inflammation in relation to prognosis and treatment in acute stroke.

Over the past years, evidence has accumulated that inflammation plays a role in the pathophysiology of acute stroke. Body temperature and classic acute-phase reactants are modified by this inflammatory reaction, and both may be useful in the prediction of the prognosis after stroke and as therapeutic targets.

BODY TEMPERATURE IN ACUTE STROKE: IMPLICATIONS FOR PROGNOSIS AND TREATMENT

Interpretation of the results of the PAIS trial

Lowering body temperature and the prevention of fever may improve functional outcome after stroke, as discussed in Chapter 2.

The main aim of this thesis was to assess whether early treatment with high-dose paracetamol improves functional outcome in patients with acute stroke by reducing body temperature and preventing fever. To answer this question, we performed a large randomized placebo-controlled multicenter trial: the PAIS trial.[1] We found that more patients on high-dose paracetamol than patients treated with placebo improved beyond expectation, but the difference was not statistically significant. In a post-hoc subgroup analysis, we found that in patients with a baseline body temperature between 37ºC and 39ºC, treatment with paracetamol was significantly associated with improved outcome.[1]

One could comment that the lack of an overall benefit of paracetamol may have been caused by the inclusion of 1400 instead of the planned 2500 patients.[2] However, the effect of adopting the sliding dichotomy approach might be almost the same as a doubling of sample size.[3]

Although explanations for this finding should be interpreted with caution, the benefit of paracetamol in patients with higher body temperatures is biologically plausible. Two phase II trials in patients with acute ischemic stroke showed that high-dose paracetamol lowered body temperature by about 0.30ºC[4, 5], which was confirmed in the PAIS trial.[1] Paracetamol induces larger temperature reductions if the initial body temperature is higher.[6] Accordingly, we found that treatment with high-dose paracetamol induced a larger reduction in body temperature in patients with a baseline body temperature between 37ºC and 39ºC than in those with a baseline body temperature between 36ºC and 37ºC.[1]

Furthermore, we found that an early rise in body temperature rather than an initially elevated body temperature was a risk factor for poor functional outcome. (Chapter 3.3) This supports the notion that prevention of an early rise in body temperature could reduce death and improve functional outcome. These results are in contrast with those of the

frequently cited Copenhagen Stroke Study that showed that the odds of poor outcome doubled for every degree increase in initial body temperature within 12 hours of stroke onset.[7] We also found that patients with severe stroke had relatively low body temperatures at baseline (Chapter 3.3), possibly because of a faster decrease in body temperature before hospital admission as a result of diminished muscle activity. This finding might explain in part that baseline body temperature was not associated with clinical outcome, and stresses the importance of considering acute stroke as a dynamic process.

The change in body temperature after onset was associated with initial stroke severity. (Chapter 3.3) One may argue that increased body temperature does not accelerate the ischemic cascade, but merely reflects extensive cerebral damage and, thereby, poor outcome. However, the relation between a rise in body temperature and poor outcome was independent of baseline stroke severity. Furthermore, the potential benefit of paracetamol in patients with higher body temperatures suggests that increased body temperatures following stroke are not an innocent consequence of brain infarction, but may increase ischemic damage.

Paracetamol is widely known as an antipyretic and analgesic, but may also have other effects. We assessed the effect of high-dose paracetamol on blood pressure in patients with acute stroke and showed for the first time that treatment with high-dose paracetamol is associated with a reduction in systolic blood pressure of approximately 5 mm Hg within the first two days of stroke onset. (Chapter 3.4) A plausible cause of this reduction in systolic pressure by paracetamol is pain relief, but this remains speculative.

The management of blood pressure in patients with acute stroke is uncertain.[8-10] However, prognostic studies have indicated that patients with systolic blood pressures of 10 mmHg above 150 mmHg may have a 4% increased risk of recurrent stroke and a 4% increased risk of death or dependency.[11, 12] Also, preliminary studies suggest that antihypertensive treatment lowers blood pressure by 10-15 mm Hg after stroke.[10, 13, 14] The effect of paracetamol on systolic blood pressure may therefore be large enough to be of clinical significance and provides an additional argument for further studies of high-dose paracetamol in acute stroke.

Implications of the PAIS trial

I do not think that a recommendation for routine use of paracetamol in acute stroke can be considered good scientific and clinical practice at this moment. Although the benefit of paracetamol in patients with higher temperatures is supported by biological plausibility, these results should be interpreted with caution, as they are derived from a subgroup analysis. Furthermore, guidelines for the treatment of acute ischemic stroke or intracerebral hemorrhage recommend administration of antipyretic medication in case of fever or a temperature above 37.5˚C.[8, 9] However, paracetamol likely improves functional outcome through the prevention of a rise in body temperature, and not through treat-

ment of high body temperature after it has developed in stroke patients. Finally, a daily dose of 6 g of paracetamol used in PAIS is 50% higher than the recommended maximum dose. Although high-dose paracetamol was safe in the controlled setting of our trial, it may still lead to acute liver failure, especially in patients with chronic liver disease.[1] In addition, it may mask fever, which could lead to delayed detection of pneumonia, urinary tract infections, and sepsis.

A new trial on high-dose paracetamol in patients with acute stroke and a baseline body temperature of 37˚C or above should be the next step. Once this trial proves that paracetamol is effective, a cheap and simple therapy will be available for many patients with acute stroke.

Suggestions for additional studies in PAIS II

Further research also needs to focus on the mechanisms of action of paracetamol in acute stroke. As I have discussed in Chapters 2 and 3, it has been suggested that COX-2 is involved in inflammation following ischemic stroke and contributes to tissue damage.[15] As a consequence, the COX-2 inhibitor paracetamol may mediate this inflammatory response. Therefore, it would be interesting to collect blood samples for the assessment of inflammatory cytokines and acute-phase reactants and analysis of inflammation-related genes.

As the mechanisms of action of paracetamol have not been fully clarified, animal studies may support in expanding our knowledge on the effect of paracetamol in acute stroke. To the best of my knowledge, only one animal study has been performed to determine the effect of paracetamol on body temperature and infarct volume in animals.[16] In this study, a trend towards a reduction in infarct volume by paracetamol was observed. Further animal studies should focus on prevention of a rise in body temperature by paracetamol in acute stroke, and may thereby contribute to better understanding of our findings in the post-hoc subgroup analysis of PAIS.

To further explore the effect of paracetamol on blood pressure, I recommend to measure blood pressure and serum levels of catecholamines and cortisol, in particular on the first day of stroke onset.

Issues concerning temperature reduction and trials on temperature-lowering therapy in acute stroke

Body temperature or brain temperature?

In a study of 8 patients with traumatic brain injury, the correlation between temperatures measured rectally or in the bladder and those measured in the brain was investigated.[17] Brain temperature was found to be 1-2°C higher than rectal and bladder temperatures. Differences were largest when the rectal or bladder temperatures were higher than 38°C. As temperatures in all trials of temperature reduction were assessed with rectal, bladder, or tympanic thermometry, the brain temperature in these patients may have

been underestimated. Magnetic resonance thermometry might be a valuable approach for non-invasive monitoring of brain temperature.[18, 19] This technique has shown useful in an experimental setting, but has to be further explored before it can be applied in the clinical setting.[18, 19] On the other hand, all clinical prognostic studies and most animal experiments that have suggested a benefit of temperature reduction reported body temperatures and not brain temperatures. In addition, in another small study of 20 patients with traumatic brain injury or subarachnoid hemorrhage and fever, the mean difference between body and brain temperature was only 0.3°C (SD 0.3°C).[20]

Is temperature lowering effective in ischemic and hemorrhagic stroke?

In ischemic stroke, temperature-lowering therapy may reduce tissue damage via several pathophysiological routes, such as protection of the blood-brain barrier, suppression of the release of excitatory amino acids and free radicals, lowering of the cerebral metabolic rate, and anti-inflammatory actions.[21, 22] The mechanisms underlying a possible benefit of temperature-lowering therapy in intracerebral hemorrhage are less clear. In animal studies, temperature lowering reduced edema, but not lesion volume.[23, 24] These findings warrant further studies. However, observational studies have shown an association between increased body temperatures and poor outcome both in patients with ischemic stroke, as well as in patients with intracerebral hemorrhage.[21] (Chapter 3.4) Therefore, we included both ischemic and hemorrhagic stroke patients in the PAIS trial.[1] The subgroup analysis based on main stroke subtype did not indicate any differences in treatment effect.[1]

What is the optimal duration of therapy?

Uncertainty exists on the optimal duration of temperature-lowering therapy. In animal models of focal cerebral ischemia, pathophysiological processes exert their deleterious effects anywhere between the first minutes and several days after vessel occlusion.[25] On the other hand, longer duration of treatment was not associated with improved outcome in a meta-analysis of hypothermia in animal models of focal cerebral ischemia.[26] Moreover, as the rise in temperature occurs mainly within the first 24 hours after stroke onset, this period might be regarded as the critical treatment period.[27, 28] In addition, most observational studies have shown that the relation between body temperature and clinical outcome is probably limited to the first 12 to 24 hours after stroke onset.[7, 29, 30] Furthermore, the risk of side effects may increase with longer durations of treatment.

Is physical temperature-lowering therapy a promising approach to stroke treatment?

Larger temperature reductions by physical cooling methods are an alternative approach to antipyretic treatment. In animal studies of focal cerebral ischemia, efficacy was highest with temperatures below 32°C, but infarct volume was still reduced by about one-third after cooling to 35°C.[26] In trials of cardiac arrest, the target temperature was 32 - 34°C.[31, 32] however, because of patient comfort monitoring, and the prevention of shivering,

temperature-lowering therapy to such levels generally requires sedation, mechanical ventilation, and therefore admission to an intensive care unit. Given the limited availability of intensive care beds in most countries, cooling to temperatures of about 33°C may be impractical. In addition, sedation and mechanical ventilation may increase the risk of side effects. Therefore, moderate hypothermia might be a better choice in patients with acute stroke. On the other hand, a local approach of selective brain cooling might have less systemic complications and might permit larger temperature reductions. Hence, further large randomized clinical trials are needed to study the feasibility, safety, optimal duration and the effectiveness of physical temperature reduction in patients with acute stroke. Two phase II trials on the combination of mild hypothermia and treatment with rt-PA are underway.[33, 34]

Furthermore, a European collaboration aiming for multi-center randomized trials of interventions involving systemic hypothermia in acute ischemic stroke (EURO-COOLS) will be launched this year. This project aims to assess the feasibility and safety of surface and endovascular cooling to 33ºC, as well as to 35ºC.

The "sliding dichotomy" approach

Because of the drawbacks of dichotomization of ordinary outcome scales and to increase statistical power, as I discussed in Chapter 3.1, we changed the primary effect estimate of the PAIS trial to the odds ratio for "improvement beyond expectation", according to the sliding dichotomy approach.[3, 35]

This approach appears to be more efficient, as the original primary effect measure did not show an effect of paracetamol on functional outcome whereas the sliding dichotomy approach did suggest a trend towards a beneficial effect. Furthermore, I believe that this approach is more relevant for clinical practice. It is also informative to show whether treatment moves patients from any category of disability to a less severe one, and not only to demonstrate differences in the numbers of patients on either side of an arbitrary cut-off point.

Patient recruitment

A key to success in any clinical trial is the achievement of a study population of an adequate sample size. In the PAIS trial, we did not meet the planned target of 2500 patients.[1] Patient recruitment is one of the greatest barriers in the conduct of randomized controlled trials. Lack of eligible patients is an important cause of an inadequate inclusion rate in clinical trials, but did not prove to be a major hurdle in the PAIS trial. PAIS was an investigator-driven trial, and therefore the success was completely dependent on the efforts of the investigators. Although it was designed as a simple trial, the workload, small as this has been compared to that of industry-driven trials, may have caused many hard-pressed neurologists to gradually lose their enthousiasm for recruiting patients. The

low recruitment rate may also have been caused by simultaneous competitive trials, in particular if these studies offered attractive financial incentives.

Various strategies may help to achieve an increase in recruitment rate in future investigator-driven trials. One option is to offer small incentives for every patient inclusion. Second, collaboration with other European countries may enhance recruitment. However, nowadays funding for trials tends to be granted at the level of an individual country and there is limited funding at the European level. Furthermore, the rules and requirements for clinical trials may be different in the participating countries. An alternative approach is to combine the results of similar trials in a meta-analysis. Standardization of clinical outcomes and designs in trials could make it easier to compare their results.[36, 37]

INFLAMMATION IN ACUTE STROKE: IMPLICATIONS FOR PROGNOSIS AND TREATMENT

Prognosis

Elevated levels of CRP can be a reflection of the inflammatory reaction following acute stroke. We found that an increased level of CRP in the very early phase of acute stroke was an independent prognostic factor for poor outcome. (Chapter 4.1) Furthermore, common variation in the CRP gene was associated with increased levels of CRP in acute stroke. (Chapter 4.2)

The use of biomarkers as predictors of stroke lesion evolution and prognosis is becoming increasingly important, as they may be valuable tools in the search for an optimal management of stroke patients. Animal studies may help us to understand the role of biomarkers in the disease process. Furthermore, biomarkers can also be used to link animal studies with human studies. Animal models may not produce clinical symptoms similar to humans. In this case, a biomarker may serve as a surrogate endpoint in the animal.

CRP is an easy to measure and readily available inflammatory marker. It may provide important prognostic information beyond conventional clinical parameters. Further studies are needed to assess whether CRP is a predictor of outcome also after stroke.

Interestingly, animal studies have found that CRP might exacerbate brain tissue damage following ischemic stroke, possibly via a complement-mediated exacerbation of tissue injury.[38] This could stimulate research into the underlying pathogenetic mechanisms and the development of new medical treatments for acute ischemic stroke. Furthermore, stroke is a heterogeneous disease and more insight in genotype–phenotype associations may provide a basis for development of tailored therapeutic strategies in ischemic stroke and intracerebral hemorrhage.

The accumulating evidence that inflammation-mediated damage plays a role in ischemic stroke suggests that immunomodulatory agents represent a promising class of drugs. As discussed in Chapter 5, the complement system plays an integrated role in the initiation and regulation of the inflammatory response and might be a promising target. We have recently started a study in 20 patients with ischemic stroke to assess the concentration of complement activation products and the remaining function of the three pathways of complement activation in peripheral blood. (Chapter 5) If brain ischemia indeed induces a strong complement activation which is reflected in the peripheral blood, further research should be aimed at the assessment of the relation between this activation of the complement system and infarct size and functional outcome. Other interesting research questions are whether activation of the complement system is associated with reocclusion after treatment with rt-PA, whether complement activation is also found in patients with intracerebral hemorrhage, and whether genetic variants contribute to activation of complement in patients with acute stroke. The nature of the substances in the infarcted area that start activation of complement system is unknown. CRP could be involved as an activator. Further experimental studies are needed to unravel the mechanisms by which the complement system is activated and to further explore the role of the complement cascade in tissue damage following acute stroke.

Furthermore, clinical and experimental studies suggest that the inflammatory reaction following stroke induces immunological changes that increase the susceptibility for infections. Further research is needed to determine the clinical consequences of these immunological changes.[39-41]

CONCLUSION

Acute stroke is a challenging and heterogeneous disease. A pivotal objective of treatment of acute ischemic stroke is lysis of the clot to restore blood flow to ischemic tissue. Reperfusion induced by intravenous administration of rt-PA has shown to be effective when initiated up to 4.5 hours of ischemic stroke onset.[42] Further research should be aimed at increasing the therapeutic window for thrombolysis by identifying patients who benefit from rt-PA beyond a 4.5-hour interval by means of diffusion–perfusion MRI or perfusion CT techniques and by applying intra-arterial thrombolytic therapy and mechanical reperfusion methods.

The studies described in this thesis show that lowering body temperature and the prevention of fever is a promising approach to improve functional outcome after stroke and provide directions for further research. It is too early for routine use of high-dose paracetamol in acute stroke. A new trial on the effect of high-dose paracetamol in patients

with body temperatures of 37°C or above is needed. Moreover, future research should focus on larger temperature reductions by physical cooling methods in acute stroke. The studies presented in this thesis also suggest that inflammation plays a prognostic role in acute stroke, and may further guide therapeutic choices in acute stroke.

Finally, it is quite likely that early recanalization or reperfusion is a prerequisite for effectiveness of neuroprotective and anti-inflammatory strategies. Combination of thrombolysis with rt-PA and neuroprotective and anti-inflammatory strategies aimed at limitation of tissue damage following ischemic stroke might be an attractive therapeutic approach.

REFERENCES

1. den Hertog HM, van der Worp HB, van Gemert HM et al. The Paracetamol (Acetaminophen) In Stroke (PAIS) trial: a multicentre, randomised, placebo-controlled, phase III trial. *Lancet Neurol* 2009; 8:434-440
2. van Breda EJ, van der Worp HB, van Gemert HM et al. PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial [ISCRTN 74418480]. *BMC Cardiovasc Disord* 2005; 5:24.
3. Murray GD, Barer D, Choi S et al. Design and analysis of phase III trials with ordered outcome scales: the concept of the sliding dichotomy. *J Neurotrauma* 2005; 22:511-517.
4. Dippel DWJ, van Breda EJ, Van Gemert HMA et al. Effect of paracetamol (acetaminophen) on body temperature in acute ischemic stroke: a double-blind, randomized phase II clinical trial. *Stroke* 2001; 32:1607-1612.
5. Dippel DWJ, van Breda EJ, van der Worp HB et al. Effect of paracetamol (acetaminophen) and ibuprofen on body temperature in acute ischemic stroke PISA, a phase II double-blind, randomized, placebo-controlled trial [ISRCTN98608690]. *BMC Cardiovascular Disorders* 2003; 3:2.
6. Van Esch A, Van Steensel-Moll HA, Steyerberg EW et al. Antipyretic efficacy of ibuprofen and acetaminophen in children with febrile seizures. *Arch Pediatr Adolesc Med* 1995; 149:632-637.
7. Reith J, Jorgensen HS, Pedersen PM et al. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet* 1996; 347:422-425.
8. Adams HP, del Zoppo G, Alberts MJ et al. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. *Circulation* 2007; 115:e478-e534.
9. Broderick JP, Adams HP, Barsan W et al. Guidelines for the management of spontaneous intracerebral hemorrhage: A statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. *Stroke* 1999; 30:905-915.
10. Geeganage CM, Bath PM. Interventions for Deliberately Altering Blood Pressure in Acute Stroke. *Stroke* 2009 Epub.
11. Willmot M, Leonardi-Bee J, Bath PM. High blood pressure in acute stroke and subsequent outcome: a systematic review. *Hypertension* 2004; 43:18-24.
12. Leonardi-Bee J, Bath PM, Phillips SJ et al. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke* 2002; 33:1315-1320.
13. Anderson CS, Huang Y, Wang JG et al. Intensive blood pressure reduction in acute cerebral haemorrhage trial (INTERACT): a randomised pilot trial. *Lancet Neurol* 2008; 7:391-399.
14. Sare GM, Gray LJ, Wardlaw J et al. Is lowering blood pressure hazardous in patients with significant ipsilateral carotid stenosis and acute ischaemic stroke? Interim assessment in the 'Efficacy of Nitric Oxide in Stroke' trial. *Blood Press Monit* 2009; 14:20-25.
15. Vongpatanasin W, Thomas GD, Schwartz R et al. C-reactive protein causes downregulation of vascular angiotensin subtype 2 receptors and systolic hypertension in mice. *Circulation* 2007; 115:1020-1028.
16. Legos JJ, Mangoni AA, Read SJ et al. Programmable microchip monitoring of post-stroke pyrexia: effects of aspirin and paracetamol on temperature and infarct size in the rat. *J Neurosci Methods* 2002; 113:159-166.

17. Henker RA, Brown SD, Marion DW. Comparison of brain temperature with bladder and rectal temperatures in adults with severe head injury. *Neurosurgery* 1998; 42:1071-1075.
18. Karaszewski B, Wardlaw JM, Marshall I et al. Measurement of brain temperature with magnetic resonance spectroscopy in acute ischemic stroke. *Ann Neurol* 2006; 60 :438-446.
19. Vogel MW, Pattynama PM, Lethimonnier FL et al. Use of fast spin echo for phase shift magnetic resonance thermometry. *J Magn Reson Imaging* 2003; 18:507-512.
20. Rossi S, Zanier ER, Mauri I et al. Brain temperature, body core temperature, and intracranial pressure in acute cerebral damage. *J Neurol Neurosurg Psychiatry* 2001; 71:448-454.
21. den HH, van der Worp B, van Gemert M et al. Therapeutic hypothermia in acute ischemic stroke. *Expert Rev Neurother* 2007; 7:155-164.
22. Karaszewski B, Wardlaw JM, Marshall I et al. Early brain temperature elevation and anaerobic metabolism in human acute ischaemic stroke. *Brain* 2009; 132(Pt 4):955-964.
23. Fingas M, Clark DL, Colbourne F. The effects of selective brain hypothermia on intracerebral hemorrhage in rats. *Exp Neurol* 2007; 208:277-284.
24. MacLellan CL, Davies LM, Fingas MS et al. The influence of hypothermia on outcome after intracerebral hemorrhage in rats. *Stroke* 2006; 37:1266-1270.
25. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999; 22:391-397.
26. van der Worp HB, Sena ES, Donnan GA et al. Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. *Brain* 2007; 130:3063-3074.
27. Boysen G, Christensen H. Stroke Severity Determines Body Temperature in Acute Stroke. *Stroke* 2001; 32:413-417.
28. Wong AA, Davis JP, Schluter PJ et al. The time course and determinants of temperature within the first 48 h after ischaemic stroke. *Cerebrovasc Dis* 2007; 24:104-110.
29. Castillo J, Davalos A, Marrugat J et al. Timing for fever-related brain damage in acute ischemic stroke. *Stroke* 1998; 29:2455-2460.
30. Jorgensen HS, Reith J, Pedersen PM et al. Body temperature and outcome in stroke patients. *Lancet* 1996; 348:193.
31. Bernard SA, Gray TW, Buist MD et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002; 346:557-563.
32. Holzer M, Bernard SA, Hachimi-Idrissi S et al. Hypothermia for neuroprotection after cardiac arrest: systematic review and individual patient data meta-analysis. *Crit Care Med* 2005; 33:414-418.
33. Guluma KZ. Intravenous thrombolysis plus hypothermia for acute treatment of ischemic stroke (ICTuS-L). *Stroke* (International Stroke Conference 2006 Abstracts) 2006; 37:706
34. Takasato Y. Combined local-intraarterial thrombolysis /brain hypothermia in acute occlusion of cerebral main trunk arteries. *Journal of Stroke and Cerebrovascular Diseases* 2000; 9:63-64
35. den Hertog HM, van der Worp HB, van Gemert HM et al. Correction: PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial. [ISCRTN74418480]. *BMC Cardiovasc Disord* 2008; 8:29.
36. Bath PM, Gray LJ, Collier T et al. Can we improve the statistical analysis of stroke trials? Statistical reanalysis of functional outcomes in stroke trials. *Stroke* 2007; 38:1911-1915.
37. Peto R. Why do we need systematic overviews of randomized trials? *Stat Med* 1987; 6:233-244.
38. Gill R, Kemp JA, Sabin C et al. Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats. *J Cereb Blood Flow Metab* 2004; 24:1214-1218.
39. Chamorro A, Urra X, Planas AM. Infection after acute ischemic stroke: a manifestation of brain-induced immunodepression. *Stroke* 2007; 38:1097-1103.

40. Dirnagl U, Klehmet J, Braun JS et al. Stroke-induced immunodepression: experimental evidence and clinical relevance. *Stroke* 2007; 38:770-773.
41. Emsley HC, Hopkins SJ. Acute ischaemic stroke and infection: recent and emerging concepts. *Lancet Neurol* 2008; 7:341-353.
42. Hacke W, Kaste M, Bluhmki E et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; 359:1317-1329.

Chapter 7

Summary

Nederlandse samenvatting

SUMMARY

Stroke ranks second as a cause of death worldwide and is the main cause of disability in high-income countries. Treatment of ischemic stroke and intracerebral hemorrhage has remained unsatisfactory. Apart from stroke unit care, intravenous thrombolysis with recombinant tissue-plasminogen activator (rt-PA) and aspirin are efficacious in patients with ischemic stroke, and there is no treatment for intracerebral hemorrhage with proven efficacy. Safe, cheap, and broadly applicable therapies for acute stroke are needed.

Inflammation plays a role in the pathophysiology of acute stroke. Body temperature and classic acute-phase reactants are modified by this inflammatory reaction, and may be useful in the prediction of the prognosis after stroke and as therapeutic targets.

This thesis focused on body temperature and temperature-lowering therapy in acute stroke. A secondary aim was to further expand the knowledge on inflammation in relation to prognosis and treatment in acute stroke.

Chapter 1, the general introduction, describes the background and the rationale for the research described in this thesis.

In **Chapter 2**, we give a general review of the literature concerning temperature-lowering therapy and discuss the existing evidence in terms of feasibility and effectiveness of this treatment strategy in acute stroke. There is currently no evidence from randomized trials to support routine use of physical or pharmacological strategies to reduce temperature or preventing fever in patients with acute stroke. Large randomized clinical trials are needed to study the safety and the effectiveness of both physical and pharmacological temperature reduction in patients with acute stroke.

Based on the existing evidence, we started the Paracetamol (Acetaminophen) In Stroke (PAIS) trial. In this multicenter, randomized, double-blind, placebo-controlled trial, we assessed whether early treatment with high-dose paracetamol (6 g daily) for 3 consecutive days improves functional outcome in 1400 patients with acute stroke.

For the PAIS trial, we decided to change the planned analysis for the primary outcome measure from a fixed dichotomy of the modified Rankin Scale (mRS) to a sliding dichotomy analysis. **Chapter 3.1** describes the protocol change. Instead of taking a single definition of good outcome for all patients, the definition was tailored to each individual patient's baseline prognosis on entry into the trial. This protocol change was initiated because of both advances in statistical approaches and to increase the efficiency of the trial by improving statistical power.

Chapter 3.2 describes the results of the PAIS trial. We found that more patients on high-dose paracetamol than patients treated with placebo improved beyond expectation, but the difference was not statistically significant (adjusted odds ratio 1.20, 95% CI 0.96–1.50). In a post-hoc subgroup analysis, we found that in patients with a baseline body temperature between 37°C and 39°C, treatment with paracetamol was significantly associated with improved outcome (adjusted odds ratio 1.43, 95% CI 1.02-1.97). The re-

sults do not support routine use of high-dose paracetamol in patients with acute stroke. Further study is needed to confirm the beneficial effects of paracetamol in patients with a baseline body temperature between 37°C and 39°C.

Subfebrile temperature or fever is present in about a third of patients on the first day after stroke onset and is associated with poor outcome. However, the temporal profile of this association is not well established. In **Chapter 3.3**, we assessed the relationship between body temperature, on admission as well as the change in body temperature from admission to 24 hours thereafter and functional outcome and death in 1332 patients included in the PAIS trial. We found that an early rise in body temperature rather than elevated body temperature on admission is a risk factor for poor functional outcome in patients with acute stroke. This provides a rationale for the hypothesis that prevention of an early rise in body temperature could reduce death and improve functional outcome.

Paracetamol is widely known as an antipyretic and analgesic, but may also have other effects. In **Chapter 3.4**, we assessed the effect of high-dose paracetamol on blood pressure in 540 patients with acute stroke. We showed that paracetamol also reduces blood pressure at 12 hours from the start of treatment. This finding provides an additional argument for further studies of the effect of prophylactic high-dose acetaminophen on functional outcome in acute stroke.

Elevated levels of C-reactive protein (CRP) can be a reflection of the inflammatory reaction following acute stroke. In **Chapter 4.1,** we evaluated the prognostic value of CRP obtained within 12 hours of symptom onset in 561 patients of ischemic stroke. An increased level of CRP in the very early phase of acute stroke was an independent prognostic factor for poor outcome. Furthermore, we found that common variation in the CRP gene was associated with increased levels of CRP in 185 patients with acute ischemic stroke in **Chapter 4.2**. Further studies are needed to investigate the relation between variation in the CRP gene, levels of CRP and clinical outcome after acute ischemic stroke.

In **Chapter 5**, a first step into further research in acute stroke aimed at integrating basic and clinical science is presented. **Chapter 5.1** gives a short overview of the different animal models of ischemic stroke that have been used to date, the advantages and disadvantages of these models and the role of animals models in relation to stroke research.

Recently, it has been shown that synthetic oligopeptides related to human chorionic gonadotropin (hCG) and various other synthetic oligopeptides may have immunomodulatory effects. In **Chapter 5.2**, a pilot study to assess the effects of two promising synthetic anti-inflammatory tetrapeptides on infarct volume and on inflammatory gene expression in an animal model of focal cerebral ischemia is described. We found no immunomodulatory effects of these synthetic tetrapeptides in a mouse model of ischemic stroke.

Complement activation may be one of the key pathways in the onset and reinforcement of the inflammation process following acute stroke. The protocol of a pilot study on complement activation in acute ischemic stroke is presented in **Chapter 5.3**.

The implications of the studies described in this thesis and future research are discussed in **Chapter 6**. It is concluded that lowering body temperature and the prevention of fever is a promising approach to improve functional outcome after stroke and provides directions for further research. It is too early for routine use of high-dose paracetamol in acute stroke. A second trial on the effect of high-dose paracetamol in patients with body temperatures of 37°C is needed. Moreover, future research should focus on larger temperature reductions by physical cooling methods in acute stroke. Inflammation plays a prognostic role in acute stroke, and may further guide therapeutic choices in acute stroke.

NEDERLANDSE SAMENVATTING

Een beroerte (herseninfarct of hersenbloeding) is de tweede doodsoorzaak en de belangrijkste oorzaak van invaliditeit in de westerse wereld. Hoewel de behandeling van het acute herseninfarct de laatste jaren is verbeterd door de komst van stroke units en behandeling met aspirine en trombolyse is de functionele uitkomst voor veel patiënten nog steeds slecht. Voor de acute hersenbloeding is behoudens de stroke unit geen enkele bewezen effectieve therapie beschikbaar. Het is daarom van belang om het onderzoek naar nieuwe behandelingen voor beroerte voort te zetten.

De laatste jaren wordt steeds meer duidelijk dat ontsteking een belangrijke rol speelt in de pathofysiologie van een beroerte. Naast het gegeven dat ontsteking betrokken is bij het herstel van weefselschade draagt het waarschijnlijk ook bij aan de weefselschade. De lichaamstemperatuur en acute fase eiwitten worden beïnvloed door deze ontstekingsreactie en zouden nuttig kunnen zijn bij het voorspellen van de prognose na een beroerte en als aangrijpingpunt voor therapie.

Het in dit proefschrift beschreven onderzoek is gericht op lichaamstemperatuur en temperatuurverlagende therapieën bij patiënten met een beroerte. Daarnaast hebben we de rol van ontsteking met betrekking tot de prognose en de behandeling van patiënten met een beroerte bestudeerd.

In **hoofdstuk 2** wordt de achtergrond geschetst van temperatuurverlaging of het voorkómen van koorts bij patiënten met een beroerte en wordt een overzicht gegeven van het bewijs van de effectiviteit en veiligheid van deze therapieën. Er zijn twee manieren om de lichaamstemperatuur te verlagen, namelijk medicamenteus met bijvoorbeeld paracetamol en door middel van fysiek koelen met bijvoorbeeld koelmatrassen, koude infuusvloeistof of koelhelmen. De conclusie is dat er onvoldoende bewijs is voor de effectiviteit en veiligheid van temperatuurverlagende therapieën bij patiënten met een beroerte. Grote gerandomiseerde trials zijn nodig om de veiligheid en effectiviteit van zowel temperatuurverlaging of het voorkomen van koorts te bestuderen.

Om na te gaan of vroege behandeling met een hoge dosis paracetamol door middel van het voorkomen van koorts en het verlagen van de lichaamtemperatuur de functionele uitkomst na een beroerte verbetert, hebben wij de Paracetamol (Acetaminophen) In Stroke (PAIS) trial uitgevoerd.

In **Hoofdstuk 3.1** wordt een wijziging op het protocol van PAIS beschreven. Voor de primaire uitkomstmaat wordt in de meeste trials met patiënten met een beroerte de modified Rankin Scale gebruikt. Deze frequent gebruikte schaal om de uitkomst na een beroerte te meten wordt vaak in twee categorieën ingedeeld, namelijk een gunstige of ongunstige uitkomst. Deze methode heeft als nadeel dat het minder overeenkomt met de klinische praktijk en minder efficiënt gebruik maakt van de beschikbare gegevens. Om deze redenen hebben wij de primaire uitkomstmaat van de PAIS gewijzigd van deze grove tweedeling naar verbetering volgens de zogenoemde "sliding dichotomy analysis". Hierbij wordt rekening gehouden met de verwachte prognose van een patiënt.

In **hoofdstuk 3.2** worden de hoofdresultaten van de PAIS besproken. In deze gerandomiseerde placebo-gecontroleerde dubbelblinde trial werden in 29 Nederlandse centra 1400 patiënten met een beroerte en een lichaamstemperatuur van 36-39 °C binnen 12 uur na aanvang van de symptomen gerandomiseerd voor behandeling met paracetamol (6 gram per dag) of placebo gedurende 3 dagen. Meer patiënten die behandeld waren met paracetamol verbeterden dan patiënten die een placebo hadden gebruikt, maar dit effect was niet statistisch significant (geadjusteerde odds ratio, 1.20; 95% BI, 0.96–1.50). In een post-hoc subgroepanalyse had paracetamol wel een significant gunstig effect bij patiënten met een lichaamstemperatuur van 37-39°C (geadjusteerde odds ratio 1.43, 95% BI 1.02–1.97). De resultaten zijn veelbelovend, maar bieden nog onvoldoende basis om een hoge dosis paracetamol routinematig te geven aan patiënten met een acute beroerte. Paracetamol verbetert mogelijk het herstel bij patiënten met een lichaamstemperatuur van 37°C of meer. Dit moet worden bevestigd in een onafhankelijke studie.

Een verhoogde lichaamstemperatuur op de eerste dag na een beroerte komt voor bij eenderde van de patiënten en is geassocieerd met slecht functioneel herstel. Het is echter onduidelijk wat de meeste voorspellende waarde heeft, de lichaamstemperatuur bij opname of de stijging van de lichaamstemperatuur gedurende de eerste 24 uur. In **hoofdstuk 3.3** zijn we nagegaan bij 1332 patiënten die geïncludeerd waren in PAIS wat de prognostische waarde was van de lichaamstemperatuur gemeten op 2 momenten, namelijk bij opname en 24 uur later op het functionele herstel en overlijden. We vonden dat de stijging in de lichaamstemperatuur en niet een verhoogde lichaamstemperatuur bij opname gerelateerd was met een slechter functioneel herstel en overlijden. Dit ondersteunt de gedachte dat het belangrijk is om temperatuurstijging te voorkómen bij patiënten met een beroerte.

Paracetamol heeft naast een pijnstillende en temperatuurverlagende werking mogelijk ook andere effecten. In **hoofdstuk 3.4** wordt een onderzoek beschreven waarin we bij 540 patiënten met een beroerte het effect van paracetamol op de bloeddruk hebben

bestudeerd. We vonden dat paracetamol ook een bloeddrukverlagend effect heeft in de eerste 12 uur na het starten van de behandeling. Deze bevinding is een extra ondersteuning voor nieuwe trials naar het effect van paracetamol bij patiënten met een acute beroerte.

Tijdens de acute fase van een herseninfarct worden in het serum van patienten verhoogde concentraties van C-reactive protein (CRP), een acuut fase eiwit, gevonden. Deze verhoogde concentraties zijn mogelijk een weerspiegeling van de ontstekingsreactie die na het herseninfarct op gang komt. In **hoofdstuk 4.1** hebben we bij 561 patiënten met een herseninfarct de prognostische waarde van CRPconcentraties op de klinische uitkomst onderzocht. Een verhoogde CRP-waarde was een onafhankelijke voorspeller voor een ongunstige klinische uitkomst.

De mate van de ontstekingsreactie bij patiënten met een acuut herseninfarct kan sterk wisselen, mogelijk op basis van erfelijke eigenschappen. In **hoofdstuk 4.2** vonden wij in 185 patiënten met een herseninfarct dat genetische variatie in het CRP-gen gerelateerd is aan hogere CRP-concentraties. Als in toekomstig onderzoek blijkt dat deze genetische variatie in het CRP-gen ook gerelateerd is aan de klinische uitkomst zou dit kunnen leiden tot een nieuw aangrijpingspunt voor therapie .

In **hoofdstuk 5** worden de eerste stappen voor vervolgonderzoek zowel in een diermodel als in patiënten gepresenteerd. In **hoofdstuk 5.1** worden de verschillende diermodellen van het herseninfarct, de voor- en nadelen van deze modellen en de rol van het diermodel binnen het onderzoek naar het herseninfarct besproken.

In **hoofdstuk 5.2** beschrijven we een eerste studie die we hebben verricht in een muismodel voor het herseninfarct. Eerder onderzoek heeft laten zien dat synthetisch verkregen tetrapeptides (moleculen die bestaan uit 4 aminozuren en veelal bouwstenen vormen voor eiwitten) die gerelateerd zijn aan het zwangerschapshormoon humaan Chorion Gonadotrofine (hCG) en diverse ander synthetisch verkregen tetrapeptides veranderingen in het immuunsysteem ter weeg kunnen brengen. Wij hebben onderzocht of deze tetrapetides een gunstige invloed hadden op de grootte van het herseninfarct en de ontstekingsreactie die na het herseninfarct op gang komt. We vonden geen aanwijzingen voor een gunstig effect van de tetrapeptides in een muismodel van het herseninfarct. Vervolgonderzoek is nodig om de rol van deze tetrapeptides op de ontstekingsreactie in de acute fase van het herseninfarct nader te bestuderen.

Een belangrijke factor bij de ontstekingsreactie is het complementsysteem. Het complementsysteem kan zorgen voor het initiëren en verergeren van een ontstekingsreactie. **Hoofdstuk 5.3** beschrijft een protocol van een studie waarin we bij patiënten met een acuut herseninfarct willen nagaan of een activatie van het complementsysteem te meten is in het serum van deze patiënten. Het is een eerste stap om de rol van het complementsysteem in de acute fase van het herseninfarct nader te bestuderen.

In hoofdstuk 6 worden de klinische implicaties van de verschillende studies in dit proefschrift besproken en worden suggesties gedaan voor toekomstig onderzoek.

Temperatuurverlaging of het voorkomen van temperatuurstijging blijft een veelbelovende behandeling, maar het is te vroeg om patiënten met een beroerte routinematig te behandelen met een hoge dosis paracetamol. Een nieuwe trial naar het effect van hoge dosis paracetamol bij patiënten met een beroerte en een temperatuur van 37°C of hoger is nodig. Ook zouden toekomstige trials zich moeten richten op fysiek koelen. Met deze methode kunnen lagere temperaturen worden bereikt. Daarnaast speelt ontsteking een rol bij de prognose van patiënten met een beroerte en kan dit een aangrijpingspunt zijn voor nieuwe therapieën.

LIST OF ABBREVIATIONS

AOR	adjusted OR
BI	Barthel Index
CI	confidence interval
COX	cyclooxygenase
CRP	C-reactive protein
DNA	deoxyribonucleic acid
ELISA	Enzyme-Linked Immuno Sorbent Assay
EuroQol-5D	European Quality of Life-5 Dimensions
Fmoc	fluorenylmethoxycarbonyl
HCG	human chorionic gonadotropin
HE	hematoxylin and eosin
ICAM	intercellular adhesion molecule
IQR	interquartil range
IL	interleukin
LCCA	left common carotid artery
LECA	left external carotid artery
LICA	left internal carotid artery
MAC	membrane attack complex
MCAO	middle cerebral artery occlusion
MRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
OR	odds ratio
PAIS	Paracetamol (Acetaminophen) In Stroke
PAPAS	Paracetamol (Acetaminophen) in Patients with Acute Stroke
PCR	Polymerase Chain Reaction
PISA	Paracetamol (Acetaminophen) and Ibuprofen in Acute Stroke
RNA	ribonucleic acid
Rt-PA	tissue-plasminogen activator
SD	Standard deviation
SNP	single-nucleotide polymorphism
STAIR	Stroke Therapy Academic Industry Roundtable
TOAST	Trial of ORG 10172 in Acute Stroke Therapy
TNF-α	tumor necrosis factor-α
TTC	2,3,5 triphenyltetrazolium chloride

Appendices

APPENDIX I: THE MODIFIED RANKIN SCALE

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

APENDIX II: BARTHEL INDEX

FEEDING
0 = unable
1 = needs help cutting, spreading butter, etc., or requires modified diet
2 = independent

BATHING
0 = dependent
1 = independent (or in shower)

GROOMING
0 = needs to help with personal care
1 = independent face/hair/teeth/shaving (implements provided)

DRESSING
0 = dependent
1 = needs help but can do about half unaided
2 = independent (including buttons, zips, laces, etc.)

BOWELS
0 = incontinent (or needs to be given enemas)
1 = occasional accident
2 = continent

BLADDER
0 = incontinent, or catheterized and unable to manage alone
1 = occasional accident
2 = continent

TOILET USE
0 = dependent
1 = needs some help, but can do something alone
2 = independent (on and off, dressing, wiping)

TRANSFERS (BED TO CHAIR AND BACK)
0 = unable, no sitting balance
1 = major help (one or two people, physical), can sit
2 = minor help (verbal or physical)
3 = independent

MOBILITY (ON LEVEL SURFACES)
0 = immobile or < 50 yards
1 = wheelchair independent, including corners, > 50 yards
2 = walks with help of one person (verbal or physical) > 50 yards
3 = independent (but may use any aid; for example, stick) > 50 yards

STAIRS
0 = unable
1 = needs help (verbal, physical, carrying aid)
2 = independent

APPENDIX III: THE STAIR RECOMMENDATIONS FOR STANDARDS REGARDING PRECLINICAL NEUROPROTECTIVE DRUG DEVELOPMENT

STAIR recommendations
1. Adequate dose-response studies should be conducted with serum concentrations measured to define minimally and maximally effective doses
2. Time-window studies to confirm efficacy are required
3. Physiological monitoring should be undertaken
4. Randomized, blinded studies that give reproducible effects should be conducted, involving at least one independent laboratory
5. Both infarct volume and functional response should be assessed, including short- and long-term assessment
6. Efficacy should be evaluated in both transient and permanent middle cerebral artery occlusion rat models
7. For novel, first-in-class agents, studies in larger species/primates are required to confirm efficacy
8. Studies should be published in peer-reviewed journals

Epilogue

ACKNOWLEDGEMENTS

De kracht van onderzoek ligt in het samen doen. Zonder de hulp van velen was dit proefschrift niet tot stand gekomen. Een aantal mensen zou ik graag in het bijzonder willen noemen.

Promotor

Prof.dr P.J. Koudstaal
Beste Peter, bedankt dat je me de kans hebt gegeven om mijn promotie-onderzoek te doen binnen je onderzoeksgroep. Je bood me de mogelijkheid om vorm te geven aan mijn ambities. Werken aan meerdere onderzoeksprojecten, congressen, stages in het buitenland, cursussen, het kon allemaal. Ik heb veel geleerd van je inzicht en scherpe blik en het vermogen om te relativeren op het juiste moment. Wat velen voor me al geschreven hebben is echt waar: niemand reageerde zo snel op mijn emails of op mijn manuscripten als jij! Ik hoop dat we onze samenwerking nog lang kunnen voort zetten.

Co-promotor

Dr. D.W.J. Dippel
Beste Diederik, door je optimisme, enthousiasme en gedrevenheid is het een groot genot om met je samen te werken. Ik heb veel geleerd van onze discussies en je relativeringsvermogen was een grote steun bij frustraties. Hartelijk dank voor je intensieve begeleiding en voor het sneltreintempo van de laatste maanden van mijn promotie mogelijk te maken. Je moest even wennen toen de inhoud van het proefschrift een iets andere wending kreeg, maar je hebt me hier altijd vrij in gelaten. Ik hoop in de toekomst nog veel van je te mogen leren!

Co-promotor

Dr. H.B. van der Worp
Beste Bart, bedankt voor de goede samenwerking, de betrokkenheid en je hulp bij de totstandkoming van dit proefschrift. Elk manuscript werd door jouw bijdrage beter. Ik heb veel geleerd van je kritische blik, die jij vaak bestempelde als een wat donkere kijk op het leven, en je net wat andere kijk op de zaak. Ik hoop dat dit promotieonderzoek de basis vormt voor een verdere wetenschappelijke samenwerking.

Overige leden stuurgroep PAIS
H.M.A. van Gemert, prof.dr. J. van Gijn, prof.dr. L.J. Kappelle en prof.dr. A. Algra

Beste Maarten, in vele opzichten heb je me de afgelopen jaren gesteund. Allereerst ben je de topincludeerder van PAIS met 297 inclusies. Daarnaast heb je me zowel geholpen met verzamelen van de gegevens als commentaar gegeven op een groot deel van de hoofdstukken van dit proefschrift. Ook was je praktische kijk binnen PAIS van groot belang en wist je humor me altijd weer op te beuren. Wat mij betreft ben je veel meer dan een waterdrager!
Beste professor van Gijn, uw bijdrage aan de manuscripten was van grote waarde. U gaf de manuscripten altijd net een beetje meer dat ze nodig hadden. Ook zal ik aan de nuttige tips met betrekking tot de Engelse taal in de toekomst nog veel hebben.
Beste Ale, het was een voorrecht om je in de stuurgroep van PAIS te hebben.
Je enthousiasme, betrokkenheid en nuttige kritieken zijn bewonderingswaardig.
Beste Jaap, bedankt voor je betrokkenheid bij het PAISonderzoek en voor je adviezen en commentaar op de manuscripten. Je goede klinische blik in combinatie met je wetenschappelijke kwaliteiten waren binnen PAIS essentieel.

De overige leden van de leescommissie

Prof.dr. D.J.G.M. Duncker, prof.dr. J.D. Laman, prof.dr. E.W. Steyerberg
Allereerst wil ik jullie bedanken voor het beoordelen van het manuscript.
Beste Dirk, bedankt voor de mogelijkheid die je me hebt geboden om een diermodel voor het herseninfarct op te zetten in je lab en de ondersteuning die je me hierin hebt gegeven. Onze gesprekken waren zeer motiverend en je enthousiasme werkt aanstekelijk. Ik hoop, nu het model er is, dat we in de toekomst nog veel zullen samenwerken.
Beste Jon, bedankt voor de avonden die je hebt opgeofferd om ons bij te scholen in de immunologie. De discussies die we hebben gevoerd over nieuwe onderzoeksvoorstellen waren voor mij zeer inspirerend en hebben een basis gelegd voor de toekomst.
Beste Ewout, bedankt voor je begeleiding in de statistiek en je deelname aan de datamonitoring commissie van PAIS de afgelopen jaren. Misschien ga ik in de toekomst het proportional odds model toch nog een keer toepassen!

De overige leden van de promotiecommissie

Prof.dr. M. Limburg ,dr. A.H. van den Meiracker en prof.dr. F.W.G. Leebeek
Beste professor Limburg, hartelijk dank voor het plaatsnemen in de de Grote Commissie.
Beste Ton, bedankt voor het plaatsnemen in de Grote Commissie en de kennis die je me hebt bijgebracht van de vasculaire interne geneeskunde. Hier ga ik nog veel profijt van hebben.
Beste Frank, hartelijk dank voor de leerzame discussies tijdens de afgelopen jaren en het plaatsnemen in de Grote Commissie.

Overige leden datamonitoring commissie

Prof.dr. P.A.G. Sandercock, prof.dr. M. Vermeulen, prof.dr. M. Correia
Thank you for chairing the datamonitoring and safety committee of PAIS.

De overige co-auteurs van mijn manuscripten
De overige co-auteurs van mijn manuscripten wil ik bedanken voor hun intellectuele bijdrage. Een speciaal woord van dank gaat uit naar prof.dr. M.P.M. de Maat, prof.dr. R. Benner en dr. B.C. Jacobs. Beste Moniek, bedankt voor je begeleiding van de SNPbepalingen, discussies en je laagdrempeligheid om even langs te kunnen lopen bij vragen. Beste professor Benner, bedankt dat u me de mogelijkheid heeft gegeven om een eerste onderzoek uit te voeren met het herseninfarctmodel in Berlijn. Beste Bart, bedankt voor je belangstelling, goede adviezen en betrokkenheid bij toekomstig onderzoek.

Het trialbureau

Naziha el Ghannouti en Esther van der Heijden
Het includeren van patiënten en het verzamelen van de gegevens uit zoveel ziekenhuizen in Nederland is een aanzienlijke klus. Naziha en Esther, zonder jullie hulp was dit niet gelukt. Ik heb goede herinneringen aan onze treinreizen naar alle uithoeken van Nederland en aan onze autoritten. Door de vele kilometers heb ik ook nog eens mijn rijvaardigheid op peil kunnen houden. Daarnaast bedankt voor jullie volhardendheid waardoor we uiteindelijk slechts één lost to follow-up hadden in de PAISstudie.

Deelnemende centra PAISstudie
Zonder de neurologen en arts-assistenten uit de 29 deelnemende ziekenhuizen was PAIS nooit een succes geworden. Bedankt voor jullie inzet de afgelopen jaren.
Daarnaast wil ik alle onderzoeksassistenten, verpleegkundigen en secretaressen die ons hierin hebben bijgestaan bedanken voor hun hulp.

Verpleging van 6 noord en 6 midden
Bedankt voor jullie bijdrage aan de PAISstudie! Dorien, bedankt voor je samenwerking en belangstelling de afgelopen jaren.

Hester Lingsma
Hester, bedankt voor je bereidheid om me te helpen bij de "sliding dichotomy" analyse en de jaarlijkse datacontrole van PAIS.

Neurovasculaire onderzoeksgroep
Mijn collega's uit de vasculaire groep, bedankt voor de gezelligheid, steun en de goede herinneringen aan onder andere de congressen in Brussel, Glasgow, Nice en Stockholm. Maaike en Lonneke, ook heb ik bijzonder goede herinneringen aan de summer school in Barcelona.
Marjolein, bedankt voor je tips bij het geven van mijn presentaties.
Marieke, bedankt voor je kritische blik en het uitpluizen van de tekst op typefouten.
Daarnaast wil ik in het bijzonder de stroketelefoonhouders Annemarie, Maaike, Ewoud, Lisette, Lonneke, Mathieu, Mary-Lou en Niels bedanken voor dat jullie door weer en wind tijdens weekenden en avonden patiënten in PAIS hebben geïncludeerd.

Minah Ngoredjo
Bedankt voor de samenwerking en het maken van alle onderzoeksafspraken.

Elza van Deel, Monique de Waard, Maaike te Lintel Hekkert, Patricia Specht
Beste Elza, Monique, Maaike en Patricia, bedankt voor jullie ondersteuning in het lab en met resultaat; het herseninfarctmodel is er!

Studenten

Joram van Rossum, Evita van den Herik en Arezo Mohamad
Beste Joram, je volhardenheid en enthousiasme hebben geleid tot mooi resultaat. Succes met je verdere carriere!
Evita, je altijd goede humeur, enthousiasme en gedrevenheid zijn opvallend.
Inmiddels ben je al bijna weer een jaar promovendus, succes met het vervolg!
Arezo, bedankt voor je ondersteuning bij het verzamelen van de data in Amersfoort, zodat het me toch nog lukte om tijdens de laatste dagen van mijn onderzoek alles nog compleet te krijgen.

Patrick Hessels
Beste Patrick, heel erg bedankt voor de mooie omslag!

Vrienden en collega's
Velen verdienen dank voor het zorgen voor de nodige afleiding tijdens de promotietijd. Allereerst mijn cordialgenoten en studievrienden. Bedankt voor jullie nooit aflatende interesse in mijn werkzaamheden. Het woord stroke is de afgelopen jaren vaak gevallen. De vakanties, weekenden weg, uitstapjes en vele etentjes waren een welkome afwisseling. Ireen, bedankt dat je altijd voor me hebt klaar gestaan en voor je luisterend oor. Je altijd goede humeur en je zeer positieve kijk op het leven bewonder ik.

Maaike, je bent me in dit traject voor gegaan en je wist me altijd op de juiste momenten weer op te beuren en zoals jezelf al zei: het gaat echt lukken!
Ilse, het sporten zorgde voor een goede ontstresser. Bedankt voor je interesse en steun. Volgend jaar zul jij hier staan.
Mijn collega's van de neurologie, dank jullie wel voor jullie steun zowel op de werkvloer als bij de borrels. Ik weet dat ik jullie de laatste maanden van dit proefschrift heb verwaarloosd, gelukkig heb ik nog voldoende tijd om het goed te maken.

Paranimfen Rosalie Kemps en Janet de Beukelaar
Rosalie, ook al begrijp je mijn voorliefde voor het onderzoek niet helemaal, ik ben superblij dat je bereid bent om me naast alle steun van de afgelopen jaren ook in dit laatste traject en op de dag van de promotie bij te willen staan.
Janet, je nimmer aflatende enthousiasme en organisatietalent zijn opvallend.
Bedankt voor je steun en relativerende vermogen tijdens de afgelopen jaren.
Ik prijs me gelukkig met jullie als paranimfen!

Mijn familie
Lieve papa en mama, jullie hebben me altijd volop de ruimte gegeven om me persoonlijk te ontwikkelen. Jullie hebben me de kans gegeven om naast de studie geneeskunde ook biomedische wetenschappen af te maken. Ik ben jullie dankbaar voor jullie vertrouwen en voor alles wat ik van jullie heb meegekregen.
Zusje, lieve Annemieke, bedankt voor je onvoorwaardelijke steun.
Oma en opa Schoemaker, bedankt voor jullie liefde, vertrouwen en interesse in alles wat mij bezig houdt. Jullie hebben me altijd gestimuleerd om me te ontwikkelen en door te zetten. Helaas kan opa er op deze promotiedag niet bij zijn.
Oma den Hertog bedankt voor uw betrokkenheid en liefde.
Mijn schoonfamilie, bedankt voor jullie belangstelling, liefde en begrip.

Michiel Proper
Lieve Michiel, ik kan je niet genoeg bedanken. Je was een enorme steun de afgelopen jaren. Bedankt voor je altijd luisterend oor, liefde en geduld. Je wist me altijd weer op te beuren,"it is not what happens, but what you do that makes the difference".

ABOUT THE AUTHOR

Heleen den Hertog was born on July 10th, 1978 in Utrecht, the Netherlands. She attended secondary school at the Sint Laurens College in Rotterdam, from which she graduated in 1996. The same year, she started studying Biomedical Sciences at the University of Leiden. One year later, she also started her medical degree at the same University. During these studies, she conducted research on the effect of deep inspirations on reversibility of airway resistance by salbutamol in mild asthmatics (prof.dr. P.J. Sterk, Department of Pulmonology, University of Leiden) and on the role of the orexin system in human narcolepsy (dr. G.J. Lammers, Department of Neurology, University of Leiden and prof.dr. D.F. Swaab, Institute for Brain Research, Amsterdam). She joined the fraternity Augustinus in Leiden and was president of the Amnesty International student group and a board member of a student yacht club. Heleen obtained her medical degree (cum laude) as well as a master's degree in Biomedical Sciences in 2004. Hereafter, she started her residency at the Department of Neurology at the Erasmus MC University Medical Center in Rotterdam (head prof.dr P.A.E. Sillevis Smitt).

From July 2005 she combined her residency with research underlying this thesis at the Department of Neurology of the Erasmus MC University Medical Center under supervision of prof.dr. P.J. Koudstaal and dr. D.W.J. Dippel, and dr. H.B. van der Worp (University Medical Center Utrecht). She was trial coordinator of the Paracetamol (Acetaminophen) In Stroke (PAIS) trial from 2005 to 2008. As part of her research training, she attended a summer school in neurovascular diseases at the University of Barcelona (Spain). She went to the Hospital Clinic, Barcelona, Spain and the Charite, Berlin, Germany to set up an animal model of acute ischemic stroke in 2007, respectively 2008. From 2008 onwards, she is a board member of the Junior Doctors' Association at Erasmus MC University Medical Center.

In 2009, she received the young investigator's award at the European Stroke Conference. As of February 2009, she is continuing her residency in Neurology at Erasmus MC University Medical Center.

COMPLETE LIST OF PUBLICATIONS AND MANUSCRIPTS

1. **HM den Hertog**, HB van der Worp, HMA van Gemert, A Algra, LJ Kappelle, J van Gijn, PJ Koudstaal, DWJ Dippel, PAIS investigators. High-dose paracetamol in acute stroke: the Paracetamol (Acetaminophen) In Stroke trial (PAIS). *Lancet Neurology* 2009; 8:434-440.
2. **HM den Hertog**, HB van der Worp, HMA van Gemert, A Algra, LJ Kappelle, J van Gijn, PJ Koudstaal, DWJ Dippel, PAIS investigators. High-dose paracetamol in stroke: new trials are necessary and feasible. *Lancet Neurology* 2009; 8:700-701
3. **HM Den Hertog**, HB van der Worp, M Tseng, DWJ Dippel. Cooling therapy for acute stroke (Cochrane systematic review). *the Cochrane Database Syst Rev* 2009; 1: CD001247.
4. **HM Den Hertog**, HB van der Worp, M Tseng, DWJ Dippel. Temperature-lowering therapy for acute stroke. *Stroke* 2009 Epub.
5. **HM den Hertog,** JA van Rossum**,** HB van der Worp, HMA van Gemert, R de Jonge, PJ Koudstaal, DWJ Dippel. C-reactive protein in the very early phase of acute ischemic stroke: association with poor outcome and death. *J Neurol* 2009 Epub.
6. **HM den Hertog**, HB van der Worp, HMA van Gemert, A Algra LJ Kappelle, J van Gijn, PJ Koudstaal, DWJ Dippel, PAIS investigators. Correction: PAIS: paracetamol (acetaminophen) in stroke; protocol for a randomized, double blind clinical trial. [ISRCTN 74418480]. *BMC Cardiovasc Disord* 2008; 8:29.
7. **HM den Hertog**, HB van der Worp, HMA van Gemert, DWJ Dippel. Review Neurotherapeutics: Therapeutic hypothermia in acute ischemic stroke: background, feasibility, and effectiveness. *Expert Rev Neurother* 2007; 7:155-164.
8. **HM den Hertog**, HB van der Worp, HMA van Gemert. Acetaminophen for temperature reduction in acute stroke: potential but unproven benefits. *Stroke* 2007; 38:e131.
9. J Hofmeijer. GJ Amelink, **HM den Hertog**, A Algra, LJ Kappelle, HB van der Worp, Hamlet investigators, PAIS investigators Appreciation of the informed consent procedure in a randomised trial of decompressive surgery for space occupying hemispheric infarction. *J Neurol Neurosurg Psychiatry* 2007; 78:1124-1128.
10. LM de Lau, **HM den Hertog**, EG van den Herik and PJ Koudstaal. Predicting and preventing stroke after transient ischemic attack. *Expert Rev Neurother* 2009; 9:1159-1170.
11. **HM den Hertog**, HB van der Worp, HMA van Gemert, A Algra LJ Kappelle, J van Gijn, PJ Koudstaal, DWJ Dippel, PAIS investigators. An early rise in body temperature is related to unfavorable outcome after stroke. *Submitted.*
12. **HM den Hertog**, HB van der Worp, HMA van Gemert, J van Gijn, PJ Koudstaal, DWJ Dippel. High-dose acetaminophen reduces systolic blood pressure in acute stroke. *Submitted.*
13. **HM den Hertog**, EG van den Herik, DWJ Dippel, PJ Koudstaal, MPM de Maat. Variation in the C-reactive protein (CRP) gene is associated with serum levels of CRP in patients with acute ischemic stroke. *Under revision.*

14. **HM den Hertog**, WA dik, DJ duncker, NA Khan, R benner and PJ Koudstaal. No effectiveness of synthetic anit-inflammatory tetrapeptides in a mouse model of ischemic stroke: a pilot study. *In preparation*.
15. **HM den Hertog**, CW Ang, DWJ Dippel. Rhombencephalitis due to Listeria monocytogenes. *Ned Tijdschr Geneesk* 2007; 151:1885-1890.
16. S Battaglia, **HM den Hertog**, M Timmers, S Lazeroms, A Vignola, K Rabe, V Bellia, P Hiemstra, and P Sterk. Small airways function and molecular markers in exhaled air in mild asthma. *Thorax* 2005; 60: 639–644
17. S Overeem, JJ Verschuuren, R Fronczek, L Scheurs, **HM den Hertog**, IM Hegemankleinn, SG van Duinen, UA Unmehopa, DF Swaab, GJ Lammers. Immunohistochemical screening for autoantibodies against lateral hypothalamic neurons in human narcolepsy. *J Neuroimmunol 2006*; 174:187-191.

PHD PORTFOLIO-SUMMARY OF PHD TRAINING AND TEACHING ACTIVITIES

Research School: COEUR

Work load	Year	(ECTS)
Research skills		
Classical methods for data analysis, Nihes, Rotterdam, NL	2005	5.0
Regression analysis, Nihes, Rotterdam, NL	2007	1.4
Repeated measurements, Nihes, Rotterdam, NL	2008	1.4
Advanced analysis of prognostic studies, Nihes, Rotterdam, NL	2008	1.4
Animal experimentation, Experimental Animal Center, Rotterdam, NL	2007	3.0
In-depth courses		
Cochrane course, Cochrane Center, Amsterdam, NL	2006	0.4
PhD courses (5x) and seminars (5x) at COEUR, Rotterdam, NL	2005-2009	9.5
Summer school neurovascular diseases, Barcelona, Spain	2007	2.0
Neuroimmunology, Department of immunology, Rotterdam, NL	2008	1.5
SNP Course V, MolMed, Rotterdam, NL	2008	1.5
Animal models for the study of neurological diseases, DIMI, Barcelona, Spain	2008	2.0
Neurovascular seminar (3x), Utrecht, NL	2005-2008	2.0
Neurovascular meetings (3x) and seminars (2x), Utrecht NL		
Grant applications introduction and advanced course MolMed, Rotterdam, NL	2008-2009	1.5
NWO talent class "grant applications"	2009	1.0
NWO talent day, Utrecht, NL	2009	0.4

(Inter)national conferences: participation and presentations		
European Stroke Conferences, Brussel, Belgium; Glasgow, UK; Nice, France; poster presentations "Paracetamol (Acetaminophen) In Stroke (PAIS study), ongoing trial."	2006-2008	3.0
COEUR seminar, Erasmus MC, Rotterdam, NL; oral presentation "Neuroprotection in acute stroke"	2007	0.4
International meeting, Charite, Berlin, Germany; oral presentation "Acute stroke and candidate peptides."	2007	1.5
COEUR seminar, Erasmus MC, Rotterdam, NL; oral presentation "Stroke induced immunodepression: from clinical experiments to animal experiments"	2008	0.4
European Stroke Conference, Nice, France; oral presentation "Cooling therapy for acute stroke."	2008	1.0
European Stroke Conference, EURO-COOLS meeting, Nice, France; Oral presentation "Cooling therapy for acute stroke: lessons from a cochrane review."	2008	1.0
European Stroke Conference Nice, France; poster presentation "C-reactive protein in acute ischemic stroke: relationship with short term outcome."	2008	1.0
World Stroke Conference, Vienna, Austria; oral presentation late breaking news session "Paracetamol (acetaminophen) In Stroke (PAIS) trial: A multicenter randomized double blind placebo-controlled trial."	2008	1.5
European Stroke Conference, Stockholm, Sweden; oral presentation "An early rise in body temperature rather than initial body temperature is related to functional outcome and death after stroke."	2009	1.0
European Stroke Conference, Stockholm, Sweden; poster presentation "Safety and feasibility of treatment with metformin in patients with TIA or minor ischemic stroke and impaired glucose tolerance: a randomized, open-label phase II trial."	2009	1.0

Research projects abroad		
Animal models of ischemic stroke		
Research group of prof.dr. M. Endres, Charite, Berlin, Germany	2007	4.0
Research group of dr. A.M. Planas, Hospital clinic, Barcelona, Spain	2008	2.0
Teaching activities		
Supervising		
Research projects of medical students at Erasmus MC		
"C-reactive protein acute ischemic stroke: relationship with short term outcome."	2007	6.0
"Hemostatis and inflammation in stroke."	2007-2008	6.0
Lecturing		
Teaching PhD students at COEUR "Vascular clinical epidemiology", Erasmus MC, Rotterdam, NL	2008	0.4
Other		
Organization and coordination of research seminars, Department of Neurology, Erasmus MC, Rotterdam, NL	2009	0.5

Stroke ranks second as a cause of death worldwide and is a leading cause of disability in high-income countries. Treatment of ischemic stroke and intracerebral hemorrhage has remained unsatisfactory. Apart from stroke unit care, intravenous thrombolysis with recombinant tissue-plasminogen activator and aspirin are efficacious in patients with ischemic stroke, and there is no treatment for intracerebral hemorrhage with proven efficacy. Safe, cheap, and broadly applicable therapies for acute stroke are needed.

Over the past years, evidence has accumulated that inflammation plays a role in the pathophysiology of acute stroke. Body temperature and classic acute-phase reactants are modified by this inflammatory reaction, and both may be useful in the prediction of the prognosis after stroke and as therapeutic targets. This thesis focuses on body temperature and temperature-lowering therapy in acute stroke. A secondary aim is to further expand the knowledge on inflammation in relation to prognosis and treatment in acute stroke.