Paracetamol (Acetaminophen) in stroke 2 (PAIS 2): Protocol for a randomized, placebo-controlled, double-blind clinical trial to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke and a body temperature of 36.5°C or above

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Rationale In the first hours after stroke onset, subfebrile temperatures and fever have been associated with poor functional outcome. In the first Paracetamol (Acetaminophen) in Stroke trial, a randomized clinical trial of 1400 patients with acute stroke, patients who were treated with high-dose paracetamol showed more improvement on the modified Rankin Scale at three-months than patients treated with placebo, but this difference was not statistically significant. In the 661 patients with a baseline body temperature of 36.5°C or above, treatment with paracetamol increased the odds of functional improvement (odds ratio 1.31; 95% confidence interval: 1.02–1.97). This relation was also found in the patients with a body temperature of 37.0°C or above, treatment with paracetamol increased the odds of functional improvement (odds ratio 1.31; 95% confidence interval 1.01–1.68). These findings need confirmation.

Aim The study aims to assess the effect of high-dose paracetamol in patients with acute stroke and a body temperature of 36.5°C or above on functional outcome.

Design The Paracetamol (Acetaminophen) In Stroke 2 trial is a multicenter, randomized, double-blind, placebo-controlled clinical trial. We use a power of 85% to detect a significant difference in the scores on the modified Rankin Scale of the paracetamol group compared with the placebo group at a level of significance of 0.05 and assume a treatment effect of 7%. Fifteen-hundred patients with acute ischemic stroke or intracerebral hemorrhage and a body temperature of 36.5°C or above will be included within 12 h of symptom onset. Patients will be treated with paracetamol in a daily dose of six-grams or matching placebo for three consecutive days. The Paracetamol (Acetaminophen) In Stroke 2 trial has been registered as NTR2365 in The Netherlands Trial Register.

Study outcomes The primary outcome will be improvement on the modified Rankin Scale at three-months as analyzed by ordinal logistic regression.

Discussion If high-dose paracetamol will be proven effective, a simple, safe, and extremely cheap therapy will be available for many patients with acute stroke worldwide.

Key words: body temperature, functional outcome, inflammation, paracetamol, stroke

Introduction

Subfebrile temperatures and fever are common in the first hours after stroke onset. Within the first day after stroke onset, a third of the patients has a body temperature higher than 37.5°C. High body temperatures have been related to poor functional outcome and death (1). In the Copenhagen study, the risk of poor outcome doubled with every degree Celsius increase in body temperature measured within 12 h from stroke onset (2). Increased body temperature may be either a direct effect of stroke or of concurrent infections. Several mechanisms may account for the potential detrimental effect of high body temperature on clinical outcome. Animal studies have suggested that a rise in temperature results in increased ischemic damage through increased cerebral metabolic demands, increased blood–brain barrier permeability, acidosis, and an increased release of excitatory amino acids (3). Vice versa, induced hypothermia reduced infarct volume and improved functional outcome in animals (4). Lowering body temperature and prevention of fever are therefore likely candidates to improve functional outcome after stroke in human beings as well.

Based on observational studies, guidelines for the treatment of patients with acute ischemic stroke (5,6) or intracerebral hemorrhage (ICH) (7,8) advocate the use of antipyretic drugs, such as paracetamol (acetaminophen) in a daily dose of four-grams, in patients with a body temperature higher than 37.5 or 38.0°C. However, routine prescription of antipyretics may lead to unrec-
ognized infections. Paracetamol is one of the most commonly prescribed antipyretic drugs. Its antipyretic properties are probably conferred by potent inhibition of prostaglandin production in the central nervous system (9). Moreover, paracetamol is well tolerated by patients with acute stroke and has virtually no side effects in doses up to six-grams daily, although in patients with chronic liver failure this dose may lead to fatal complications (10).

In patients admitted with acute ischemic stroke, it was shown to reduce body temperature by about 0·3°C within four-hours after treatment onset when prescribed at a daily dose of six-grams (10).

In the first Paracetamol (Acetaminophen) in Stroke (PAIS) trial, a double-blind, placebo-controlled, randomized clinical trial of 1400 patients with acute stroke, paracetamol reduced body temperature at 24 h after start of treatment by 0·26°C [95% confidence interval (CI): 0·18–0·31]. Patients treated with paracetamol showed more improvement on the modified Rankin Scale (mRS) at three-months than those treated with placebo, but this difference was not statistically significant [adjusted odds ratio (OR) 1·21; 95% CI: 0·97–1·51] (11). In the 661 patients with a baseline temperature between 37·0 and 39·0°C, paracetamol reduced body temperature more effectively than in those with a baseline temperature lower than 37·0°C (0·30°C versus 0·19°C), increased the odds of improvement (OR 1·43; 95% CI: 1·02–1·97), and was associated with a 9% (95% CI: 1–16%; P = 0·02) absolute decrease in the risk of poor outcome, defined as a score on the mRS > 2. The relation between treatment with paracetamol and functional outcome was also found in the 1080 patients with a body temperature of 36·5°C or higher (OR 1·31; 95% CI 1·01–1·68). The large majority of these patients had a baseline temperature between 36·5 and 37·5°C, and would not have received paracetamol according to the aforementioned guidelines. Although the observed benefit of paracetamol in patients with temperatures higher than 36·5°C is biologically plausible, this should be interpreted with caution, as this concerns a subgroup analysis within a randomized clinical trial in which no overall statistically significant beneficial effect could be demonstrated. Confirmation of this observation in an independent study is therefore needed (12). For this reason, the aim of PAIS 2 is to assess the effect of high-dose paracetamol on functional outcome in patients with acute stroke and body temperature of 36·5°C or above in the first 12 h after stroke onset.

Methods

Design

The Paracetamol (Acetaminophen) In Stroke 2 (PAIS 2) trial (Fig. 1) is a multicenter, randomized, double-blind, placebo-controlled clinical trial of high-dose paracetamol.

Patients

Patients are eligible for inclusion if they have a diagnosis of ischemic stroke or ICH and a body temperature of 36·5°C or higher. Other inclusion and exclusion criteria are listed in Table 1.

Randomization

Patients are randomly allocated to paracetamol or an identical placebo. The study drug is provided in white paper boxes, with

![Fig. 1 The PAIS 2 trial logo.](image)
values. The randomization procedure will be simple. Patients who meet the inclusion criteria and who have given written informed consent are assigned a box containing the study medication. The box is labeled with a unique study number. The local investigator will have to complete a short web-based form to include the patient into the study. The anonymous patient data will be automatically added to the study database. An automatically updated log of the randomized patients will be available for each participating center.

Baseline data will include demographics, time of stroke onset, stroke severity [measured by the National Institutes of Health Stroke Scale (NIHSS)], body temperature at inclusion, pulse rate and blood pressure, results of CT scanning, and vascular history and vascular risk factors. In selected centers, a blood sample will be taken on admission and 24 h later, to assess markers of inflammation. During hospital stay (serious) adverse events will be reported to the trial office. Discharge data will include discharge destination, study compliance, body temperature and blood pressure at 24 h, laboratory findings at days 2–3, stroke subtype and NIHSS at discharge. Follow-up will be carried out by the trial office and will be conducted through telephone interviews. Outcome assessments will be made through structured interviews by experienced research nurses of the trial center who will be stationed at the trial office of the neurovascular division of the department of neurology of the Erasmus MC University Medical Center Rotterdam (Fig. 2). Patient data will be entered through a web-based form. Local investigators will be asked to fill out a one-page web-form at inclusion and a one-page web-form at one-week or at discharge. All data are stored in central database, which is continuously updated.

**Primary outcomes**

The primary outcome will be the shift on the score on the mRS at three-months. No dichotomization is necessary, as we will analyze shifts on the whole scale.

**Secondary outcomes**

Secondary outcome measures will include: poor outcome, defined as mRS > 2 at three-months; score on the Barthel index, Telephone Interview for Cognitive Status score, and Euroqol 5D score at three-months, body temperature and markers of inflammation at 24 h after start of treatment.

**Data monitoring and safety committee**

An independent data monitoring and safety committee (DMSC), consisting of two experienced neurologists and clinical investigators and an independent statistician, will meet at least annually. During the period of recruitment into the study, unblinded but strictly confidential interim analyses of in-hospital mortality and of any other information that is available on major outcome events, including serious adverse events, will be supplied every year to the chairman of the DMSC, along with any other analyses that the committee may request. In the light of these analyses, the DMSC will advise the chairman of the steering committee about continuation of the trial. These interim analyses will be prepared by the trial statistician, who is unblinded. However, the trial statistician is otherwise not involved with the execution of the trial as long as it runs.

**Statistical analyses**

Statistical analyses will be performed according to the intention-to-treat principle. The primary effect estimate will be the common OR of improvement on the mRS assessed by means of multiple ordinal logistic regression, and be expressed as an OR.
with 95% CI. In order to increase the power of the study, adjustments will be made for chance imbalances between prognostic factors, including stroke severity (NIHSS), stroke type, ischemic stroke subtype (lacunar versus nonlacunar), treatment with alteplase, previous stroke, atrial fibrillation, and diabetes mellitus.

We will test for heterogeneity of treatment effect across important clinical subgroups of patients: stroke type (ischemic vs. hemorrhagic stroke), severity (dichotomized at the median NIHSS score), ischemic stroke subtype (lacunar versus nonlacunar ischemic stroke), time of onset of treatment (within six-hours versus between 6 and 12 h after onset of symptoms), treatment with recombinant tissue plasma activator (rtPA) and body temperature (13,14).

**Sample size**

The study will be powered to detect a statistically significant shift in the distribution of the scores on the mRS at three-months, assuming an effect that leads to a 7% absolute increase in the cumulative proportion of patients with mRS between 0 and 2 in the paracetamol group, compared with patients on placebo. We base the expected distribution of outcome categories on the placebo arm of the PAIS trial (11). Figure 3 shows the expected distributions of mRS categories. A total study size of 1410 patients (2 x 705 patients) allows for a power (1-beta) of 85% to detect a significant difference in the scores on the mRS of the paracetamol group compared with the placebo group at a level of significance of 0.05 (15). The total study size that will be needed is rounded to 1500 patients.

**Study organization and funding**

PAIS 2 is an independent academic trial. This study is run by an executive committee that consists of members of the steering committee (SC) who are actually involved in carrying out the study on a daily basis (Table 2). The recruitment period will be 4.5 years. The central coordination is performed by the SC. They will meet at least once a year, and monitor the progress of the study. Decisions regarding continuation of the trial, amendments to the protocol, and publication of its results (taking into account the advice of the DMSC) will be taken by the SC. The SC strives for consensus decisions, but may have to settle for majority votes. The trial is funded by the Stichting Neurovasculair Onderzoek Rotterdam (Foundation for Neurovascular Research Rotterdam); additional support has been requested.

**Public disclosure and publication policy**

The investigators aim at public disclosure and publication of the research data in highly-ranked, international peer-reviewed, scientific journals. Results of this research project will be disclosed unreservedly. All authors will have to comply with the Vancouver protocol for authorship.

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**Table 2: PAIS 2 study group**

| Principal investigators | Diederik W. J. Dippel, neurologist, Erasmus Medical Center University Hospital, Rotterdam  
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| | H. Maarten A. van Gemert, neurologist, Meander Medical Center, Amersfoort  
| | A. H. C. M. L. (Tobien) Schreuder, neurologist, Atrium Medical Center, Heerlen  
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| | Ewoud J. van Dijk, assistant professor of neurology, Radboud University Nijmegen Medical Center, Nijmegen  
| Study coordinator | Inger R. de Ridder, junior researcher, Erasmus Medical Center University Hospital, Rotterdam  

The steering committee consists of the principal investigators, local principal investigators, other study group members, and the study coordinator. The executive committee consists of the principal investigators, study coordinator, and trial manager. The core writing committee consists of the principal investigators and the study coordinator. The writing committee consists of the core writing committee, the local principal investigators, and the other study group members.
Discussion

Several observational studies have demonstrated a strong relationship between increased body temperature in the first hours after symptom onset and poor functional outcome after stroke (1,11). It remains unclear whether this relation is causal, and more importantly, whether lowering of body temperature and prevention of fever increases the likelihood of a favorable functional outcome. In the first PAIS trial, treatment with paracetamol increased the odds of improvement at three-months in patients with a baseline body temperature between 37.0 and 39.0°C (OR 1.43; 95% CI: 1.02–1.97) and was associated with a 9% (95% CI: 1–16%, P = 0.02) absolute decrease in the risk of unfavorable outcome. Because these findings are based on subgroup analyses, confirmation is needed. We updated a Cochrane systematic review of temperature-lowering therapy for acute ischemic stroke with the results of PAIS (16). The results of the review are now dominated by the results of this study, and the estimate of the treatment effect leaves room for a substantial reduction in poor outcome (OR 0.93; 95% CI: 0.57–1.50). It should be noted, however, that this systematic review combined physical and pharmacological strategies to reduce body temperature and included several phase 2 studies that had a long time window after symptom onset for the start of treatment. The last issue is crucial because the association of body temperature with clinical outcome is likely limited to the first 12–24 h of stroke onset, and the amount of salvageable brain tissue will rapidly diminish over time (1). We have previously shown that paracetamol reduces body temperature in patients with acute stroke within four-hours after start of treatment (17). It seems therefore rational to include patients up to 12 h after onset of stroke symptoms.

Whether increased body temperature in acute stroke contributes to poor outcome or is just a marker of severe stroke remains unclear. Hyperthermia has been suggested to be an epiphenomenon of the extent of cerebral damage. Animal studies suggest that hyperthermia is associated with larger infarct size (18). Body temperature may also be increased because of stroke-associated infections and central dysregulation.

Increased body temperature may also be an indicator of local cerebral inflammation and neuronal tissue injury. Cyclooxygenase-2 (COX-2) isoenzymes play an important role in temperature regulation at the level of the hypothalamus (19). In animal studies, COX-2 mRNA and protein are up-regulated 12 to 24 h after the onset of focal cerebral ischemia (20). COX-2 is expressed in neurons and vascular cells at the border of the ischemic territory (21). COX-2 has also been found in the human brain after stroke (22). COX-2 inhibition reduces neuronal damage in animal models of focal and global cerebral ischemia (23). These observations raise the possibility that COX-2 reaction products, such as prostaglandin 2 (PGE2), contribute to cerebral ischemic injury, either directly, or through temperature elevation. It has been shown that paracetamol is effective in blocking cerebral COX-2 and lowering cerebral PGE2 production (24). This indicates the possibility of a direct neuroprotective effect of paracetamol in case of ischemic stroke. Early after ICH also, a strong proinflammatory response has been observed. Therefore, our hypothesis is that lowering cerebral COX-2 also has a neuroprotective effect in ICH.

With the PAIS trial, we showed that a large phase III trial with high-dose paracetamol is feasible in the Netherlands, although the recruitment rate was lower than expected. Fourteen hundred patients were included instead of the 2500 patients that were aimed for. Lessons learned from successful participating study centers in the PAIS trial were in particular that inclusion of patients has to be made as simple as possible. The PAIS 2 trial will therefore use a simple web-based randomization and data entry procedure. In addition, to enhance compliance, suppositories will be made available for rectal administration for the first 24 h, after which time paracetamol will be administered orally. With oral administration, peak plasma levels are reached after 30 mins to one-hour; with rectal administration after one-hour. Differences in pharmacokinetics between oral and rectal administration are therefore negligible. If oral administration is applied whenever possible, this reflects daily clinical practice best. This is the most important reason why intravenous administration of paracetamol has not been considered in this trial. In addition, intravenous administration is much more expensive and peak plasma levels are reached just slightly earlier (10–20 mins after administration) when compared with oral administration.

We use ordinal logistic regression to estimate a treatment effect on the mRS. A common OR for improvement on the scale will be estimated. Simulation studies have indicated that ordinal logistic regression is a more powerful method for analysis of trials with ordered categorical outcome data when compared with the more commonly used sliding dichotomy approach, and have proven its robustness against violations of the proportional odds assumption (25).

The PAIS trial also showed that treatment with high-dose paracetamol is safe. The number of adverse events was similar in the treatment and placebo groups. We will closely monitor the adherence to inclusion and exclusion criteria in order to prevent inclusion of patients at increased risk of liver failure.

In conclusion, PAIS 2 will be a simple but highly relevant clinical trial. The treatment strategy tested is safe and inexpensive. If high-dose paracetamol will be proven beneficial, a simple, safe, and extremely cheap therapy will be available for many patients with acute stroke worldwide.

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