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TREATMENT OF ACUTE ISCHEMIC STROKE IN PATIENTS WITH AND WITHOUT ATRIAL FIBRILLATION

De behandeling van het acute herseninfarct bij patiënten met en zonder boezemfibrilleren

PROEFSCHRIFT

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'I see nobody on the road', said Alice.
'I only wish I had such eyes', the King remarked in a fretful tone.
'To be able to see nobody. And at that distance, too!
Why, it's as much as I can do to see real people, by this light.'

Lewis Carroll,
Through the Looking-Glass

Aan mijn vader en grootvader,
die mij vanaf het begin hun liefde
voor de wetenschap hebben bijgebracht

Contents

Chapter 1

General introduction

Introduction

In the Netherlands, 21.000 patients per year are struck by an ischemic stroke. Stroke is a major cause of death and an important cause of hospital admission and long-term disability. Although case-fatality rates have steadily declined over the past 25 years, this is mainly due to improved general stroke management but not to new strategies to halt or reverse the harmful effects of brain ischemia. Stroke as a clinical syndrome was recognized even before the time of Hippocrates, but it was not until 1995 that the first effective treatment, thrombolysis with recombinant tissue plasminogen activator, became available. However, this treatment can be applied only to a small percentage of patients. For all others cases no effective treatment is available.

In this thesis I shall describe and discuss two types of treatment for acute ischemic stroke, that were studied in randomized controlled trials. Firstly, we evaluated the safety, efficacy and pharmacodynamics of the use of a hemoglobin solution, diaspirin cross-linked hemoglobin (DCLHb) in a multicenter trial of patients with acute ischemic stroke. Secondly, we studied the effect of heparin in patients with acute ischemic stroke in combination with atrial fibrillation (AF) entered in a large clinical study, the International Stroke Trial (IST). We had specific interest in the prevention of early stroke recurrence.

Contents of this thesis

Chapter 2 describes the rationale for using DCLHb and heparin in the treatment of acute stroke. The first part provides the background of the use of hemoglobin solutions with a more in-depth look at DCLHb in particular and reviews the available information, mostly based on experimental animal models. It continues with an evaluation of clinical studies that assessed the merits of anticoagulant treatment in acute stroke in combination with AF and justifies why we analysed a subgroup of 3169 acute stroke patients with AF from the International Stroke Trial (IST).

Chapter 3 covers the design, conduct and main results of the DCLHb in acute ischemic stroke (DIAS) trial in which we studied the safety and efficacy of the use of DCLHb in acute ischemic stroke in humans.

In *Chapters 4,5 and 6* we attempt to elucidate the pharmacodynamics of DCLHb and try to explain why DCLHb was not successful in reversing or halting the harmful effects of cerebral ischemia. In chapter 4 the induced elevation of blood pressure by DCLHb in acute ischemic stroke patients is described. In chapter 5 the role of vasoactive hormones in general and ET-1 in particular was explored by measuring BP and ET-1 plasma concentrations before and during administration of DCLHb. Chapter 6 focuses on the cardiovascular effects of DCLHb in our population.

Chapter 7 provides the introduction to the second part of this thesis. One of the feared complications in the acute phase of ischemic stroke is early recurrence of stroke. This chapter aims to review the definition, epidemiology of and risk factors i.e. AF for early recurrent stroke and to focus on the benefits and risks of antithrombotic therapy in the prevention of this complication.

In *chapter 8* the additional diagnostic yield of long term Holter monitoring compared to repeated ECG recordings is calculated, in the identification of paroxysmal AF in the acute phase of stroke.

Chapter 9 describes the effects of treatment with heparin in 3169 patients with atrial fibrillation and acute ischemic stroke from the IST.

Finally *chapters 10 and 11* provide a general discussion and summary of the studies presented in this thesis.

Chapter 2

Treatment of acute ischemic stroke: the background and rationale of the studies presented in this thesis

Introduction

The first adequate account of 'apoplexy' or stroke appeared in the Hippocratic writings. Apoplexy in Greek meant *struck with violence*, resulting in paralysis of some or all parts of the body. According to Hippocrates the causes of apoplexy varied, but in general, related to heating of the blood vessels of the head, which thereby attracted phlegm or caused the flow of black bile to the head. In the centuries to follow, many descriptions and concepts of apoplexy were presented, but little was added to the ancient descriptions. In the Eighteenth Century, Morgagni was the first to correlate pathological lesions on one side of the brain to clinical signs on the opposite side of the body. In 1828 Abercrombie analyzed into great detail forty cases of apoplexy and suggested possible causes, amongst which were narrowing of the arteries.[1]

Animal experimentation began early in the systematic investigation of cerebrovascular disease. In 1836 Cooper used the method of extrinsic ligation of the carotid and vertebral arteries in dogs, and by 1847 Flourens introduced intravascular injection techniques. It was also in 1847 that Virchow first reported occlusion of arteries of the brain by thrombi that seemed to have originated in the heart. Since then it became more and more apparent that obstruction to blood flow to the brain caused signs of apoplexy. All subsequent animal experiments on stroke have been variations of these two basic themes, either embolism or extrinsic compression of cerebral vessels. The next most significant advance in the study of apoplexy came with the elucidation of areas of vascular supply to the brain and in the demonstration of the common sites of lesions in the brain and specific symptoms following vascular occlusion. This was largely carried out by Henri Duret in 1873.

In the Sixteenth Century the various form of treatment generally used in clinical medicine were also used in neurological diseases, and patients with apoplexy were treated with phosphorus, purgation or blood-letting. Since then, there has been very little progression.[1] In the Twentieth Century, causes and consequences of stroke were largely elucidated, and strategies to for secondary prevention of stroke with aspirin and heparin proved to be successful, but there has been a almost complete lack of progression in finding a treatment for stroke.

In the past decades numerous compounds have been shown to reduce infarct volumes in animal stroke models. Unfortunately these results could not be reproduced in human trials, except for thrombolysis with tissue plasminogen activator (tPA) within three hours after the onset of symptoms in carefully

selected patients has convincingly shown to improve outcome. This treatment however, is applicable to a small percentage of patients only.

Background and rationale for using a blood substitute, diaspirin cross-linked hemoglobin (DCLHb) in acute ischemic stroke

Viral contaminations, recurring shortages, cross-matching, typing, limited shelf life and problems in storage are some of the reasons for developing new oxygen carriers as a safe and effective alternative to blood. These blood substitutes can temporarily augment the oxygen-carrying capacity of the patient's red blood cells.

Diaspirin cross-linked hemoglobin (DCLHb, HemAssist®) was originally developed as a blood substitute. When it was discovered that DCLHb enhanced oxygen delivery while increasing blood pressure and organ perfusion, studies with animal stroke models were undertaken and showed favorable results. This eventually led to the start of our safety study: DCLHb in Acute Ischemic Stroke (DIAS).

History of blood substitutes

The search for an alternative to blood began almost as soon as people realized blood circulates through the body. Physicians performed the first successful blood transfusion into a human in 1667, but stopped the practice when subsequent transfusion recipients died. Researchers have infused solutions containing hemoglobin, extracted from red blood cells with varying degrees of success since 1868.

A breakthrough in the treatment of blood loss came in 1883, when it was found that blood pressure could be restored by infusion of with Ringer's solution. Further blood-transfusion research stalled until scientists had a better understanding of issues critical to successful transfusion, including coagulation, bacterial contamination and the incompatibility of different blood types. By the early 1920s, health-care professionals widely practiced transfusion therapy, and interest in the search for a blood substitute waned. World War II rekindled interest in research on blood and blood storage, culminating in the establishment of American Red Cross blood banks in 1947.

As blood must be refrigerated and can be stored for no more than six weeks it cannot be stockpiled for immediate use in disaster relief or for combat-casualty

care, nor can it be readily carried aboard ambulances or by field medical personnel. The need for typing and cross-matching of patients' blood means blood transfusions rarely can be given when such therapy might have its greatest value, during the first "golden hour" after a trauma event. Type O negative units are saved for such emergencies, but are always in short supply. It was during the Vietnam conflict, that these limitations on the use of blood for modern combat casualty care became apparent, and research on both hemoglobin solutions and synthetic oxygen carriers was renewed.

Finally, safety remains a concern. Viral inactivation of red blood cells is not yet possible. Immune suppression and transfusion reactions, though often minor, still occur in 1 in 10 transfused patients. In the mid-1980s, the rising incidence of HIV and hepatitis infections, led scientists to intensify their work to improve the safety of the human blood supply.[2]

Physical, chemical and pharmaceutical properties and formulation

DCLHb is prepared from packed red blood cells. The erythrocytes are lyzed to release hemoglobin and filtered to produced stroma-free hemoglobin and to remove contaminating viruses. The purified human hemoglobin is chemically stabilized to enhance intravascular persistence and oxygen delivery. Stabilization of hemoglobin as a tetrameric protein is achieved by cross-linking the α subunits by means of a reaction with bis(3,5-dibromosalicyl) fumarate (DBBF). The heat treatment that follows results in virus inactivation. To achieve physiologic compatibility, the pH is adjusted and the final DCLHb solution is prepared in a balanced electrolyte solution.

The solution has the following properties at 37°C: pH 7.4, oncotic pressure of 42-44 mmHg (hyperoncotic compared with whole plasma), oxygen P50 affinity of 32 mmHg and a viscosity of <1.5 centistokes. Oxygen equilibrium binding measurements, as well as binding kinetic studies demonstrate that oxygen transport and release by DCLHb solutions is comparable to that of fresh human red cells containing an equivalent amount of hemoglobin.[3]

Nonclinical studies regarding the pressor and perfusion effects

Although the exact mechanism of action was unknown, historically hemoglobin solutions have been associated with a vasopressor action in both animals and man.[4,5] Consistent with this action DCLHb produces a predictable, rapid and sustained rise in mean arterial pressure (MAP), primarily through an increase in diastolic pressure. The pressure effect is reversible, non-linear, increases with the dose of DCLHb , and reaches a maximum at higher concentrations.[6,7] The

pressure increase can be readily controlled by standard anti-hypertensive agents, such as nitroglycerine, labetolol, nicardipine and prazosin.[8]

In a reciprocal response to the rise in blood pressure (BP), the heart rate (HR) decreases. Contrary to typical hypertensive effects of catecholamines, the pressor response of DCLHb is accompanied by a rapid increase in perfusion as indicated in organ-flow measurements in hypervolemic (volume-load)[7,9] as well as hypovolemic (hemorrhagic), animal studies.[10] In the latter, perfusion to vital organs such as the heart and brain exceeded pre-hemorrhage levels.[11]

Mechanism of the pressor effect

The pressor response observed after hemoglobin infusion is not a unique property of DCLHb, but rather a general property of hemoglobin solutions. When conscious swine were given an infusion of 2000 mg/kg unmodified pig stroma-free hemoglobin, this produced a rapid rise in MAP with a temporal profile and magnitude similar to DCLHb.[12]

The pressor effect is maintained when administered to cervical spine-sectioned rats, suggesting that DCLHb primarily interacts with the peripheral vascular autoregulatory system, rather than the central nervous system. A simple explanation of the pressor effect would be that DCLHb stimulates the release of catecholamines from the adrenal glands. When administered to bilateral adrenal demedullated rates, however, DCLHb produced a pressor and heart rate response similar to that observed after infusion into normal rats.[13]

At the local level, vascular tone is normally maintained, at least in part, as a balance between the vasoconstrictive effects of endogenous catecholamines and endothelin (ET), and the vasodilatory effects of endothelium-derived relaxant factor, which has been identified as nitric oxide (NO).[14,15] Hemoglobin in general, and DCLHb in particular have been shown to interact with NO and to induce contraction of vascular smooth muscle cells.[16,17] Studies in rats have shown that infusion of DCLHb leads to an increase of ET-1 levels along with an increase in BP.[18,19] This pressor effect was significantly attenuated by pretreatment with the ET-receptor antagonist, BQ-123, thus providing evidence for the hypothesis that the pressor effect of DCLHb is mediated, at least in part, through the increase of ET-1 levels.[20]

A study in rats with various pharmacological agents DCLHb potentiates adrenergic vasoconstriction, presumably through sensitization of both α_1- and α_1-adrenoceptors in the peripheral vascular system.[21,22]

Perfusion studies

A rapid increase in BP as a result of vasoconstriction, could result in decreased tissue perfusion resulting in tissue ischemia and insufficient oxygenation. However, this effect does not occur with DCLHb as evidenced by several studies. While the commonly used pressor agents, such as the sympathomimetic amines, at best, divert blood from less vital to more vital tissues, DCLHb increases perfusion to above baseline levels in almost all organs, including the brain. The ability of DCLHb to increase tissue perfusion has been demonstrated in several volume-load and hemorrhage resuscitation animal studies.

Administration of DCLHb in rats produced significant increases in blood flow to the heart, gastrointestinal tract, portal system and skin. The blood flow to kidney and brain increased slightly, but not significantly, while blood flow to the musculoskeletal system was not affected.[7]

Rats that were lethally hemorrhaged demonstrated superior whole body perfusion after a small volume of DCLHb was infused. Infusion of 20% of the shed blood volume restored brain perfusion to pre-hemorrhage baseline levels and infusion of 50% of the shed blood volume the perfusion to the brain increased to far above baseline values. Comparative studies have shown that neither autologous blood nor clinically used oncotically matched colloids have the same beneficial effect.[11]

DCLHb in animal stroke models

As DCLHb offered the potential advantage of enhancing oxygen delivery while increasing blood pressure and organ perfusion, this naturally led to experiments with animal stroke models.

Cerebral blood flow (CBF) was assessed in a stroke-reperfusion model in rats undergoing temporary middle cerebral artery (MCA) occlusion. DCLHb was administered to achieve hemodilution and maintain hematocrits (HCT) of 37%, 30%, 32%, 16% or 9%. Control animals received donor blood to achieve a 44% HCT. After 10 minutes of ischemia, brain sections were evaluated for CBF with radioactive microspheres. CBF to the ischemic area increased significantly in the DCLHb treated groups at HCT of 30% and lower. The greatest increase in CBF was observed in the non-affected hemisphere in the 30% and lower HCT groups. The same experiment showed that at HCT 30% and lower, oxygen delivery to the affected hemisphere increases significantly.[23]

The effect of hemodilution with DCLHb on brain injury and edema was assessed during middle cerebral artery (MCA) occlusion (180 minutes) and

reperfusion (120 minutes) in rats. Prior to MCA occlusion animals received either donor blood to maintain a HCT of 44%, 10% albumin to reduce HCT to 30%, or DCLHb to reduce HCTs to 30 or 9%. The percentage of ipsilateral hemispheric brain injury was greater in the blood (42±4%) and albumin (38±3%) groups versus the two DCLHb groups: 27±4% in the 30% HCT group versus 18±3% in the 9% HCT group. Cerebral edema, assessed by microgravimetry, was less in all hemodiluted groups versus the blood control group.[24]

In a similar reversible stroke model, spontaneously hypertensive rats were given either donor blood to maintain a HCT of 45%, or DCLHb to achieve hemodilution and maintain HCTs of 30%, 16% or 9%. In addition, at HCTs of 30 and 16%, some groups of animals were maintained normotensive (by giving DCLHb initially as an exchange, followed by a slow bolus), while other corresponding groups were allowed to become hypertensive (by giving DCLHb initially as a rapid bolus, followed by an exchange). Both edema and brain injury, as a percentage of the ischemic hemisphere were significantly reduced in a dose-dependent fashion 2 hours after DCLHb infusions (31±4% in 30% HCT group, 20±3% in 16% HCT group, 19±4% in 9% HCT group) compared to the blood 44% HCT group (43±5%). In HCT-matched groups, the most effective reductions in ischemic injury occurred when DCLHb was given in a manner that allowed the inherent hypertensive response to be manifested but reduced in all the groups of rats treated with DCLHb. Edema was not affected by hypertension.[25]

Cerebral edema (measured via MRI) and brain tissue oxidation (measured via cytochrome aa_3), were assessed in cats given either DCLHb 15ml/kg, Dextran-40, perfluorocarbon (PFC) emulsion or no treatment 3 hours after stroke induction (MCA ligation plus 2 hours of carotid artery clamping followed by 1 hour of stabilization). Post-stroke cerebral edema in the DCLHb-treated animals was 74% less than the Dextran-treated animals, 52% less than the PFC-treated animals and 73% less than the non-treated animals. DCLHb-treated animals showed relative tissue oxidation of the affected hemisphere that was significantly greater than the other groups except for the PFC group. However, the PFC group was maintained at an FiO_2 of 1.0 while DCLHb-treated animals received an FiO_2 0.4.[26]

Finally, in a rabbit model of spinal stroke, DCLHb treatment before clamping of the aorta, significantly reduced the likelihood of paralysis, thus improving functional outcome.[27]

In the early stage of cerebral infarction the ischemic area is thought to consist of a central core of densely ischemic tissue surrounded by a rim with less dense

ischemia, known as the ischemic penumbra. This penumbra is characterized by metabolic and ionic disturbances and a loss of functional activity, but with preserved structural integrity and the possibility to recover when blood flow is restored and/or so-called neuroprotective agents are administered. Restoration of blood flow is essential for recovery and reduces the volume of the infarct if instituted with undue delay. However, reperfusion may paradoxically lead to further tissue damage, which has been termed reperfusion damage. Nitric oxide (NO) is a free radical with vasodilatory effects, that plays a dual role in brain ischemia. NO is protective or destructive depending on the stage of evolution of the ischemic process and on the cellular source of NO.[28] Since hemoglobin in general and DCLHb in particular is an extremely effective scavenger of nitric oxide (NO), inactivation of this endogenous vasodilator is one of the most likely mechanisms by which hemoglobin solutions induce a rise in BP.[15,18] thereby possibly inhibiting NO-related neurotoxicity.[18,29]

In summary, in animal stroke models, hemodilution with DCLHb induced a hypertensive response and resulted in significant reductions in the extent of the brain injury and improved clinical outcome. It was anticipated that similar effects would occur in patients suffering acute ischemic stroke.

Background and rationale for using heparin in acute ischemic stroke associated with atrial fibrillation

Regardless of the lack of convincing evidence of safety or efficacy physicians have been administering heparin to stroke patients for more than 50 years. Heparin is given to promote early clot lysis and to prevent thromboembolic events such as deep venous thrombosis, pulmonary embolism, stroke progression and early stroke recurrence.[30] Since the recurrence rate is presumed by some to be even larger in patients with atrial fibrillation (AF),[31] clinicians are even more inclined to treatment with heparin in ischemic stroke in the presence of AF.[32,33] However, there are no conclusive data on this recurrence rate and on the effects of heparin to prevent these recurrences.

Early stroke recurrence

One of the feared complications in the acute phase of ischemic stroke is early recurrence of stroke.[34,35] Although nearly all patients who survive an acute ischemic stroke eventually show some degree of clinical improvement, neurological symptoms are often unstable during the early phase. The diagnosis of early recurrent stroke is not always easy in patients with acute ischemic stroke, since 20-50% of these show spontaneous fluctuations,[36-38] some of which may be caused by repeated embolism, but many of which have other causes, including systemic disorders, such as myocardial ischemia and metabolic disturbance.[39]

Very few studies have attempted to define recurrent stroke. Some have included worsening of a preexisting deficit, while others have accepted only new deficits that occurred in a different anatomic or vascular territory or were of a different stroke subtype than the index stroke. [32,40-44] It is obvious that the lack of an uniform definition of recurrent stroke strongly influences the existing data. The incidence of, risk factors for and prevention of early recurrent stroke are discussed at length in chapter 7.

Treatment of acute ischemic stroke with heparin

Several surveys have shown that heparin is widely used to treat acute ischemic stroke, but without a clear consensus on whether it is effective or not. For instance a survey among physicians in the United Kingdom about 45% of physicians used heparin regularly in patients with acute ischemic stroke, whereas only 10-20% thought that heparin had been proven to be effective.[33,45]

In the past few years several 4 randomized controlled trials, of which the primary aim was to demonstrate a beneficial effect on stroke outcome of anticoagulation in acute ischemic stroke have been reported All these trials also included patients with cardioembolic stroke. The only trial that showed a positive effect was published in 1995 by Kay et al.[46] In this study, 312 Chinese patients with acute ischemic stroke were treated during 10 days with subcutaneous low molecular weight heparin (LMWH), either low-dose or high-dose, or placebo. The main measure of outcome, clinical condition at 6 months, was significantly improved in both actively treated groups. Early recurrent stroke during the treatment period occurred in 3 patient receiving the high-dose, 3 in the low-dose and 6 in the placebo group. Early hemorrhagic transformation was found in 5 patients in the high-dose group, 7 in the low-dose, and 9 of the placebo patients. The risk of recurrent stroke per day during the treatment period was 0,1%, 0,2% and 0,5% per day.

The 3 trials that failed to show a positive effect from heparin were the TOAST-trial,[47] the IST [48] and the FISS-bis study.[49] In the trial of ORG 10172 in Acute Stroke Treatment (TOAST),[47] danaparoid administered intravenously was tested against placebo in 1275 patients with acute ischemic stroke, and turned out not to be associated with an improvement in favorable outcome at 3 months. The rate of recurrent stroke during the 7 day treatment period was 1,5% for both treatment groups, equaling a risk of 0.21% per day. In patients with AF the recurrence rate did not differ from those in sinus rhythm. In the FISS-bis study LMWH, again either low- or high-dose was tested against placebo in 766 patients, treated during 10 days, starting within 24 hours of stroke-onset, also showed no positive effect from heparin.[49] The International Stroke Trial (IST) [48] was the largest study to evaluate heparin in acute stroke. A total of 19435 patients were randomized to receive heparin (5000 or 12500 IU s.c. twice daily) or to "avoid heparin" and in a factorial design half were allocated aspirin and half to "avoid aspirin". Treatment was started within 48 hours after stroke onset and continued for 14 days or until hospital discharge. At 6 months neither heparin regimen offered any clinical advantage. The 14 day recurrence rate for all patients was 2,9% (0.21% per day) in the heparin treated group vs. 3,8% (0.27% per day) in the "avoid heparin" group, but there was an equally large increase in hemorrhagic strokes in the group treated with heparin.

A recent systematic review by Gubitz et al[50] included 23.427 patients (most of whom were from the trials mentioned earlier) also showed that immediate anti-coagulant therapy in patients with acute ischemic stroke is not associated with net short- or long-term benefit, as the advantage of fewer recurrent ischemic strokes is completely offset by a similar sized increase in hemorrhagic strokes.

Stroke and atrial fibrillation

Non-valvular (non-rheumatic) AF is the most common cardiac arrhythmia, and the prevalence of AF in developed countries increases rapidly with age.[51] In patients aged 50 to 59 years, it occurs in only 0.5% of the population.[52] Between 60 and 69 years, the prevalence is about 3.8% for men and slightly less for women, in those older than 70 years, the estimated prevalence is 9%.[53] In a community-based Minnesota study, 16.1% of men and 12.2% of women older than 75 years had AF.[54] A Dutch study of patients seen in a general practice setting confirms these findings.[55]

AF is found in about 15% of all strok\e patients [56,57], and is the most common cause of cardioembolic stroke as well as an independent and powerful risk factor for ischemic stroke and increased mortality.[53] In the Framingham Cohort

Study, the risk of stroke was 5.6 times greater in patients with AF than that in comparably aged patients in sinus rhythm.[57].

In developing countries, rheumatic heart disease accounts for most cases of AF, and the predominant incidence is in the young. In Western societies, however, when AF occurs in the young it is usually an isolated phenomenon, without predisposing structural heart disease, hypertension, or diabetes. These patients, usually younger than 60 years, have what is termed *lone atrial fibrillation* and are at low risk for systemic embolism.[58]

The overall risk of stroke among patients with AF without prior stroke or transient ischemic attack (TIA) is about 4% per year.[59] The risk of developing a stroke varies with age. The cumulative incidence of stroke among patients 60 years or younger with lone AF is not significantly different from that in a control population matched for age and sex: 0.5%/y.[60] In the elderly group, however, the risk is much higher, often exceeding 10%/y.[61]

Following an initial stroke patients are at an increased risk for recurrent cardioembolic stroke. The reported risk of recurrent stroke varies between 10 and 20% during the first year[62-66] and the risk of very early recurrence has been investigated in several studies,[31,34,42,43,63,64,67-69] and varies between 0.1% and 1.3% per day during the first two weeks after the initial event.[31,44,63,64,67,68] The consequences of these embolic strokes are often devastating, with either death or persistent neurological deficits in 40-70% of the affected patients.[42,64,67,70-72]

Primary prevention of stroke in patients with atrial fibrillation

Five studies have investigated the effect of anti-coagulation on the primary prevention of stroke in patients with AF. These studies were independently designed and include the Atrial Fibrillation, Aspirin, Anticoagulation Study from Copenhagen, Denmark (AFASAK),[71] the Stroke Prevention in Atrial Fibrillation (SPAF I) study,[73] the Boston Area Anticoagulation Trial in Atrial Fibrillation (BAATAF),[74] the Canadian Atrial Fibrillation Anticoagulation (CAFA) study,[75] and the Veterans Affairs Stroke Prevention in Non-rheumatic Atrial Fibrillation (SPINAF) study.[76] Patients requiring anticoagulation for other reasons or having contraindications to warfarin or aspirin were excluded. The average length of follow-up ranged between 1.2 and 2.3 years. All trials, except the Canadian trial, were terminated early because of the benefit demonstrated with warfarin. The Canadian trial was terminated because of the definitive results of the other studies.

In a formal pooled analysis of these trials, conducted collaboratively by the principal investigators of each of the trials,[59] the value of warfarin was

consistent among trials and, in aggregate, decreased the risk of stroke by 68% (4.5% to 1.4%/y) with virtually no increase in the frequency of major bleeding (the rates were 1.2%, 1.0%, and 1.0%/y for warfarin, aspirin, and placebo groups, respectively). It was further determined that increasing age and a history of hypertension, diabetes, and previous TIA or stroke were independent risk factors for developing a stroke among patients taking placebo. For patients younger than 65 years without these risk factors, even without anticoagulation, the stroke risk was 1%/y. These patients would not benefit from warfarin therapy. All other warfarin-eligible patients would benefit from warfarin treatment (event rate reduction from between 3.5%-8.1%/y to 1.1%-1.7%/y). Aspirin was evaluated in 2 of these studies but in different doses: 75 mg/d for the AFASAK study[71] and 325 mg/d for SPAF I.[73] In the BAATAF study,[74] patients in the control group were allowed to take 325 mg/d of aspirin. Both the CAFA[75] and the SPINAF[76] studies excluded patients who used aspirin or nonsteroidal anti-inflammatory drugs.

In the AFASAK study, the incidence of thromboembolic complications and vascular mortality among patients taking aspirin was not significantly different from the incidence of these complications in the placebo group. In the SPAF I trial, aspirin use was associated with a 42% reduction in stroke. In the BAATAF study, patients in the control group were allowed to use aspirin, but no benefit was seen. Overall, the reduction of stroke afforded by aspirin compared with placebo was 36%.[59] Hence, the primary prevention trials proved warfarin's superiority over both aspirin and placebo.

Secondary prevention of stroke in patients with atrial fibrillation

The only published randomized controlled secondary prevention trial is the European Atrial Fibrillation Trial (EAFT).[66] The cohort consisted of 1007 patients from 108 centers with non-rheumatic AF with a recent TIA or minor ischemic stroke. A total of 669 warfarin-eligible patients (group 1) were randomized to either open anticoagulation or further randomized to double-blind treatment with either 300 mg/d of aspirin or placebo. The 338 patients with contraindications to anticoagulation (group 2) were randomized to receive only aspirin or placebo. The main outcome measures were death due to vascular disease, any stroke, myocardial infarction, or systemic embolism. Patients with chronic and poorly controlled hypertension, history of hemorrhagic cerebral infarction, retinopathy, chronic alcoholism, non-compliance, or refusal to use anticoagulants were not included in the study.

During a mean follow-up of 2.3 years, the annual rate of outcome events was 8%/y in patients in the anticoagulant group and 17%/y in the placebo group (in

group 1). Warfarin use reduced the risk of stroke from 12%/y to 4%/y (66% reduction). Among all patients assigned to aspirin, the incidence of outcome events was 15%/y compared with 19%/y among the patients receiving placebo (in group 2). The incidence of major bleeding complications was low in this study: 2.8%/y in the anticoagulant group, 0.9%/y in the aspirin group, and 0.7%/y in the placebo group. Fatal intracerebral haemorrhage occurred in 3 patients: 1 in the placebo and 2 in the aspirin group.

This study shows that in patients with non-rheumatic AF and recent TIA or minor stroke, anticoagulant treatment reduces the risk of recurrent stroke by two thirds. The incidence of recurrent stroke was 12%/y in the placebo group, almost 3 times as high as in the placebo group of the primary prevention trials.[59] The EAFT does not provide information on the balance between the risk and benefit of anticoagulant therapy in the early period after onset of symptoms.

Treatment with heparin of acute ischemic stroke associated with atrial fibrillation

Given the high efficacy of anticoagulation and the reported risk for early recurrence of 0.1-1.3% per day in the first 14 days after the initial event[63,67,68,77] should treatment be started as soon as possible? The risk of hemorrhagic transformation of the cerebral infarct has been stressed in patients with cardioembolic stroke.[62] The dilemma whether to withhold anticoagulants for a few days or start treatment immediately is a long-standing subject for debate. Some have recommended withholding anticoagulants during the first few days after suspected stroke, especially if the infarct is large, to prevent hemorrhagic transformation.[78]

To our knowledge there have been only 2 studies that focused solely on treatment with anticoagulants in the acute phase of an ischemic stroke of presumed cardioembolic origin, mostly AF. In 1983 an open randomized study compared immediate treatment with intravenous UFH to delayed (14 days) anticoagulation in patients with cardioembolic stroke. 45 Patients were included and of the 24 patients randomized to immediate heparin only 14 had AF. The study was terminated prematurely because of 2 recurrent strokes and 2 asymptomatic hemorrhagic transformations occurred in the control group. In a recent study Berge et al investigated whether low-molecular-weight heparin (LMWH, dalteparin 100IU/kg sc bid) is superior to aspirin for the prevention of recurrent stroke during the first 14 days in patients with AF. Treatment was started within 30 hours of stroke onset and cerebral tomographic (CT) scanning was mandatory before inclusion to exclude intracranial hemorrhages. AF had to be present on admission or documented within the 24 months before the stroke.

The results for the primary outcome event, recurrent ischemic stroke within the first 14 days were: 19/244 (8,5%) in dalteparin-allocated patients vs. 17/225 (7.5%) in aspirin allocated patients. The secondary events such as symptomatic and/or cerebral hemorrhage, progression of symptoms within the first 48 hours, death and functional outcome at 14 days or 3 months also revealed benefit nor harm of dalteparin. The authors concluded that while the data could not provide any evidence that LMWH is superior to aspirin in this setting, the study could not exclude the possibility of smaller, but still worthwhile effects of either of the trial drugs. The trial was also not large enough to reliably estimate a significant increase in cerebral hemorrhage on dalteparin.

Since 3169 patients with AF were randomized in the IST[48], we decided to study this subgroup in more detail. Even though this is a subgroup analysis, we considered it worthwhile still since many questions regarding AF in association with acute ischemic stroke are unanswered. We aimed to describe the effect of AF on early case fatality and the risk of recurrent stroke. We also wished to identify the main causative factors of the early case fatality, and study the effect of treatment with heparin compared with "no heparin" on these events.

Diagnosis of paroxysmal atrial fibrillation in patients with acute ischemic stroke

Both persistent and paroxysmal AF are a major source of cardiogenic embolism, thus an independent risk factor for acute ischemic stroke.[53,57,59] The diagnosis of AF has therapeutical consequences since several large studies have demonstrated the superiority of warfarin compared to anti-platelet treatment in primary and secondary stroke prevention studies.[66,71,73-76] In acute stroke patients the ECG is sufficient to detect patients with persistent AF, but generally unable to diagnose paroxysmal AF.

Two retrospective studies failed to show any additional value of Holter monitoring.[79,80] One prospective study in 184 consecutive stroke and TIA patients did find a larger number of patients with (paroxysmal) AF with 48-hour automated arrhythmia monitoring (55 patients had an additional Holter registration) than with the combination of anamnesis, physical examination and ECG.[81] The current practice in the Netherlands is to perform ambulatory ECG monitoring in those patients with a history of cardiac disease, suspicious

standard ECG or complaints of palpitations or loss of consciousness preceding or during the stroke.[82] It is not known how many patients with PAF are missed with this practice. Other authors do recommend making a Holter in all stroke patients.[83]

As a part of the safety study we monitored cardiac function by means of 84 hour Holter recording in the DCLHb in Acute Stroke Trial (DIAS) . We wanted to determine whether ECG-monitoring for 84 hours instead of repeated ECG's, 3 in 7 days, increased the likelihood of diagnosis of PAF in patients with acute ischemic stroke.

The main questions addressed in this thesis

Part 1

- Is DCLHb safe when used in the acute phase of ischemic stroke? Are there indications that it is effective in halting or reversing the ischemic damage caused by the stroke?
- DCLHb causes the BP to rise. Is this increase in BP tolerated by these stroke patients?
- What are the specific cardiac effect of DCLHb?
- What is the role of various vaso-active hormones, such as catecholamines and ET-1, in the pressor effect of DCLHb?

Part 2

- What is the definition of early recurrent stroke? How often does it occur and what are the risk factors associated with early recurrences? Do acute ischemic stroke patients with AF have a higher chance of early recurrent stroke? What are the risks and benefits of antithrombotic therapy in the prevention of this complication.
- What is the extra yield of long-term Holter-monitoring in the diagnosis of PAF?
- How does the risk of early death and recurrent stroke and effect of heparin in 3169 patients with acute ischemic stroke and atrial fibrillation in international stroke trial compare to these risks in patients in sinus rhythm?

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Chapter 3

Design, conduct and main results of the DIAS trial: a controlled safety study of a hemoglobin based oxygen carrier, DCLHb, in acute ischemic stroke

Adapted from: Saxena R, Wijnhoud AD, Carton H, Hacke W, Kaste M, Przybelski RJ, Stern KN, Koudstaal PJ. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. Stroke 1999; 30:993-996.

Abstract

Diaspirin cross-linked hemoglobin (DCLHb) is a purified cell-free human hemoglobin solution. In animal stroke models it produced both an increase in blood pressure and tissue perfusion and a significant reduction in the extent of brain injury. The primary objective of this study was to evaluate the safety of DCLHb in patients with acute ischemic stroke.

This multi-center, randomized, single-blind, controlled safety trial consisted of 3 parts. Twelve doses of 25, 50 and 100mg/kg DCLHb or equal volumes normal saline were administered over 72 hours to 85 patients with acute ischemic stroke in the anterior circulation, within 18 hours of symptom onset.

DCLHb caused a rapid rise in mean arterial blood pressure. The duration of which was dose-dependent. The pressor effect was not accompanied by complications, nor an excess need for anti-hypertensive treatment. All laboratory abnormalities were clinically asymptomatic and disappeared within a week. Two patients, in the 100 mg/kg group, had an adverse event which was possibly drug-related: one of fatal brain and pulmonary edema, the other of transient renal and pancreatic insufficiency. Jaundice and hemoglobinuria were minor adverse drug reactions that predominantly occurred in the 100 mg/kg group. Although patients in the DCLHb group tended to have more severe strokes at baseline than the control group, multivariate logistic regression analysis showed that a severe stroke at baseline (odds ratio (OR) 20.9;confidence interval (CI) 4.1-102.4) and treatment with DCLHb (p=0.015; OR 3.9; CI 1.4-12.0) were independent predictors of a worse outcome (Rankin 3-6) at 3 months.

In conclusion, outcome scale scores were worse in the DCLHb group and more serious adverse events and deaths occurred in DCLHb-treated patients than in controls. We recommend that additional safety studies be performed, preferably with a second generation, genetically engineered hemoglobin.

Introduction

Diaspirin cross-linked hemoglobin (DCLHb) is a cell-free hemoglobin based oxygen carrying solution. In animal studies DCLHb causes an increase in blood pressure (BP) and tissue perfusion.[1,2] In animal stroke models, hemodilution with DCLHb resulted in significant reductions in the extent of the brain injury and cerebral edema. These reductions were most profound when DCLHb was given in a manner that induced its inherent hypertensive response.[3,4] The viscosity of DCLHb is lower than that of whole blood and it offers the potential advantage of hemodilution without a decrease in oxygen delivery.[5] In addition, experimental data suggests that DCLHb scavenges nitric oxide (NO),[6] thereby possibly inhibiting NO-related neurotoxicity.[7] Hypertension has been used in the treatment of stroke to increase blood flow, but it has not been widely adopted.[8,9] An increase in BP and improved perfusion with an oxygen carrier in tissues at risk of ischemia may improve outcome for these patients.

In the phase I study 24 healthy volunteers received a single dose of DCLHb (25, 50 or 100 mg/kg), dose-dependent increases in mean arterial pressure (MAP).[10] In another small study 18 subjects receiving chronic hemodialysis therapy were given the same dose.[11] No significant adverse events nor toxicity occurred in either study. At the time our study took place, DCLHb was being developed as a hemoglobin therapeutic for abdominal aortic aneurysm surgery,[12] sepsis,[13] cardiac surgery[14] and trauma.[15]

The aim of our study was to assess the safety and tolerability of repeated low dose infusions of DCLHb in acute ischemic stroke patients started within 18 hours of symptom onset.

Subjects and methods

The trial was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice for Trials on Medicinal Products. For participation written informed consent from the patients or their family was required. The Medical Ethics Committees of the participating hospitals approved the protocol. The participating centers were the departments of Neurology in the University Hospitals of Heidelberg, Helsinki, Leuven and Rotterdam. Patients received all standard care and treatment with increased clinical monitoring and assessment, including prophylactic medication such as acetylsalicylic acid and heparin.

Patients

Patients with clinical symptoms of an acute ischemic stroke, with motor disturbance, consistent with localization in the anterior cerebral circulation were eligible to participate in the study if they were older than 20 years; could be treated within 18 hours after start of symptoms; and were likely to survive for at least 3 months. Patients had to be alert or at least arousable by stimulation to obey, answer or respond to verbal commands and a brain computed tomography (CT) scan had to be normal or compatible with a recent infarction. Exclusion criteria were: any major disabling disorder interfering with the assessments; pregnancy or lactation; an evident hematological cause of the symptoms; congestive heart failure or acute myocardial infarction; systolic BP > 230 mmHg or diastolic BP >130 mmHg; renal or liver disease; spontaneous improvement of symptoms by at least 2 grades on the modified Rankin scale;[16] and previous enrollment in this study or enrollment in another investigational trial within 30 days. Eighty-five patients were enrolled at four centers between August 1994 and November 1996.

Drugs

DCLHb is a hemoglobin based oxygen carrier derived from human erythrocytes. Outdated human erythrocytes are washed, filtered and lysed. DCLHb is produced by cross-linking of molecular hemoglobin between the alpha- subunits by means of a reaction with the diaspirin compound, bis (3,5-dibromosalicyl) fumarate[5] and is subjected to rigorous viral inactivation and removal procedures.[17] The cross-linking gives the hemoglobin biochemical stability and a favorable oxygen dissociation curve. DCLHb is provided as a solution that is electrolyte balanced and has the following properties at 37°C: pH 7.4, oncotic pressure of 42-44 mmHg (hyperoncotic compared with whole plasma), oxygen P50 affinity of 32 mmHg and a viscosity of <1.5 centistokes.[5] The solution was kept frozen at -20°C until needed, when it was passively thawed to 5°C. DCLHb was prepared and provided by Baxter Healthcare Corp., Deerfield, IL, USA (lot numbers 94A21AD11 through 95L08AD11).

Treatment regimen

Patients were randomly assigned to DCLHb or equal volume of 0.9% normal saline (placebo) in a 1:1 ratio. The study was single blind because of the

prominent color of the drug and the difficulty to manufacture a proper placebo. Three doses were tested: 25, 50 and 100mg/kg 10% DCLHb (n=10, 10 and 20 respectively) or equal volume of saline (n=45) (17, 35 and 70ml per infusion in a 70kg person) every 6 hours for 72 hours (12 doses) intravenously at a rate of 2 ml/minute. Each successive increased-dose segment was initiated only after 10 patients had received the preceding dose of DCLHb.

Assessments

Baseline assessment consisted of a medical history, general physical and neurological examinations, ECG, CT scan, urine analysis, hematological and biochemical tests.

Neurological status was assessed by means of the modified National Institutes of Health Stroke Scale (NIHSS).[18] Functional ability was scored by means of the Barthel Activities of Daily Living (ADL) index.[19] The Modified Rankin scale was used to score handicap.[16] BP and HR and digital pulse oximetry were recorded immediately prior to each infusion and 15, 30 and 45 minutes after start (where applicable), at the end of infusion and 30 minutes and 1,2,3,4 and 5 hours after each infusion. Baseline BP and HR were measured during one hour, also every 15 minutes, and these values were averaged. We used an automatic, oscillometric blood pressure device (Accutor 3SAT, Datascope Corp. Paramus, NJ).

The physical examination was repeated at day 3, 7, 14 and at the 3 month follow-up. The NIHSS was assessed again at day 1, 3, 14 and 3 months. Rankin and Barthel scores were measured at day 14 and at the 3 month follow-up.

Blood samples for complete blood count (CBC) and sedimentation rate (ESR) were collected pre-infusion (before the first infusion) and day 1,2,3,7 and 14. Hematocrit, fibrinogen and blood viscosity were measured preinfusion and twice daily before infusions and on day 7. Blood urea nitrogen, serum creatinine, total creatine kinase (CK, total and fractionated), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total serum bilirubin, electrolytes, serum amylase, lactate dehydrogenase (LDH, total and fractionated) and glucose were measured prior to infusion and on day 1,2,3 and 7. Transferrin was measured preinfusion and on day 7. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured preinfusion and on day 1,3 and 7. Plasma hemoglobin concentrations were determined when possible immediately prior and at the end of each infusion for all twelve infusions and 2, 4, 6, 12, 24 and 36 hours after the final infusion.

Antibody titers to DCLHb were measured once at the 3-month visit. Urinalysis was performed pre-infusion and on day 1, 2 and 3. Fluid intake and output was measured for the first three days.

ECG's were repeated on day 1 and once between day 3 and 7. Continuous Holter ECG monitoring was started before the first infusion and continued for 84 hours.

The CT scan was repeated approximately 14 days after the first infusion and a Magnetic Resonance Imaging (MRI) scan was done once between 7 and 14 days.

Statistics

The analysis included all randomized subjects. Values are expressed as mean ± SD unless otherwise indicated. For comparison between two groups Student's unpaired t-test, chi-square or Fisher's exact test were used as appropriate. Regression analysis was used to identify factors independently related to outcome at 3 months. A p value<0.05 was considered statistically significant.

Results

Patient characteristics are presented in table 1. The groups were well-matched with respect to all baseline variables, although patients receiving control treatment patients tended to have less severe strokes. The average time to treatment was the same for both groups: 11±5 hours from onset of symptoms.

Adverse events

Two patients in the 100mg/kg group had unexpected adverse events, possibly related to DCLHb. The first was a 65-year old male with a severe cortical stroke (NIHSS 23), cardiomegaly, slightly elevated SGOT (41, normal 5-37), SGPT (55,normal 5-40) and GGT (128, normal 11-50), and obstructive breathing at baseline. The patient received 5 doses DCLHb of 80ml each. Subsequently scleral icterus, hypertension, fever, two episodes of pulmonary and cerebral edema developed, leading to death. The other patient was a 53-year-old female with a moderately severe lacunar stroke (NIHSS 9), and a medical history of untreated hypertension. Baseline examinations showed a slightly elevated amylase 171 U/l (normal 30-130 U/l). She was treated 12 doses DCLHb of 50ml each. She developed renal and pancreatic insufficiency,

Table 1 Characteristics of patients with acute ischemic stroke randomized to DCLHb or normal saline.

	Saline	**DCLHb**	**P-value**
Number of patients	45	40	
Age (years)	65 ± 15	68 ± 13	0.40
Female/Male ratio	22/23 (49/51)	24/16 (60/40)	0.42
Stroke subtype			
Cortical	32 (71)	26 (67)	0.84
Lacunar	13 (29)	12 (33)	0.84
Stroke side (R/L)	19/26 (42/58)	25/15 (63/37)	0.10
Rankin score pre-infusion			
2	2 (4)	2 (5)	0.69
3	16 (36)	7 (17)	0.10
4	11 (24)	17 (43)	0.12
5	16 (36)	14 (35)	0.86
Medical History			
Angina pectoris	4 (9)	8 (20)	0.25
Atrial fibrillation	7 (16)	6 (15)	1.00
Diabetes mellitus	9 (18)	9 (23)	0.79
Hypercholesterolemia	1 (2)	1 (3)	1.00
Hypertension	18 (40)	20 (50)	0.48
Myocardial infarction	6 (13)	3 (8)	0.49
Smoking	19 (42)	12 (30)	0.35
Stroke	12 (27)	13 (33)	0.73
Systolic BP	155±20	158±24	0.52
Diastolic BP	85±13	84±13	0.72
Heart rate	75±15	80±13	0.18

Values in parentheses are percentages

nausea and anemia within 24 hours. Additional tests did not reveal the cause of these symptoms. All signs and symptoms resolved within 7-10 days, except for the amylase level, which was still asymptomatically, elevated (215 U/l) at the 3-month follow-up.

Various adverse events occurred more frequently in the DCLHb treated patients, as summarized in Table 2. Multivariate analysis however, showed that the adverse events of cerebral coning, nausea and pneumonia were related to stroke severity rather than with DCLHb. Insomnia occurred more often in the control group. Adverse events that were independently related to DCLHb were jaundice and hemoglobinuria. Jaundice occurred in 0/10, 1/10 and 17/20 patients in the 25, 50 and 100mg/kg groups respectively versus 0/45 in controls (p=0.00). Jaundice typically appeared within 24 hours after start of treatment and disappeared without sequelae around day 5. There was no evidence of associated hepatotoxicity and the jaundice was considered to be a discoloration due to the extravasation of DCLHb and the rise in bilirubin, which is a metabolic product of DCLHb. Hemoglobinuria was found in 22/40 patients in the treatment group vs. 13/45 in controls (p=0.03). There were no associated renal function changes and the signs resolved by day 7.

Table 2 Univariate analysis of adverse events in the DCLHb and control group

	Saline	**DCLHb**	**P-value**
Cerebral coning	0	4 (10)	0.05
Hemoglobinuria*	13 (40)	22 (69)	0.03
Insomnia	8 (18)	1 (3)	0.03
Jaundice*	0	17 (43)	0.00
Nausea	3 (7)	11 (28)	0.02
Pneumonia	3 (7)	10 (25)	0.04

Values in parentheses are percentages
* Differences still statistically significant in the regression analysis

Laboratory parameters

Dose-dependent increases of LDH, CPK, bilirubin, AST and plasma hemoglobin were found. Amylase was elevated in the 100mg/kg group only.

LDH levels were elevated by 39, 61 and 77% respectively for the increasing doses of DCLHb; increases of CPK were 128, 222 and 280%; for bilirubin: 100, 181 and 263%; for serum ASAT: 44, 63 and 166% and for amylase only in the 100mg/kg group 170%. The subfractions LDH-1 and CPK-MB were not increased. All laboratory abnormalities were clinically asymptomatic and disappeared within a week. Maximum plasma hemoglobin concentrations were achieved around 24 hours after start of treatment for doses 25 and 50 mg/kg and around 42 hours for 100 mg/kg and were 74 (52-160), 229 (149-267) and 419 (331-632) vs. 3.9 (0-139) mg/dl (median and range).

All other laboratory measurements (BUN, creatinine, electrolytes, ALT, glucose, CBC, ESR, PT, APTT, transferrin, blood viscosity, hematocrit, fibrinogen, APTT, PT) showed no difference between groups. At 3 months no DCLHb anti-bodies were found.

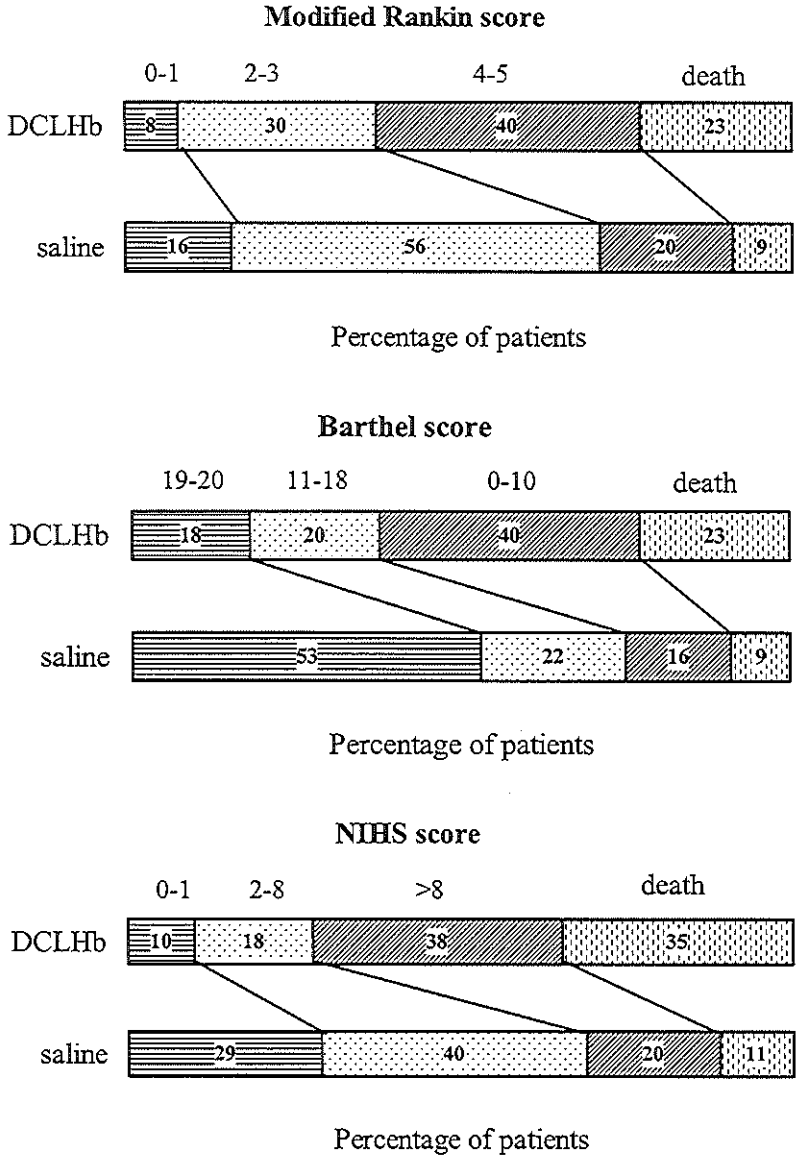
Blood pressure

DCLHb produced a rapid rise in mean arterial pressure (MAP), from 113±14 at baseline to 134±20, vs. 109±16mmHg in controls. The magnitude of the increases caused by the different doses was similar, but the duration of the pressor was dose-dependent.[20] The hypertensive reaction was not accompanied by adverse events. The hypertensive reaction was not accompanied by clinical signs of hypertensive encephalopathy, nor did the CT and MRI-scans show occipital edema or brain swelling. Severe hypertension that needed pharmacological intervention occurred in 3 patients treated with DCLHb vs. 3 in the saline group. Fluid intake and output during the first 3 days did not differ between treatment groups.

Outcome

Outcome, measured by means of the Rankin score at 3 months was significantly worse in the treatment group. Thirty-four (85%) patients had an unfavorable outcome (Rankin score of 3-6) vs. 23 (51%) in controls ($p=0.002$). The Rankin score at 14 days was also worse in DCLHb treated patients: 90% vs. 68% ($p=0.04$). Multivariate logistic regression analysis showed that a severe stroke at baseline (Rankin 4-5; $p<0.001$; odds ratio 20.9; confidence interval 4.1-102.4) and treatment with DCLHb ($p=0.015$; odds ratio 3.9; confidence interval 1.4-12.0) were independent predictors of a worse outcome (Rankin 3-6). Outcome was not related to the dose of DCLHb. The Barthel scale and NIHSS showed similar results (figure 1).

Figure 1 Clinical outcome at 3 months according to treatment



Discussion

Following the beneficial treatment effects of DCLHb in animal models of focal cerebral ischemia, we initiated this phase II trial, to investigate the safety of this hemoglobin therapeutic in patients with acute ischemic stroke. In keeping with animals studies as well as in healthy volunteers and hemodialysis patients DCLHb produced a rapid rise in blood pressure, the duration of which was dose-dependent. The hypertensive effect was not accompanied by complications, such as excess need for anti-hypertensive treatment, nor did we find any signs or symptoms of hypertensive encephalopathy or hemorrhagic transformation of the infarction. Immediate side effects that were independently related to the use of DCLHb were jaundice and hemoglobinuria. These were all transient and not associated with morbidity. Cerebral coning and pneumonia were related to a severe stroke at baseline, rather than with DCLHb.

The dose-dependent increases of LDH, CPK, bilirubin, AST and amylase were clinically asymptomatic and disappeared within a week. The subfraction analysis of LDH and CPK iso-enzymes revealed that the elevated enzyme levels did not originate from the heart. The rise in bilirubin was expected, since this is a metabolic product of DCLHb. The rise in ASAT was not accompanied by a rise in ALT; therefore it probably did not arise from the liver. Except for one patients, the rise in amylase was also asymptomatic and not accompanied by any evidence of pancreatic insufficiency.

However, treatment with DCLHb was an independent predictor of an unfavorable outcome at 3 months. The cause of the worse outcome is unclear. Endothelin-1 (ET-1, a potent vasoconstrictor) and NO (a vasodilator) may well have played an important role, although we cannot be sure how exactly. We have reported elevated ET-1 levels in response to DCLHb[21] which may have contributed to the ischemic damage through the potent vasoconstrictor effect of ET-1. (See chapter 5)[22] On the other hand, there is also evidence that a systemic increase in endothelin causes a vasodilator effect in the brain.[23] The dose-dependent increase in ET-1 may have been promoted by the duration of treatment of 72 hours or by the treatment delay of 18 hours. DCLHb is also a scavenger of NO,[6] a free radical that plays a dual role in brain ischemia. NO is protective or destructive depending on the stage of evolution of the ischemic process and on the cellular source of NO.[7]

Alternative explanations of the worse outcome in DCLHb patients are the play of chance in this small study, the imbalance, although not statistically significant, of stroke severity at randomization, or bias due to the single-blind

nature of the study. Furthermore, in analogy with other stroke treatments,[24,25] it may be beneficial to administer DCLHb immediately after the onset of ischemia, but harmful if given during a later phase. In most animal experiments a highly favorable response was found after a single high dose exchange transfusion of DCLHb, either before or within one hour after initiation of ischemia. After our study had finished, such high doses were found to be safe in patients after undergoing coronary by-pass surgery.[14] On the other hand, a North-American[15] and European study[26] in trauma patients were prematurely terminated due to higher mortality in the treatment group.[15]

In conclusion, this was the first study in which the effects of low doses DCLHb over three days were studied in acute ischemic stroke patients. As far as we are aware this is also the first randomized study in which the blood pressure of stroke patients was deliberately elevated. DCLHb affected outcome adversely. We recommend that the safety of a hemoglobin therapeutic in the treatment of stroke be further explored, preferably using a second generation, genetically engineered hemoglobin. We suggest that treatment should include a single high dose given earlier after stroke onset.

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Chapter 4

Induced elevation of blood pressure by DCLHb in the acute phase of ischemic stroke

Adapted from: Saxena R, van den Meiracker AH, Wijnhoud AD, Koudstaal PJ, on behalf of the DCLHb in Acute Stroke study investigators. Induced elevation of blood pressure in the acute phase of ischemic stroke in man. Stroke 2000;31:546-548. *(Letter)*

Abstract

Blood pressure (BP) is often elevated in the acute phase of stroke. Lowering BP is commonly discouraged as this may adversely affect outcome. Controlled evidence on the safety and value of induced hypertension is lacking. In healthy volunteers, infusion of diaspirin cross-linked hemoglobin (DCLHb) produced a dose-dependent increase in BP. The aim of our study was to study the safety of induced hypertension with DCLHb in the acute phase of ischemic stroke.

DCLHb in a dose increasing fashion (25, 50 and 100 mg/kg, n=10, 10 and 20, respectively) or placebo (n=45) was infused intravenously every 6 hours for 72 hours in patients with an acute ischemic stroke in the setting of a randomized controlled trial. We measured BP and heart rate every 15 minutes. Safety was further monitored by repeated physical and neurological examinations and by CT- and MRI scans.

DCLHb caused a rapid rise in mean BP from 113±14 at baseline to 129±21, 135±11 and 135±24 for the 25, 50 and 100mg/kg respectively, vs. 109±16mmHg in controls. The duration of this effect on BP was dose-dependent. The pressor effect was not accompanied by complications such as hemorrhagic transformation of the infarct, brain edema or hypertensive encephalopathy and there was no excess need for anti-hypertensive treatment. However, clinical outcome at 3 months was worse in the treatment group.

Even though the pressor effect was well tolerated, clinical outcome was worse in patients treated with DCLHb. Further studies should try and evaluate other methods of induced hypertension and explore their clinical efficacy.

Introduction

Blood pressure (BP) is often elevated in the acute phase of stroke. Possible reasons are: previous hypertension, a pathophysiological response to ensure perfusion in the ischemic penumbra through the Cushing reflex or increased catecholamines through sympathetic nervous system activation, or a reaction to the mental and physical stress of hospitalization. The course of BP following an acute stroke has been extensively studied, and typically shows a spontaneous decline during the first week.[1-5]

Decreasing BP in the acute phase of stroke can have a deleterious effect on outcome.[6,7] The general consensus among neurologists is not to treat high BP during the first week, except for a consistently elevated systolic BP over 250 mmHg or diastolic BP over 140 mmHg, which can cause hemorrhagic transformation, brain edema or hypertensive encephalopathy through failed autoregulation.[8] Other reasons for lowering BP are cardiovascular complications such as myocardial ischemia or aneurysma dissecans.[9]

While lowering BP may be harmful, the safety and efficacy of increasing BP is completely unknown. Hypervolemia and induced systemic hypertension are generally considered the standard approach to the treatment of ischemia due to vasospasm after subarachnoid hemorrhage.[10] Early reports in humans,[11,12] and recent experimental work,[13,14] have shown a beneficial effect on outcome of treatment with induced hypertension. However, this therapy is very rarely used in acute ischemic stroke[15,16] and it has never been studied in a randomized controlled fashion. The rationale for this therapy is that it may help to restore the blood flow to the ischemic area of which the perfusion is passively dependent on systemic BP because of the loss of local autoregulation, or it may induce dilatation of the leptomeningeal collateral circulation in case of an embolic occlusion.

In animal stroke models, diaspirin cross-linked hemoglobin (DCLHb), a purified cell-free human oxygen-carrying hemoglobin solution, produced both an increase in BP and in tissue perfusion and a significant reduction in the extent of brain injury. This reduction was most profound when DCLHb was given in a manner that induced its inherent hypertensive response.[17-19] We recently performed a safety study of DCLHb in patients with acute ischemic stroke (see chapter 3).[20] In the present study, we have investigated the tolerability of induced hypertension with DCLHb in the acute phase of ischemic stroke. We have specifically focused on the commonly feared complications of

this therapy, that is, hypertensive encephalopathy, hemorrhagic transformation, brain edema, myocardial infarction and congestive heart failure.[21]

Subjects and methods

DCLHb in a dose increasing fashion (25, 50 and 100 mg/kg, n=10, 10 and 20, respectively) or placebo (n=45) was infused intravenously every 6 hours for 72 hours in patients with an acute ischemic stroke in the anterior circulation, within 18 hours of onset of symptoms, in the setting of a randomized controlled, single-blind trial.[20] (See chapter 3). The volumes infused per 24 hours were approximately 70 ml for the lowest, 140 ml for the intermediate and 280 ml for the highest dose of DCLHb.

Prophylactic medication such as acetylsalicylic acid was permitted. If patients were already on anti-hypertensive medication, this medication was continued, without change, throughout the study. New anti-hypertensive therapy was not initiated unless BP exceeded 220/135 mmHg for an hour.

Assessments

During the study BP and heart rate (HR) were measured at 15-minute intervals with an automatic, oscillometric BP device (Accutor 3SAT, Datascope Corp. Paramus, NJ, USA) and a 3-lead ECG was recorded continuously. Baseline BP and HR were measured during one hour, also every 15 minutes, and these values were averaged.

CT scan was performed on admission and after 2 weeks and a MRI scan around day 7.

Physical examination were performed at baseline, day 3, 7, 14 and at the 3-month follow-up. Neurological examinations using the NIH stroke scale[22] at baseline, day 1, 3, 14 and 3 months. Patients were checked for adverse events on a daily basis while admitted and again at 3 months. Outcome at 3 months was measured by means of the Modified Rankin scale.[23]

Statistics

The analysis included all randomized subjects. Since a similar protocol was followed for all patients, the data of the three groups of patients randomized to saline infusion, referred to as the placebo group, were averaged.

Values of MAP and HR are presented as mean and 95% confidence interval. Comparison of data between the four groups of patients was done by analysis of variance (ANOVA). For comparison between two groups Student's unpaired t-test, chi-square or Fisher's exact test were used as appropriate. A p value < 0.05 was considered statistically significant.

Results

Clinical characteristics of the patients randomized to treatment with DCLHb or placebo are summarized in Table 1. There were no significant differences between groups. BP was elevated (>150/90 mmHg) in 64% of the patients on admission, whereas 45% were known to have hypertension.

In the control group (n=45), BP on admission was 155±20/85±12 mmHg. The systolic and diastolic BP were significantly lower at 24 hours: 140±25 mmHg (p=0.01) and 78±16 mmHg(p=0.01) and did not fall further during the 2 weeks of observation (see table 2). There were no significant changes in heart rate.

After the first infusion, DCLHb produced a rapid rise in blood pressure, which depended more on a elevation of the systolic than the diastolic pressure. It reached a maximum within two hours after the first infusion. For the treatment group as a whole (n=40) the systolic pressure increased from 159±24 mmHg at baseline to 172±27 mmHg, vs. 156±24 mmHg in controls (p=0.005). The diastolic pressure increased from 83±17 mmHg to 94±15 mmHg, vs. 84±17 mmHg in controls (p=0.008). The magnitude of the increases caused by the different doses was similar, but the duration of the pressor effect was dose-dependent, despite the repeated infusions (see figure 1).

The mean arterial pressure during the treatment phase was dose dependently higher in the DCLHb treated groups. Heart rates in the 4 groups were similar (see table 3 and figure 2). In the patients treated with DCLHb (n=40), the BP on admission (158±23/83±14 mmHg) did not fall during the treatment period of 72 hours. By day 7 the BP had significantly decreased to 130±7/78±9 (p=0.05) (see table 2).

Table 1 Characteristics of patients randomized to DCLHb or saline

	Saline	**DCLHb**	**P-value**
Number of patients	45	40	
Age (years)	65 ± 15	68 ± 13	0.40
Sex			
Female	22 (49)	24 (60)	0.42
Male	23 (51)	16 (40)	0.42
Stroke subtype			
Cortical	32 (71)	26 (67)	0.84
Lacunar	13 (29)	12 (33)	0.84
Blood pressure (mmHg)			
Systolic	155±20	158±24	0.52
Diastolic	85±13	84±13	0.72
Medical History			
Angina pectoris	4 (9)	8 (20)	0.25
Atrial fibrillation	7 (16)	6 (15)	1.00
Diabetes mellitus	9 (18)	9 (23)	0.79
Hypercholesterolemia	1 (2)	1 (3)	1.00
Hypertension	18 (40)	20 (50)	0.48
Myocardial infarction	6 (13)	3 (8)	0.49
Smoking	19 (42)	12 (30)	0.35
Stroke	12 (27)	13 (33)	0.73
Cause of stroke			
Large vessel	10 (22)	9 (23)	1.00
Small vessel disease	13 (29)	12 (30)	1.00
Cardioembolic	9 (20)	10 (25)	0.71
Other	13 (29)	9 (23)	0.67
Time to treatment (hours)	11±5	11±5	1.00

Values in parentheses are percentages

Table 2 Systolic and diastolic blood pressure values for the 4 treatment groups

Systolic blood pressure in mmHg

Time	Saline	DCLHb 25mg/kg	DCLHb 50mg/kg	DCLHb 100mg/kg
n	45	10	10	20
0 hours	155 ± 20	149 ± 23	164 ± 19	161 ± 26
24 hours	140 ± 25 *	146 ± 21	169 ± 23	167 ± 24
48 hours	140 ± 20 *	141 ± 22	160 ± 27	164 ± 18
72 hours	140 ± 26 *	147 ± 22	151 ± 24	163 ± 24
day 7	137 ± 20 *	130 ± 7 *	140 ± 24 *	142 ± 27 *
day 14	139 ± 20 *	136 ± 14 *	134 ± 11 *	136 ± 17 *

Diastolic blood pressure in mmHg

Time	Saline	DCLHb 25mg/kg	DCLHb 50mg/kg	DCLHb 100mg/kg
n	45	10	10	20
0 hours	85 ± 12	77 ± 11	86 ± 14	88 ± 13
24 hours	78 ± 16 *	80 ± 14	86 ± 8	92 ± 16
48 hours	79 ± 13 *	73 ± 14	80 ± 11	91 ± 11
72 hours	79 ± 15 *	85 ± 11	77 ± 12	93 ± 16
day 7	78 ± 12 *	78 ± 5	73 ± 10 *	80 ± 11 *
day 14	81 ± 12 *	85 ± 11	75 ± 9 *	81 ± 6 *

* denotes a significant change from baseline

Figure 1 Relative changes in mean arterial pressure (MAP) in relation to baseline values in percentage of baseline values.

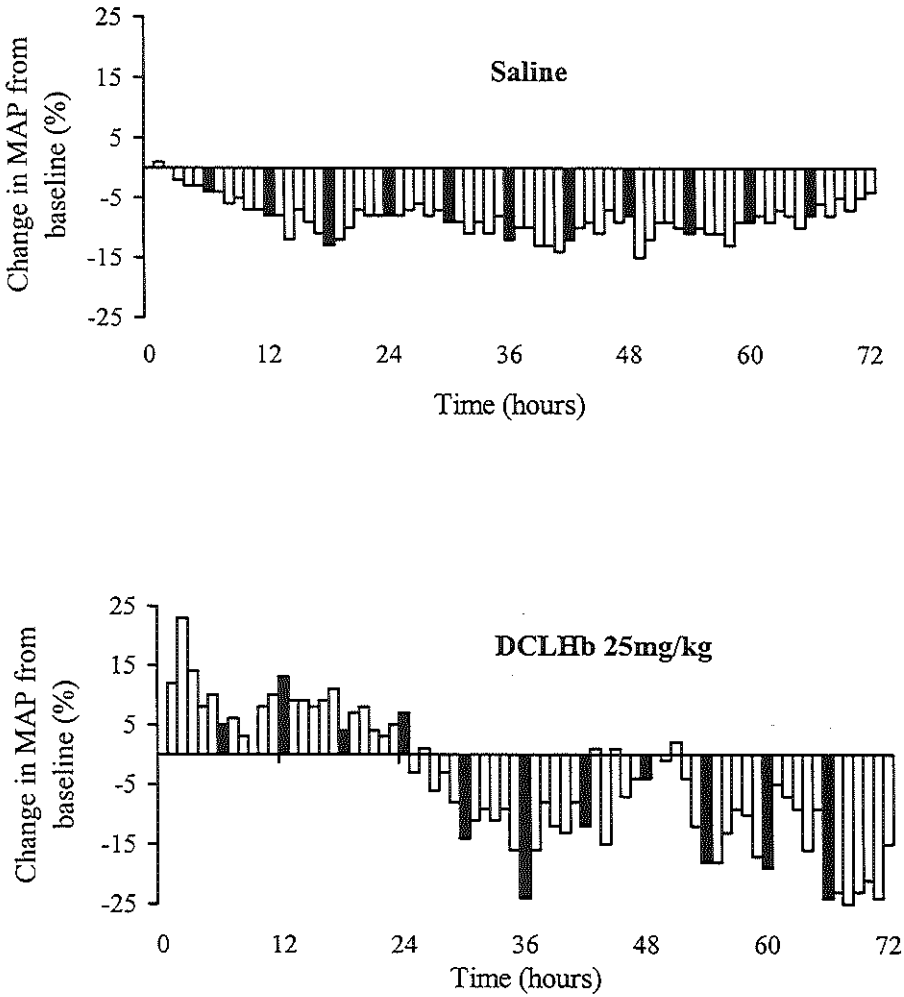
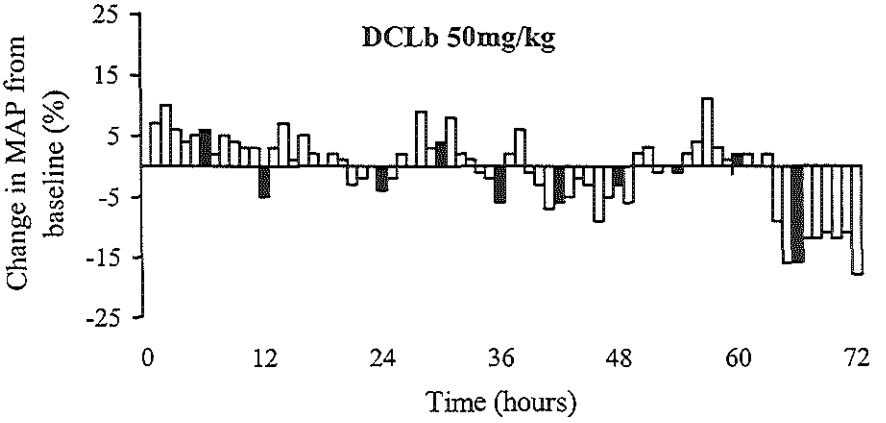


Figure 1 Relative changes in mean arterial pressure (MAP) in relation to baseline values in percentage of baseline values, *continued.*



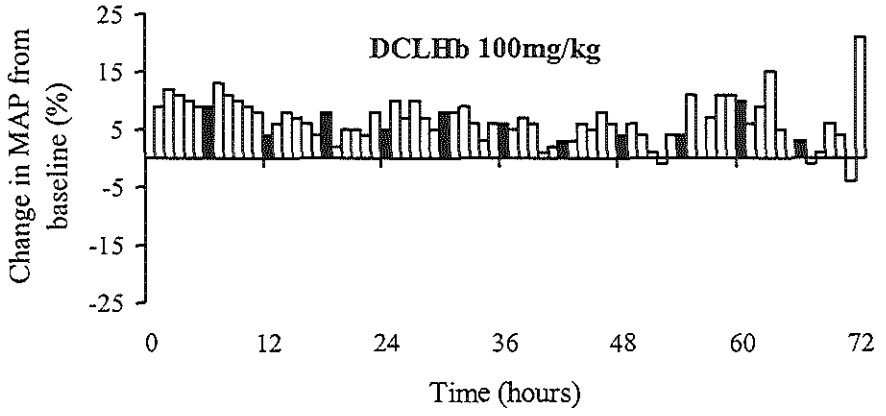


Table 3 Blood pressure in mmHg during the treatment period of 72 hours

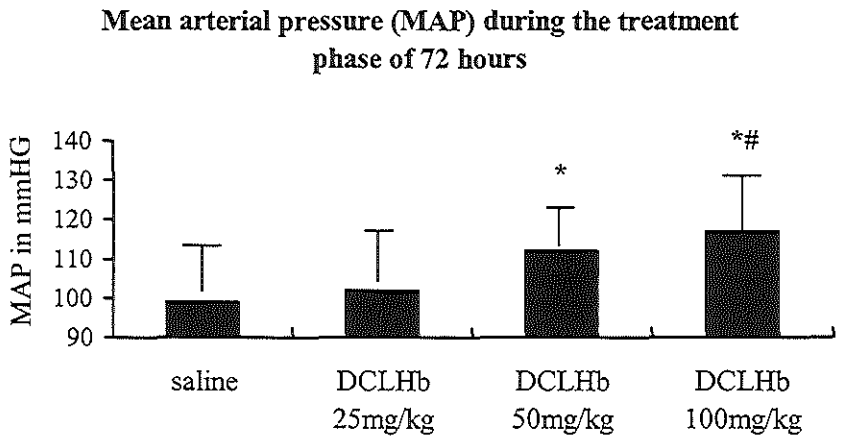
	Saline	**DCLHb 25mg/kg**	**DCLHb 50mg/kg**	**DCLHb 100mg/kg**
n	45	10	10	20
Mean MAP ± SD	99 ± 11	102 ± 9	112 ± 10 *	117 ± 13 * #
95% CI for MAP	95 - 102	96 - 108	105 - 120	111 - 123
Mean SBP ± SD	141 ± 17	146 ± 13	163 ± 21 *	166 ± 20 * #
95% CI SBP	136 - 146	137 - 155	148 - 178	157 - 176
Mean DBP ± SD	78 ± 10	80 ± 9	87 ± 7	92 ± 11
95% CI for DBP	75 - 81	74 - 86	81 - 92	87 - 97
Mean HR ± SD	73 ± 13	79 ± 17	74 ± 14	74 ± 13
95% CI for HR	69 - 77	67 - 92	64 - 84	68 - 80

* denotes a significant change from saline

# denotes a significant change from DCLHb 25mg/kg

Table 4 shows the adverse events that could be expected after induced hypertension. Cerebral edema/coning occurred in 4 patients treated with DCLHb vs. 0 in the placebo group (p=0.05), but multiple regression analysis showed this was related to a misbalance in severe stroke at baseline, rather than to treatment with DCLHb (see chapter 3).[20] Hemorrhagic transformation occurred in 2 patients treated with DCLHb vs. 6 in the saline group (p=0.17). There were no significant differences in the prevalence of myocardial ischemia or cardiac failure. Hypertensive encephalopathy, did not occur at all. Severe hypertension that needed pharmacological intervention (BP>220/135 mmHg for one an hour) occurred in 4 patients treated with DCLHb vs. 5 in the saline group.

Figure 2 Mean arterial pressure during the treatment period of 72 hours in patients treated with DCLHb and in controls



* denotes a significant change from baseline

# denotes a significant change from DCLHb 25mg/kg

Table 4 Adverse events that might be expected with induced hypertension

	Saline	DCLHb	P-value
Cerebral edema	0	4	0.05
Myocardial ischemia	*3*	0	0.10
Hypertension >220/135 for an hour	5	4	0.58
Congestive heart failure	2	3	0.44
Hypertensive encephalopathy	0	0	0.99
Hemorrhagic transformation	6	2	0.17

Discussion

Experimental data to support the value of induced hypertension in stroke are abundant.[13,14] Human data are more scarce,[11,12] and there are no reports of randomized controlled studies. We have studied the effect of DCLHb on BP in the setting of a randomized controlled trial in which the safety and efficacy of DCLHb in acute ischemic stroke was investigated. Administration of DCLHb was started within 18 hours after the onset of symptoms. DCLHb caused a pressor effect that delayed the typical fall in blood pressure in stroke patients [1-5] as also seen in our control group. The magnitude of the pressor effect was similar in the 3 treatment groups. However, the duration of this effect was dose dependent, resulting in dose-dependent higher MAP values during the treatment phase of 72 hours. The induction of hypertension did not lead to complications such as hemorrhagic transformation, brain edema or hypertensive encephalopathy.

It is noteworthy that the BP in the control group stabilized within 24 hours, which was sooner than described in other studies, in which this varied between 4,[3,4] 7,[1] and 10[5] days. One explanation might be that our patients had lower BP at stroke onset than the ones described in other studies. This could be explained by the use of an automatic oscillometric BP device, which is known to yield lower values than manual measurements, partly due to the avoidance of the white coat effect. Furthermore, we measured BP every 15 minutes during the first 72 hours, and the baseline BP was defined as the average of the first hour recordings. Finally, our study population was more homogeneous, since all patients in our study had had an ischemic stroke and were randomized within 18 hours after the insult, than those of the abovementioned studies in which patients with cerebral hemorrhages and long intervals after stroke onset were also accepted.

Our study suggests that induction of hypertension can be performed in the acute phase of stroke without complications such as hemorrhagic transformation, brain edema or hypertensive encephalopathy. However, it is impossible to draw conclusions regarding the clinical effect on outcome since DCLHb is an agent with more effects than just hypertension. In a previous paper, we reported a dose-dependent increase in endothelin-1 (ET-1) levels in our patients (see chapter 5)[24] and in another the worse outcome in the treatment group which seemed to be independently related to the use of DCLHb (see chapter 3).[20] In addition, a North-American[25] and European[26] study in trauma patients were terminated prematurely terminated due to higher mortality in the treatment group. Further studies should therefore try and evaluate other methods of induced hypertension and explore their clinical efficacy.

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Chapter 5

Diaspirin cross-linked hemoglobin: effect on endothelin-1 and blood pressure in acute ischemic stroke in man

Saxena R, Wijnhoud AD, Man in 't Veld AJ, Meiracker AH van den, Boomsma F, Przybelski RJ, Koudstaal PJ. Effect of diaspirin cross-linked hemoglobin on endothelin-1 and blood pressure in acute ischemic stroke in man. J.Hypertens. 1998; 16:1459-1465.

Abstract

For almost 50 years it has been known that hemolyzed blood can increase blood pressure (BP). Although pre-clinical studies suggest that this pressor response is due to interaction of hemoglobin with endothelium-derived vasoactive substances, its mechanism in humans is unknown. We investigated the involvement of endothelin-1 (ET-1) in the BP response to the oxygen carrier diaspirin cross-linked hemoglobin (DCLHb) in stroke patients.

In a randomized phase II study, DCLHb in a dose increasing fashion (25, 50 and 100 mg/kg, n=8, 8 and 11, respectively) or placebo (n=26) was infused intravenously every 6 hours for 72 hours to patients with an acute ischemic stroke. BP and heart rate (every 15 minutes) and plasma concentrations of ET-1, catecholamines, renin, vasopressin and atrial natriuretic peptide (before and 24 and 66 hours after the start of infusions) were measured.

In the placebo group, mean arterial pressure (MAP) of 112 (109-115) mmHg (mean and 95%CI) at baseline decreased spontaneously by 11.4 (5.4 -17.5) and 12.5 (5.4-19.5) mmHg after 24 and 66 hours, respectively. This decrease in MAP was attenuated in patients treated with DCLHb, reaching statistical significance in the highest dose group. Plasma ET-1 concentration decreased slightly in the placebo group from 4.2 (3.1-5.3) pg/ml (median and range) at baseline to 2.4(1.9-3.7) pg/ml after 24 hours ($p=0.0044$) and 2.8 (1.9-3.7) pg/ml after 66 hours ($p=0.0042$), but increased dose-dependently in response to DCLHb infusion. With the highest dose of DCLHb, plasma ET-1 concentration rose from 4.8 (0.1-7.8) pg/ml at baseline to 21.2 (13.4-53.2) pg/ml after 24 hours ($p<0.001$) and to 27.6 (11.9-47.8) pg/ml after 66 hours ($p<0.001$). Increments in plasma ET-1 concentration and in MAP were correlated ($r=0.30$, $p=0.02$). Other vasoactive hormones were not affected by DCLHb infusion.

Infusion of DCLHb in patients with acute ischemic stroke is associated with a dose-dependent increase in plasma ET-1 concentration. We suggest that this increase prevents the natural decrease in BP in these patients.

Introduction

Diaspirin cross-linked hemoglobin (DCLHb) is a hemoglobin-based oxygen carrying solution that is derived from human red blood cells osmotically lyzed to release hemoglobin. In a phase I study, performed in healthy volunteers, a single intravenous infusion of a 10% DCLHb solution in doses of 25, 50 or 100 mg/kg was associated with a dose-dependent increase in blood pressure (BP) [1]. Increments in BP have also been observed with other hemoglobin solutions, and do not appear to be unique to DCLHb.[2] Despite this consistent pharmacological effect, the mechanism has not been studied in human subjects or patients.

Since hemoglobin is an extremely effective scavenger of nitric oxide (NO), inactivation of this endogenous vasodilator is one of the most likely mechanisms by which hemoglobin solutions induce a rise in BP.[3,4] Interestingly, recent in vitro studies [5,6] and experiments in rats [4,7] and pigs [8] have provided evidence that endothelin-1 (ET-1) may also be a mediator of the pressor response observed with DCLHb.

In animal studies, DCLHb causes an increase in BP and tissue perfusion. Since the viscosity of DCLHb is lower than that of whole blood, it offers the potential advantage of hemodilution without a concomitant decrease in oxygen delivery [9]. In animal stroke models, hemodilution with DCLHb resulted in significant reductions in the extent of the brain injury and cerebral edema. These reductions were most profound when DCLHb was given in a manner that induced its inherent hypertensive response.[10-12] In addition, experimental data suggests that DCLHb scavenges NO, thereby possibly inhibiting NO-related neurotoxicity.[4,13] Considering these experimental results administration of DCLHb to patients in the acute phase of ischemic stroke may improve outcome.

In the present study the involvement of ET-1 in DCLHb-induced increase in BP was further explored by measuring BP and ET-1 plasma concentrations before and during administration of DCLHb to patients with an acute ischemic stroke. Understanding the mechanism by which DCLHb increases BP in humans will likely provide useful information concerning the optimal utilization of this hemoglobin therapeutic.

Patient and methods

Patients

Patients with clinical symptoms of an acute ischemic stroke, consistent with localization in the anterior cerebral circulation, who were admitted to the Department of Neurology of the University Hospital Dijkzigt, were eligible to participate in the study. The age of the patients had to be over 20 years, the duration of symptoms less than 18 hours, and they had to be likely to survive for at least 3 months. The patients had to be alert or at least arousable by stimulation to obey, answer or respond, and a brain computed tomography scan had to be normal or compatible with a recent infarction. Exclusion criteria were: pregnancy or lactation, an evident hematological cause of the symptoms, congestive heart failure or an acute myocardial infarction, systolic BP > 230 mmHg or diastolic BP >130 mmHg, renal or liver disease or spontaneous improvement of symptoms by at least 2 Rankin grades.[14] All patients received standard care and treatment with increased clinical monitoring and assessment, and prophylactic medication such as acetylsalicylic acid was permitted. If patients were already on anti-hypertensive medication, this medication was continued, without any change, throughout the study. New anti-hypertensive therapy was not initiated unless systolic BP increased above 240 mmHg. For participation written informed consent from the patients or their family was required. The protocol was approved by the Medical Ethics Committee of the University Hospital Dijkzigt.

Drug and study design

DCLHb was prepared and provided by Baxter Healthcare Corp., Round Lake, IL, USA (lot numbers 94A21AD11 through 95L08AD11). DCLHb is a hemoglobin based oxygen carrier derived from human erythrocytes and is produced by cross-linking of molecular hemoglobin between the alpha-subunits by means of a reaction with the diaspirin compound, bis (3,5-dibromosalicyl) fumarate and is subjected to rigorous viral inactivation and removal procedure. The cross-linking gives the hemoglobin biochemical stability and a favorable oxygen dissociation curve. DCLHb is electrolyte balanced and has the following properties at 37°C: pH 7.4, oncotic pressure 42-44 mmHg (iso-oncotic with whole blood and hyperoncotic with plasma), oxygen affinity 32 mmHg and a viscosity <1.5 centistokes. The solution was kept frozen at -20°C until needed, when it was passively thawed to 5°C.[9]

Stroke patients fulfilling the entry criteria were randomized to receive a low (25 mg/kg), intermediate (50 mg/kg) or high (100 mg/kg) intravenous dose of a

10% DCLHb solution or equal volumes of normal saline (placebo). Because this was a safety study each successive increased-dose segment was initiated only after 8 patients had received the preceding dose of DCLHb. The infusion rate was 2 ml/min. Infusions of DCLHb or saline were repeated every 6 hours for a total duration of 72 hours. The volumes infused per 24 hours were approximately 70 ml for the lowest, 140 ml for the intermediate and 280 ml for the highest dose of DCLHb.

During the study BP and heart rate (HR) were measured at 15-minute intervals with an automatic, oscillometric blood pressure device (Accutor 3SAT, Datascope Corp. Paramus, NJ, USA) and a 3-lead ECG was recorded continuously. Baseline BP and HR were measured during one hour, also every 15 minutes, and these values were averaged. Before and 24 and 66 hours after start of infusions blood was sampled through an indwelling intravenous cannula for the measurement of plasma concentrations of ET-1, catecholamines, N-terminal pro-atrial natriuretic peptide (NtproANP), renin, and vasopressin (AVP). In addition, blood was sampled for routine laboratory evaluation, including measurement of liver enzymes, BUN, serum creatinine, creatine phosphokinase and a complete blood count.

Analytical methods

Blood for measurement of ET-1, NtproANP, renin and vasopressin was collected in chilled 10-ml tubes containing EDTA (19 mg), whereas blood for measurement of catecholamines was collected into chilled heparinized 10-ml tubes containing 12 mg of glutathione. Within 30 minutes after sampling, blood was centrifuged at 3000 x g for 10 minutes at 4^0C and the plasma was stored at -80^0 C until assay.

Plasma catecholamines were determined with fluorimetric detection after HPLC separation.[15] ET-1 was measured after SepPak extraction by means of a radioimmunoassay using a commercially available kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Normal plasma values of ET-1 in healthy volunteers range from 1.6 - 4.9 pg/ml and intra- and inter-assay coefficients of variation were respectively, 4.5 and 6.8 %. In vitro addition of various concentrations of DCLHb to plasma samples had no effect on the measured concentration of ET-1. NtproANP was measured by means of a radioimmunoassay with a commercial kit (Biotop, Oulu, Finland). Normal values of NtproANP in healthy elderly subjects range from 1.6-6.6 ng/ml.[16,17] Vasopressin was measured after SepPak extraction by means of a radioimmunoassay (INCSTAR, Stillwater, MN, USA). Normal plasma values in healthy volunteers range from not detectable to 4.7 pg/ml.

Active plasma renin concentration was measured according to a previously described method.[18] Normal values range from 14-51 μU/ml.

Statistics

Since a similar protocol was followed for all patients, the data of the three groups of patients randomized to saline infusion, referred to as the placebo group, were averaged.

Values of MAP and HR are presented as mean and 95% confidence interval. Values of neurohormones were not normally distributed and are presented as median and range. Comparison of data between the four groups of patients was done by analysis of variance (ANOVA) for MAP and HR or the Kruskal-Wallis non-parametric ANOVA for the neurohormones. For comparison between two groups Student's unpaired t-test or chi-squared analysis was used as appropriate. A p value < 0.05 was considered to indicate a statistically significant difference.

Results

Clinical characteristics of the patients randomized to administration of DCLHb or placebo are given in Table 1.

Baseline values of MAP, HR and plasma concentrations of ET-1, noradrenalin, adrenalin, renin, vasopressin and NtproANP of patients did not differ between the four groups (Table 2).

In the placebo group MAP decreased from 112 (109-115) mmHg at baseline by 11.4 (5.4 -17.5) mmHg at 24 hours and 12.5 (5.4-19.5) mmHg at 66 hours after start of infusions. This decrease was attenuated in the patients treated with DCLHb, reaching statistical significance in the group randomized to 100 mg/kg (Fig.1).

In contrast to the marked fall in MAP, HR did not change in the placebo group 24 and 66 hours after start of infusions, whereas it decreased by 7.1 (0.9-13.3) bpm at 24 hours and 6.2 (-2.8-12.2) bpm at 66 hours after infusion of 100 mg/kg DCLHb (Fig.1).

Considering an ET-1 level of 4.9 pg/ml as the upper limit of normal, 16 patients had increased baseline values (median 6.5 pg/ml, range 5.0-26.1 pg/ml).

Table 1 Baseline characteristics of patients randomized to DCLHb or saline

	DCLHb	**Saline**	**P-value**
Number of patients	27	26	
Age (years)	69±12	63±16	0.69
Sex: female/male (n)	17/10	14/12	0.15
Stroke subtype			
Cortical	18 (67)	18 (69)	1.00
Lacunar	9 (33)	8 (31)	1.00
Stroke side (right/left)	18/9	10/16	0.07
Rankin score			
3	6 (22)	10 (38)	0.32
4	10 (37)	8 (31)	0.85
5	11 (41)	8 (31)	0.64
Medical history			
Hypertension	14 (52)	10 (38)	0.66
Myocardial infarction	2 (7)	4 (15)	0.32
Angina pectoris	6 (22)	1 (4)	0.06
Atrial fibrillation	5 (19)	2 (8)	0.23
Diabetes mellitus	7 (26)	4 (15)	0.27
Stroke	11 (41)	7 (27)	0.13
Hypercholesterolemia	1 (4)	1 (4)	0.75
*Smoking**	11 (41)	7 (27)	0.44
Cause of stroke			
Large vessel disease	4 (15)	7 (27)	0.46
Small vessel disease	8 (30)	8 (31)	1.00
Cardioembolic	7 (26)	4 (15)	0.54
Other	8 (30)	7 (27)	1.00
Time to treatment (hours)	11±5	11±5	1.00

Values in parentheses are percentages; other values are mean ± SD
* More than 5 cigarettes per day

Table 2 Pre-infusion values of mean arterial pressure and heart rate, and plasma concentrations of endothelin-1, adrenalin, noradrenalin, renin, N-terminal-proANP and vasopressin in patients randomized to saline or DCLHb infusions

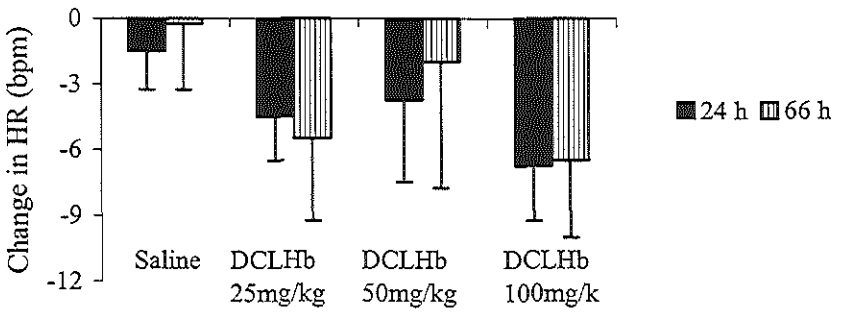
	Saline	**DCLHb 25 mg/kg**	**DCLHb 50 mg/kg**	**DCLHb 100 mg/kg**	**P-value**
Number of patients	26	8	8	11	
Mean BP (mm Hg)	112 (106-118)	106 (96-116)	123 (117-129)	116 (109-123)	0.32
Heart rate (bpm)	74 (69-79)	83 (68-98)	79 (72-86)	80 (69-91)	0.25
Endothelin-1 (pg/ml)	3.7 (0.5-10.6)	5.2 (3.2-7.4)	4.1 (2.6-26.3)	4.2 (0.1-7.1)	0.33
Adrenalin (pg/ml)	64 (7-344)	61 (16-99)	34 (23-79)	62 (3-157)	0.53
Noradrenalin (pg/ml)	277 (169-705)	423 (189-959)	314 (185-1295)	244 (119-526)	0.08
Renin (μU/ml)	11.3 (3.6-220)	6.9 (0-34.4)	7.0 (2.0-10.9)	8.4 (1.9-15.0)	0.5
NtproANP (ng/ml)	4.3 (2.6-21.5)	4.9 (2.0-23.2)	2.8 (1.6-37.8)	6.4 (3.7-23.1)	0.24
Vasopressin (pg/ml)	0.7 (0.2-8.8)	1.4 (0.2-2.8)	0.6 (0.3-9.4)	0.7 (0.2-8.8)	0.53

Values of mean BP and heart rate: mean (95% CI)

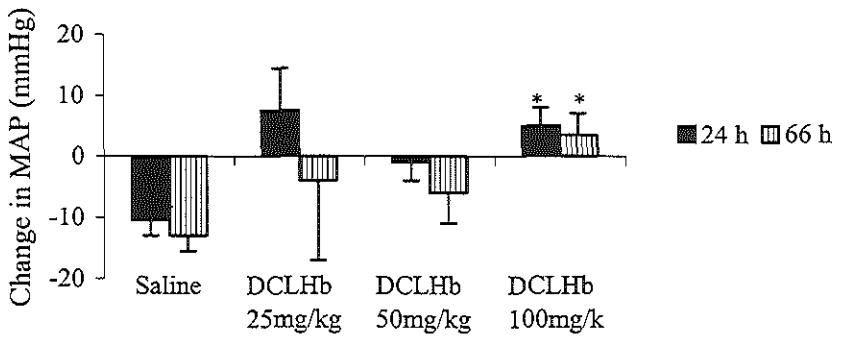
Values of neurohormones: median (ranges)

Figure 1 Bar-diagrams showing changes in MAP and HR as compared to baseline values 24 hours and 66 hours after start of infusions for the four groups of patients randomized to receive saline or DCLHb infusions

Changes in heart rate (HR) 24 and 66 hours after start of infusions compared to baseline

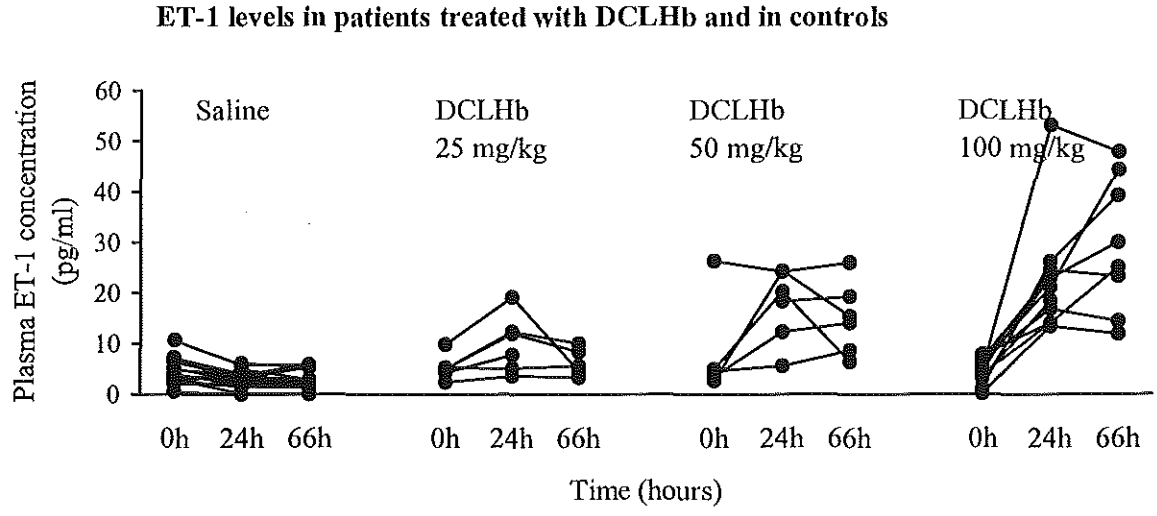


Changes in mean arterial pressure (MAP) 24 and 66 hours after start of infusions compared to baseline



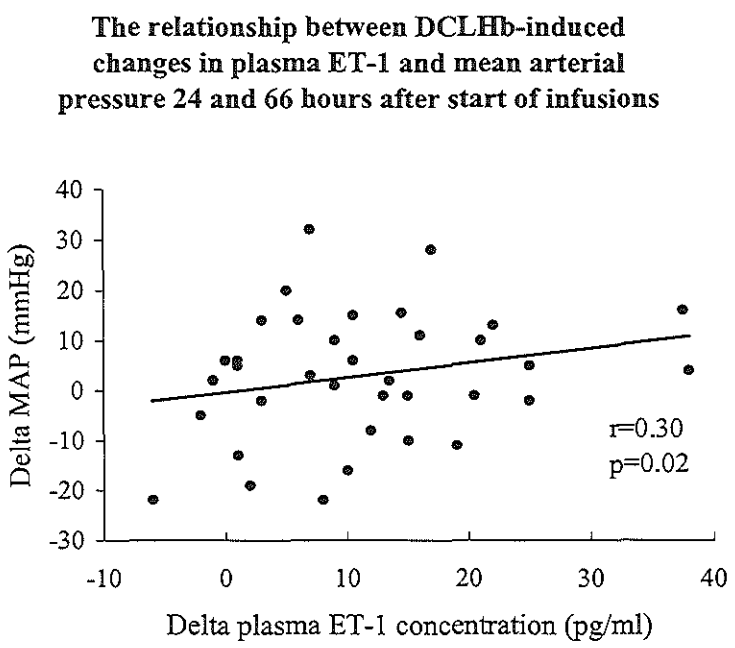
* P<0.05 for DCLHb-induced changes when compared to the placebo group

Figure 2 Line-plots showing pre-infusion values of plasma ET-1 concentration and values 24 and 66 hours after start of infusions in the individual patients of the four treatment groups



In the group of patients randomized to placebo, ET-1 values slightly decreased from 4.2 (3.1-5.3) to 2.5 (1.9-3.7) pg/ml after 24 hours (p=0.0044) and to 2.8 (1.9-3.7) pg/ml after 66 hours (p=0.0042), whereas values increased markedly and dose-dependently in response to DCLHb (Fig.2). With the highest dose of DCLHb plasma ET-1 concentration rose from 4.8 (0.1-7.8) pg/ml at baseline to 21.2 (13.4-53.2) pg/ml after 24 hours (p<0.001) and to 27.6 (11.9-47.8) pg/ml after 66 hours (p<0.001). The DCLHb-induced increments in ET-1 concentration and MAP were correlated (r =0.30, p=0.02, Fig. 3).

Figure 3 Scatterplot showing the relation between DCLHb-induced changes in plasma ET-1 concentration and mean arterial pressure 24 hours and 66 hours after start of infusions



In contrast to the marked rise in plasma ET-1 concentration, plasma concentrations of noradrenalin, adrenalin, renin, NtproANP and vasopressin did not change in response to DCLHb.

Changes in serum creatinine, BUN, liver enzymes, creatine phosphokinase, erythrocytes, leukocytes or platelets as observed in the groups of patients randomized to DCLHb treatment did not differ from changes in the control group.

Side effects that were directly related to DCLHb were transient yellow discoloration of the skin and sclerae (0/8, 1/8 and 11/11 in the low, medium and high dose group respectively) and hemoglobinuria (2/8, 6/8 and 11/11). The hemoglobinuria occurred due to excretion of DCLHb in the urine and was not associated with any impairment of renal function as assessed by serum creatinine.

The DCLHb induced effects on MAP were well tolerated. There was no excess need for anti-hypertensive treatment in comparison with the control group, nor were there any signs or symptoms of hypertensive encephalopathy or hemorrhage into the infarct. The number of fatal outcomes at three months was 3/26 (0/8, 0/8 and 3/10) in the patients randomized to placebo and 7/27 (3/8, 1/8 and 3/11) in those treated with DCLHb (dose A, B and C respectively; p=0.16). Treatment with DCLHb did not have a favorable effect on neurological outcome as assessed by the Rankin disability score.

Discussion

In this safety study, patients with an acute ischemic stroke were treated with DCLHb. DCLHb could be of potential benefit to these patients because of its ability to increase cerebral perfusion pressure as well as oxygen delivery to the brain.[9-12] DCLHb also scavenges NO, thereby possibly further reducing NO related neurotoxicity.[4,13] We investigated the involvement of ET-1 in the effect on MAP in stroke patients in response to the oxygen carrier DCLHb. Of the pressor hormones measured, only ET-1 increased progressively with increasing doses of DCLHb. In addition DCLHb-induced changes in plasma ET-1 concentration and MAP were correlated.

Observational studies have shown that hypertension occurs in three-fourths of the patients in the acute phase of a cerebral infarction.[19,20] In most instances the elevated BP decreases spontaneously in the first few days and this decrease is often most marked in the first 24 hours after the infarction.[21-23] A similar course of the BP was observed in the control patients. In the DCLHb groups this fall in

BP was attenuated and in the highest treatment group BP remained above baseline during the entire treatment period of 72 hours. The absence of fall in BP was well tolerated, but did not have a beneficial effect on survival or degree of disability.

That hemolyzed blood or hemoglobin solutions can increase BP has been known for almost 50 years,[2] but only recently evidence has accumulated that this rise in BP is mainly due to an interaction of hemoglobin with endothelium-derived vasoactive substances.[4] Oxyhemoglobin (1-3 μmol/L) has been shown to stimulate the release of ET-1 in cultured endothelial cells.[5,6,24] In awake Sprague-Dawley rats an intravenous bolus injection of DCLHb (280 mg/kg) was associated with a rapid rise in BP, which could almost completely be prevented by pre-treatment with phosphoramidon, an agent known to inhibit the conversion of inactive proendothelin to ET-1.[5] However in another study, also performed in rats, this could not be confirmed.[25] Recently, Gulati and co-investigators showed that infusion of DCLHb in rats induced a rise in the plasma and tissue concentration of ET-1. The same authors also showed that most of the hemodynamic effects of DCLHb could be blocked by the specific endothelin-A receptor antagonist BQ123.[7] These experimental studies indicate that the BP elevation induced by DCLHb, and probably also other hemoglobin solutions, is mediated at least in part by ET-1.

There is evidence from studies in man [26,27] and animals [28,29] that a modest, also called pathophysiological increase in plasma ET-1 concentration induced by exogenous administration of ET-1 is associated with a rise in BP due to a rise in vascular resistance. For example in a study performed in hypertensive subjects reported by Kaasjager et al. [27] a 4-fold increase in plasma ET-1 concentration was associated with an increase of BP of about 10 mmHg and a rise in calculated systemic vascular resistance of about 27%. With the highest infusion rate of DCLHb currently used plasma ET-1 concentration rose 4 to 5-fold. Considering the results of the aforementioned studies, it is possible that plasma ET-1 concentrations obtained in the present study were high enough to explain, at least in part, the observed rise in MAP. The fact that the correlation coefficient between DCLHb-induced increments in ET-1 and MAP was not very strong does not contradict such a contention. BP depends on numerous variables and an induced rise in BP is counteracted by the arterial baroreflex to a different extent in different patients. More information about the precise role of ET-1 in the DCLHb-induced rise in BP could have been obtained if a complementary study had been done in the absence and presence of an ET-1 receptor antagonist. Unfortunately, ET-1 receptor antagonists are not freely

available for clinical research at this moment, so we did not have the opportunity to perform such a study.

Although not investigated in the present study, interaction of DCLHb with the endothelium-derived relaxing factor NO is likely an additional mechanism by which this compound maintained BP at higher levels than in the placebo group. NO reacts rapidly with oxyhemoglobin and deoxyhemoglobin, yielding nitrite and methemoglobin and nitric oxide-hemoglobin, respectively.[30,31] This interaction of NO with hemoglobin results in a shift in vascular tone towards vasoconstriction due to an imbalance between endothelium derived vasoconstrictor and vasodilator substances. This imbalance may cause an increase in vascular resistance and BP. The rise in BP may involve ET-1 since it has been shown that this effect is blocked by ET-1 receptor antagonism.[32]

The mechanism by which DCLHb induces a rise in plasma ET-1 concentration remains to be established. It is tempting to speculate that this rise is caused by a DCLHb-induced decline in the concentration of NO. In vitro studies with cultured human endothelial cells have shown a substantial increase in ET-1 gene expression and ET-1 production after inhibition of endothelium-derived NO.[33] Furthermore, Richard et al. reported increased plasma concentrations of ET-1 after NO synthase inhibition.[34] However, these increments were only modest as compared to the striking increments in plasma ET-1 concentration observed in the present study.

Besides oxyhemoglobin, vasopressin, angiotensin II and thrombin are other factors known to stimulate the release of ET-1 from endothelial cells. In the present study plasma vasopressin levels were not affected by DCLHb, nor did the plasma concentration of renin, the rate-limiting enzyme in the biochemical cascade of the formation of angiotensin II, change. Plasma thrombin levels were not measured, but changes in the number of platelets, the main source of thrombin, did not differ from controls.

Elevated plasma ET-1 levels have been noted in serious clinical insults including eclampsia,[35] cardiogenic shock, and acute myocardial infarction.[36] The results of studies in patients in patients with acute ischemic stroke are variable. Some studies have shown a moderate increase [37,38], but in another study no rise in plasma ET-1 concentration was found.[39] In our study 16 of 53 patients (30%) had elevated baseline plasma ET-1 levels, but, with the exception of two patients, the elevations were very modest. In the patients randomized to placebo, plasma ET-1 levels significantly decreased in the successive days, which agrees with previous observations.[37,38]

The 4 to 5-fold increase in plasma ET-1 concentration, observed in the highest dose of DCLHb is considerably higher than the increase observed in patients

with an acute myocardial infarction or heart failure.[40,41] With ET-1 being a potent and long-lasting vasoconstrictor, one may wonder whether the elevated ET-1 concentrations associated with the use of DCLHb may be cardiotoxic. In our study, ECG recording and monitoring of serum creatine phosphokinase levels showed no evidence of myocardial damage. Still, we think that clinical investigators should be aware that DCLHb, and probably other hemoglobin solutions as well, can induce high plasma ET-1 concentrations. Future studies should demonstrate whether high plasma ET-1 levels are cardiotoxic, especially in subjects with a compromised coronary circulation and in situations where vasoconstrictor mechanisms are already activated as for example hemorrhagic shock, a clinical condition where treatment with a hemoglobin solution seems a logical option.

In conclusion, this study provides the first data regarding the mechanism of the hemodynamic effects in man. Infusion of DCLHb in patients with acute ischemic stroke is dose-dependently associated with high increments in plasma ET-1 levels. Though not investigated in the present study, there is experimental evidence suggesting that most likely, this increase in plasma ET-1 concentration in combination with the inactivation of NO by DCLHb underlies the observed prevention of the natural decrease in BP in these patients.

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Chapter 6

The influence of a hemoglobin based oxygen carrier, DCLHb, on cardiac parameters in patients with acute ischemic stroke

Abstract

Cardiac disease is a well-known risk factor for stroke. Conversely, cardiac disorders can be caused by stroke itself or its treatment. We have previously shown that diaspirin cross-linked hemoglobin (DCLHb), a purified human hemoglobin solution, was not beneficial when given to patients with acute ischemic stroke. The aim of our study was to determine whether the adverse effect on outcome was caused by the effect of DCLHb on cardiac parameters.

DCLHb in a dose increasing fashion (25, 50 and 100 mg/kg, n=10, 10 and 20, respectively) or placebo (n=45) was infused intravenously every 6 hours for 72 hours in patients with an acute ischemic stroke in the setting of a randomized controlled trial. We measured BP and heart rate every 15 minutes. Continuous ECG monitoring was performed during 84 hours. Standard 12 lead ECG's were made at entry, after 24 hours and once between day 3 and 7. Patients were checked for adverse events on a daily basis. A repeated physical examination was performed at 3 months. Plasma levels of CK and LDH including sub-fractions were determined regularly.

DCLHb caused a rapid rise in mean arterial blood pressure that was not accompanied by complications or excess need for anti-hypertensive treatment. Cardiac functions as assessed by ECG's, Holter registration, and physical examination were not affected. Cardiac adverse events, such as heart failure, dysrhythmias occurred equally in both groups. Dose-dependent increases of LDH and CPK were found, but without an increase in CK-MB or LDH-1 sub-fractions.

Although the clinical outcome of patients was adversely affected by treatment with DCLHb, this was not caused through adverse effect on the heart.

Introduction

Diaspirin cross-linked hemoglobin (DCLHb) is a cell-free hemoglobin based oxygen carrying solution.[1] Until recently, it was developed as a hemoglobin therapeutic for high blood loss, surgery, sepsis, hemodialysis, cardiac surgery and trauma. In several phase III clinical trials, DCLHb was not found to be efficacious and did not meet the predefined level ofsafety.[2-6] We have recently reported on our phase II study in which we assessed the safety and efficacy of DCLHb in patients with acute ischemic stroke. Outcome scale scores were significantly worse in the DCLHb group and more serious adverse events and deaths occurred in DCLHb-treated patients than in controls.[7]

A major toxicity finding in the pre-clinical studies in swine and primates had been the occurrence of focal and multi-focal myocardial lesions. These lesions were typically found 24-48 hours after volume-load doses as low as 200 mg/kg in rhesus monkeys and 700 mg/kg in pigs. Incidences in the most sensitive animal model, the rhesus monkey, were 5/5 at 700 mg/kg, 3/3 at 500 mg/kg, 3/5 at 350 mg/kg, 1/5 at 200 mg/kg and 0/5 at both 100 and 50 mg/kg. CK-MB and LDH-1 levels did not increase. No electrocardiographic (ECG) changes, dysrhythmias, decrease in cardiac output or other physiologic consequences have been associated with these lesions. Histologically the lesions are similar to those occurring after high doses catecholamines.[investigator's brochure]

In a double-blind, cross-over phase I study involving 24 healthy volunteers, participants received a single infusion DCLHb of 25 mg/kg (n=7) 50 mg/kg (n=8) and 100 mg/kg (n=7). The evaluation of cardiac function by 12-lead ECG, echocardiography and 48-hour Holter monitoring revealed no treatment related abnormalities. Dose dependent increases in mean arterial pressure (MAP), were reported with a concomitant decrease in heart rate.[8]

Recent studies in humans in different populations, such as patients undergoing cardiac surgery or abdominal aortic surgery, have shown that DCLHb increases BP, systemic vascular resistance index (SVRi) and slightly decreases cardiac output.[2,4]

The primary aim of our study was to assess whether the adverse effect of DCLHb on clinical outcome in patients with acute ischemic stroke, was mediated through adverse effects on the heart.

Patients and methods

The trial was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice for Trials on Medicinal Products. For participation, written informed consent from the patients or their family was required. The participating centers were the departments of Neurology in the University Hospitals of Heidelberg, Helsinki, Leuven and Rotterdam. The Medical Ethics Committees approved the protocol. Patients received all standard care and treatment, including prophylactic medication such as acetylsalicylic acid and heparin. If patients were already on anti-hypertensive medication, this medication was continued, without any change, throughout the study. New anti-hypertensive therapy was not initiated unless BP exceeded 220/135 mmHg for an hour.

Patients

Patients with clinical symptoms of an acute ischemic stroke, in the anterior circulation were eligible if they were older than 20 years; could be treated within 18 hours after start of symptoms; and were likely to survive for at least 3 months. Patients had to be alert or arousable by stimulation to obey, answer or respond to verbal commands. Brain computed tomography (CT) scan had to be normal or compatible with a recent infarction. Exclusion criteria were: any major disabling disorder interfering with the assessments; pregnancy or lactation; an evident hematological cause of the symptoms; congestive heart failure or acute myocardial infarction; systolic BP > 230 mmHg or diastolic BP >130 mmHg; renal or liver disease; spontaneous improvement of symptoms by at least 2 grades on the modified Rankin scale; and previous enrollment in this study or enrollment in another investigational trial within 30 days. Eighty-five patients were enrolled between August 1994 and November 1996.

Drugs

DCLHb was derived from human erythrocytes and subjected to rigorous viral inactivation and removal procedures.[1] DCLHb was prepared and provided by Baxter Healthcare Corp., Deerfield, IL, USA (lot numbers 94A21AD11 through 95L08AD11).

Treatment regimen

Patients were randomly assigned to DCLHb or saline (placebo) in a 1:1 ratio. The study was single blind because of the prominent color of the drug and the

difficulty to manufacture a proper placebo. Three doses were tested: 25, 50 and 100mg/kg 10% DCLHb (n=10, 10 and 20 respectively) or equal volume of saline (n=45) every 6 hours for 72 hours (12 doses) intravenously at a rate of 2 ml/minute. The volumes infused per 24 hours were approximately 70 ml for the lowest, 140 ml for the intermediate and 280 ml for the highest dose of DCLHb.

Assessments

Baseline assessment consisted amongst others of a medical history, general physical and neurological examinations and an ECG. Blood pressure and HR were measured every 15 minutes with an automatic, oscillometric blood pressure device during the 72-hour treatment period.

The physical examination was repeated at day 3, 7, 14 and at 3 months. At the 3 month follow-up, the presence of an irregular pulse was interpreted as the presence of AF. Patients were checked for adverse events on a daily basis during their hospitalization and again at the 3 month visit. Creatine kinase (CK, total and fractionated) and lactate dehydrogenase (LDH, total and fractionated) were measured prior to infusion and on day 1,2,3 and 7. Safety was further monitored by ECG's on day 1 and once between day 3 and 7 and 84-hour Holter monitoring, started at study entry.

The ECG's and Holter-tapes were assessed by cardiologists who were blinded to treatment. ECG's were assessed as normal or abnormal. The latter was further specified into new ischemia, non-specific ST- and T wave changes, left ventricular hypertrophy, AF, supraventricular dysrhythmias other than AF, ventricular dysrhythmias, conduction disorders and a category other. The latter consisting mainly of old infarctions and atrial enlargement. A single ECG could carry any number of diagnoses.

Holter-analysis consisted of rhythm-analysis as well as ST-analysis. Holter-registrations were also judged to be normal or abnormal. When abnormal, the results were further specified into ST-segment depressions and/or ischemia, AF, atrial or ventricular arrests flutter, Q-T interval prolongation, and a category other in which mostly of many premature atrial or ventricular complexes (PAC's or PVC's), significant sinustachycardia or sinusarrythmia. A chest X-ray was performed once during the study period.

Statistics

For statistical analysis we used SPSS, version9.0. Values are expressed as mean ± SD unless otherwise indicated. Values of MAP and HR are presented as mean and 95% confidence interval. Comparison of data between the four groups of

patients was done by analysis of variance (ANOVA) for MAP and HR. Cardiac enzymes were not normally distributed and were analyzed using the Wilcoxon signed-rank test. For comparison between two groups Student's unpaired t-test or chi-squared analysis was used as appropriate. A p-value < 0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics

Relevant patient characteristics are presented in table 1. More extensive baseline characteristics were published in an earlier paper (see chapter 3).[7] The groups were well-matched with respect to cardiovascular baseline variables.

Table 1 Characteristics of patients with acute ischemic stroke randomized to DCLHb or normal saline

	Saline	**DCLHb**	**P-value**
Number of patients	45	40	
Age (years)	65 ± 15	68 ± 13	0.40
Cause of stroke			
Large vessel	10 (22)	9 (23)	1.00
Small vessel	13 (29)	12 (30)	1.00
Cardioembolic	9 (20)	10 (25)	0.71
Other	13 (29)	9 (23)	0.67
Medical History			
Angina pectoris	4 (9)	8 (20)	0.25
Atrial fibrillation	7 (16)	6 (15)	1.00
Hypertension	18 (40)	20 (50)	0.48
Myocardial infarction	6 (13)	3 (8)	0.49
Systolic BP	155±20	158±24	0.52
Diastolic BP	85±13	84±13	0.72
Heart rate	75±15	80±13	0.18

Values in parentheses are percentages

Prior Medication

Twenty patients did not use any medication in the two weeks prior to study entry. Six had been allocated to receive DCLHb, 14 to saline. There were no significant differences in medication used at study entry, specifically no difference in use of anti-hypertensive medication, diuretics, anti-coagulants such as heparin and warfarin, platelet aggregation inhibitors, anti-arrhythmic and inotropic medication.

Medication used during the first week

Anti-hypertensive medication such as aldosterone antagonists, beta blocking agents, calcium channel blockers, clonidine, converting enzyme blockers) were used by 30 DCLHb patients and 35 saline patients and coronary vasodilators by 3 patients in each group. Diuretics for heart failure and hypertension were used at baseline in 9 DCLHb vs. 6 control patients, and during treatment in 16 versus 8, respectively ($p=0.04$). . We found no relationship between the occurrence of clinical adverse events and the use or type of co-medication.

ECG's and Holter analysis

There were no significant differences between the treatment and control group in the baseline ECG's, or in the follow-up ECG's. The results are given in table 2.

The results of the Holter analysis are summarized in table 3. Again there were no differences in incidences between the two groups. ST-dysfunction or ischemia occurred in 5 saline and 3 DCLHb patients, but mostly subclinically. See also under adverse events.

Adverse events

Cardiac arrests did not occur in either group.

Heart failure occurred in 3 DCLHb and 3 saline within the first 14 days. In all but one (saline) patient, heart failure was not present at baseline and newly developed during the study. Cardiac failure was of mild to moderate severity, resolved in 3 and persisted in 2 (one in each group) patients. Another seven patients (4 DCLHb, 3 saline) already had heart failure at the time of admission.

Myocardial ischemia or injury, defined as the development of electrocardiographic evidence of T-wave inversion, ST-segment elevation or depression in leads corresponding to or opposite the area of involvement or elevated CK with elevated CK-MB, occurred in 4 saline and 0 DCLHb patients.

Table 2 Results of the repeated ECG recordings

	ECG pre-treatment		ECG at 24 hours		ECG between day 3-7	
	Saline	**DCLHb**	**Saline**	**DCLHb**	**Saline**	**DCLHb**
Number of patients *	45	40	45	38	45	35
Result abnormal	28	27	26	23	27	25
Supraventricular rhythm disorder **	2	1	1	1	2	0
Atrial fibrillation	4	6	5	5	5	8
Ventricular rhythm disorder	0	1	1	0	0	0
Conduction delay	9	9	8	7	10	5
New ischemia	1	0	1	0	3	1
Left ventricular hypertrophy	3	3	0	1	1	2
Non-spec. ST- and T-wave changes	14	16	12	14	10	14
Other ***	14	12	12	10	11	12

* At 24 hours: ECG not done in 4 saline and 7 DCLHb patients, so last observation was carried forward. 2 DCLHb patients were dead, of whom 1 had AF. At day3-7: ECG not done in 9 saline and 4 DCLHb patients, so last observation was carried forward. 5 DCLHb patients were dead, of whom 2 had AF

** Other than atrial fibrillation

*** PVC's, PAC's, old ischemia, atrial enlargement

Table 3 Results of 84 hour Holter-registration

	Saline	DCLHb	P-value
Number of patients*	41	40	
Result abnormal	13	18	.25
ST-dysfunction, ischemia	5	3	.71
Atrial fibrillation	4	10	.08
Ventricular or atrial arrests	1	3	.35
Q-T interval prolongation	5	1	.20
Supraventricular arrhythmia	3	2	1.00
Ventricular arrhythmia**	0	5	0.02
Other disorders***	3	7	0.19

* Due to technical failure reports in four saline patients are missing, and 2 of these had AF on their ECG

** 4 Ventricular tachycardia, 1 ventricular flutter

*** Many PAC's or PVC's, marked sinustachycardia, sinus arrhythmia

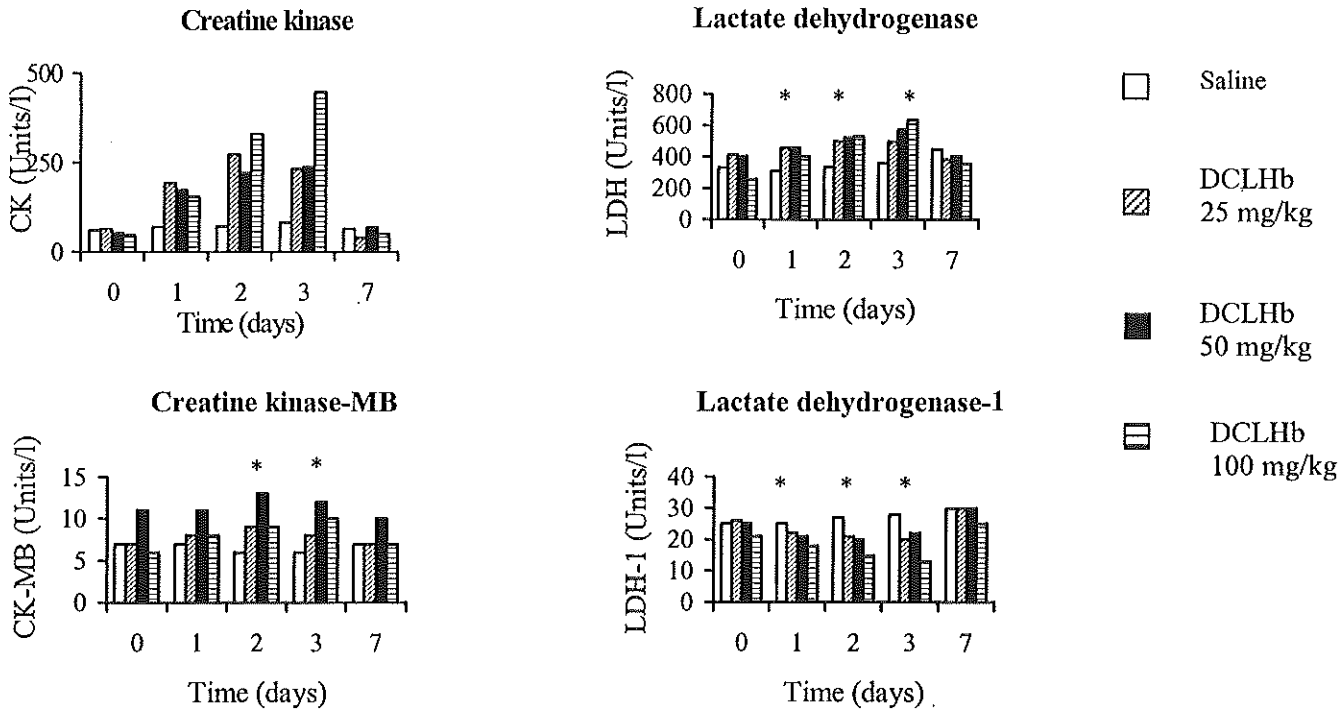
Cardiac laboratory parameters

During the first 3 days, concentrations of CPK were increased in the active treatment groups, see figure 1. CK-MB levels were also elevated, but remained within the normal range of 0-14 U/l. LDH levels were elevated pre-infusion in the low and intermediate dose group, but further increased after treatment. The LDH sub-fraction originating from the heart, LDH-1, however, was dose-dependently decreased compared with controls. All laboratory abnormalities were clinically asymptomatic and disappeared within a week.

Blood pressure and heart rate

DCLHb produced a rapid rise in mean arterial pressure (MAP). The magnitude of the increases caused by the different doses was similar, but the duration of the pressor was dose-dependent. Severe hypertension that needed pharmacological intervention occurred in 3 patients treated with DCLHb vs. 3 in the saline group. Heart rates were similar in both groups and did not change significantly from baseline. We have reported this in more detail elsewhere (see chapter 4).[9]

Figure 1 Median values of CK, CK-MB, LDH and DH-1 levels during the first week



* Denotes a significant difference between groups with ANOVA

Discussion

We conducted a safety study of DCLHb in patients with acute ischemic stroke. We have previously reported that treatment with DCLHb was an independent predictor of an unfavorable outcome at 3 months, but the cause of this unexpected adverse effect remained unclear.[7] We also showed that DCLHb caused a rise in ET-1 levels and blood pressure, but did not find evidence that this caused stagnation of recovery stagnate.[10] In the present study, we wished to determine whether the adverse effect on outcome was mediated through an adverse effect on the heart. We studied cardiac functions extensively through ECG's, 24-hour Holter monitoring, laboratory tests and patients were checked for adverse events. We found that none of the cardiac function parameters differed between treated patients and controls. The Holter-analysis revealed more ventricular arrhythmias in the treated group, but these were mostly ventricular tachycardias not leading to discomfort or sequelae. We found a dose-dependent transient increase in LDH en CPK, but without an increase of the cardiac sub-fractions. The previously reported increase in BP was well tolerated.

These results are in concordance with experimental evidence and clinical trials in humans. Lamy et al. performed a trial in which DCLHb was infused as an alternative to blood transfusion after cardiac bypass surgery in 104 patients, and the control group (n=105) received packed red blood cells. Although the number of deaths was balanced in this study, there were more adverse and serious adverse events in DCLHb recipients. However, myocardial iso-enzymes (CK-MB and LDH-1) and Troponin T increase from baseline was smaller in the DCLHb group. These results suggested that there was no evidence that DCLHb caused myocardial stress or damage. DCLHb caused a rise in systemic and pulmonary artery pressures, but no adverse clinical sequelae were reported as a consequence of the increase in blood pressure.[4]

In a controlled, randomized, dose-incremental trial by Garrioch et. al., 34 patients undergoing elective repair of an abdominal aortic aneurysm, the effects of a single infusion with 50, 100 or 200 mg/kg DCLHb or Ringer's lactate was studied. DCLHb caused an increase in arterial pressure, with a small decrease in cardiac index, while heart rate did not change.[2]

In conclusion, infusion of low doses of DCLHb over 3 days did not harm the cardiac functions as assessed in this study. Therefore, the adverse effect of

DCLHb on outcome among patients with acute ischemic stroke can not be explained by an adverse effect on the heart.

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Chapter 7

Prevention of early recurrences in acute stroke

Saxena R, Koudstaal PJ. Prevention of early recurrences in acute stroke. In: Acute stroke treatment, 2nd edition (2002). Bogousslavsky J editor. Dunitz M, Cambridge University Press. *(In press)*

Abstract

The diagnosis of early recurrent stroke is not always easy in patients with acute ischemic stroke, since up to 20% of patients show spontaneous fluctuations, some of which may be caused by repeated embolism, but many have other causes, including systemic disorders. The risk of recurrent stroke seems to be higher in patients entered in clinical trials than in population based studies or stroke data banks. The aggregate results of randomized clinical trials of heparin in acute ischemic stroke suggest that this treatment reduces the risk of early recurrence by about one quarter, but also that this benefit is completely offset by an equal risk of hemorrhage. Present data neither suggest a clear subgroup, in particular those with cardioembolic stroke, with a higher risk of early recurrence, nor one that specifically benefits from early treatment with anticoagulants. Findings from two large trials show that aspirin is effective in reducing death and early recurrence (10 events prevented per 1000 treated) and safe.

Introduction

One of the feared complications in the acute phase of ischemic stroke is early recurrence of stroke. The frequency of early recurrent stroke varies in different studies and is often believed to be higher in patients with a potential cardiac source of embolism. It has also been suggested that recurrent stroke is often more severe than the initial event and is associated with increased mortality and disability. The value of antithrombotic treatment, especially heparin and aspirin, has recently been addressed in several studies. This chapter aims to review the definition and epidemiology of early recurrent stroke and to focus on the benefits and risks of antithrombotic therapy in the prevention of this complication.

Definition of early stroke recurrence

Although nearly all patients who survive an acute ischemic stroke eventually show some degree of clinical improvement, neurological symptoms are often unstable during the early phase. Patients may show rapid deterioration ('progressive stroke'), fluctuations ('stuttering stroke') or secondary deterioration after initial improvement ('recurrent stroke'). These various forms of worsening are difficult to define and often overlap. Several studies have shown that spontaneous fluctuations occur in about 20% of carotid territory ischemic stroke and in up to 50% of vertebrobasilar ischemia.[1-3] Systemic disorders, such as myocardial ischemia and metabolic disturbance, are thought to play a role in fluctuation and late deterioration. Surprisingly, the large majority of studies focusing on early recurrent stroke reviewed in this chapter have not addressed this diagnostic problem. In fact, this issue is briefly discussed in only one study[4] and very few studies have attempted to define recurrent stroke (see table 1).[5-10] Some have included worsening of a preexisting deficit, while others have accepted only new deficits that occurred in a different anatomic or vascular territory or were of a different stroke subtype than the index stroke. It is obvious that the lack of an uniform definition of recurrent stroke strongly influences the existing data on the epidemiology and medical prevention of this complication.

Table 1 Definition of recurrent stroke in various studies

Author	Definition
CESG (1983)[5]	Clinical evidence of recurrent systemic or cerebral embolism (no further details
Ramirez-Lassepas (1986)[6]	New infarct and no extension of index cerebral infarct (no further details)
Sacco (1989)[7]	A cerebrovascular event within 30 days after the index stroke that clearly resulted in a new deficit and occurred in a different anatomic or vascular territory or was of a different subtype than the index stroke
Sandercock (1992)[8]	Clear clinical evidence of the sudden onset of a new neurological deficit, or an increase in an existing deficit, for which no explanation other than a recurrent stroke could be found
Hornig (1993)[9]	Sudden worsening of preexisting focal deficit, or new symptoms without other reasons, e.g. epileptic seizure
Berge (2000)[10]	A clinical sudden and persistent (>48h) deterioration occurring after the first 48h following stroke onset, which equals a loss of 3 or more points in the SSS, excluding cerebral hemorrhage, intercurrent illness and effect of medication

Table continued on next page

Table 1 Definition of recurrent stroke in various studies, *continued*

Author	Definition
Petty (2000)[17]	A new neurological deficit fitting the definitions of ischemic or hemorrhagic stroke, occurring after a period of unequivocal neurological stability or improvement lasting≥24h and not attributable to edema, mass effect, brain shift syndrome, or hemorrhagic transformation of the incident cerebral infarction
Other publications on the subject	Not explicitly stated

Incidence of early recurrence

The frequency of early recurrence has recently been reported in several large acute stroke trials, in which the efficacy of aspirin and/or heparin was investigated. Table 2 summarizes the early stroke recurrence rate. Because of the inherent methodological drawbacks of retrospective, non-randomized and uncontrolled studies only prospective, randomized, controlled studies, in which recurrent stroke was a predefined outcome event are shown. Data for untreated groups are presented if available or otherwise data for patients treated with aspirin. Patients were randomized within 24-48 hours of stroke onset and the observation period varied between 7 and 28 days after the index stroke. The International Stroke Trial (IST),[11] Chinese acute stroke trial (CAST)[12] and the Trial of ORG 10172 in Acute Stroke Treatment (TOAST)[13] and FISS[14] included all types of ischemic strokes. However, physicians were free to exclude patients whom they believed to require anticoagulation, for instance patients with atrial fibrillation (AF) and a presumed high risk of stroke recurrence. In the Heparin in Acute Embolic Stroke trial (HAEST) only patients with acute ischemic stroke and AF were randomized.[10] Table 2 shows that the risk of early recurrence varies between 0.09% per day in CAST patients (7% AF) and 0.68% per day in

the small CESG trial. In the HAEST study the recurrence risk was also high (0.53%/day). In that study, recurrent ischemic stroke was a primary outcome event and patients were assessed for neurological deterioration every day during the first week and every other day during the second. Another important factor is the time between stroke onset and inclusion in studies. In IST and CAST, patients could be randomized up to 48 hours after onset of symptoms, so some very early recurrences may have been missed.

Since patients from clinical trials in general represent a selected group, information from stroke data banks and population based studies are important. These are also summarized in table 2. The disadvantage of some of these studies is, however, that they were retrospective, leading to potential underreporting of recurrent stroke and to uncertainty regarding dose, duration and the clinical grounds of antithrombotic therapy. The observation period in all these studies was 30 days.

Sacco et al studied 1,273 patients with acute ischemic stroke who were entered into the NINDS-Stroke Data Bank, a prospective, observational study.[7] The risk of early recurrence was 3.3% in 30 days. Gustafsson followed 276 consecutive patients admitted to a population-based stroke unit in Stockholm.[15] Within 1 month, 2.9% had a recurrent stroke. In the Rochester Epidemiology Project (REP), 1,382 patients with first ever cerebral infarction between 1960 and 1984 were tracked.[16] The recurrence risk within 30 days was 2%. In a second report, patients between 1985 and 1989 were studied retrospectively and the 30 day recurrence rate was 4.8%.[17] In the Oxfordshire Community Stroke Project (OCSP), 545 patients with first ever ischemic stroke were followed.[8] The risk of early recurrence within 30 days was 3.5%. However, some recurrences may have occurred unnoticed as many of these patients stayed at home and were not carefully studied in the acute phase.

In conclusion, the risk of recurrent stroke seems to be higher in patients entered in clinical trials than in population based studies or stroke data banks. Patients in trials were randomized early after stroke onset and probably followed more carefully. On the other hand, they probably represent a selected group of high risk patients. It is also possible that the longer observation period in the population based and stroke data bank studies has diluted the early recurrence rate.

Table 2 Risk of early stroke recurrence in untreated or aspirin-treated patients from randomized, controlled acute stroke trials and patients from stroke data banks and community studies, with unspecified treatment

Study	Patients (n)	Observation Period (days)	Recurrences (%) During observation	Per day	Time to inclusion (h)
Clinical trials					
CESG (19830[5]	21	14	9.5	0.68	48
FISS (1995)[14]	105	10	5.7	0.57	48
IST (1997)[11]	9718*	14	3.8	0.27	48
CAST (1997)[12]	10552	28	2.5	0.09	48
TOAST (1998)[13]	631	7	1.4	0.20	24
HAEST (2000)[10]	225#	14	7.5	0.53	30
Stroke data banks and community studies†					
NINDS-SDB (1989)[7]	1273	30	3.3	0.11	unknown
Gustafsson (1991)[15]	276	30	2.9	0.10	unknown
REP '60-'84 (1992)[16]	1382	30	2.0	0.07	unknown
OCSP (1992)[8]	545	30	3.5	0.12	unknown
REP'85-'89 (2000)[17]	442	30	4.8†	0.16†	unknown

* *Half were treated with aspirin*
\# *All were treated with aspirin*
† *Procedure related strokes were excluded*

Risk factors for early recurrence

All of the abovementioned population studies have also tried to identify risk factors for early stroke recurrence. Some authors have compared the recurrence rate for specific etiological stroke subtypes. Sacco et al prospectively studied determinants of early recurrence in 1,273 patients with ischemic stroke from various causes.[7] A total of 40 patients (3.3%) had a recurrence within 30 days. Procedure related strokes were not included. Patients with atherothrombotic infarction (ATH) had the highest risk of very early recurrence (7.9%), followed by patients with presumed cardioembolic stroke (CES, 4.3%), infarction of undetermined cause (IUC, 3.0%), and lacunar infarcts (LAC, 2.2%). Petty et al reviewed records of 442 patients with first ever ischemic stroke from the Rochester Epidemiology Project and used the same etiological subclassification.[17] Twenty-five patients (5.6%) had a recurrent ischemic stroke within 30 days and there was a significant difference between stroke mechanisms and the recurrence rate: ATH: 18.5%; CES: 5.3%; IUC: 3.3% and LAC:1.4%. Procedure related strokes were included and it is noteworthy that 4 of the 13 recurrences in the ATH group were iatrogenic (angiography, carotid endarterectomy).

As the early recurrence rate is often believed to be higher in patients with a potential cardiac source of embolism, data for patients with presumed cardio-embolic stroke, compared to those without cardioembolic stroke are shown in table 3. In IST, 3,169 (17%) of 18,451 acute stroke patients had AF at study entry.[11] Patients with AF had a higher risk of early death, which could be explained by older age and larger initial strokes in AF patients, but not by a higher risk of early recurrent ischemic stroke, although slightly more patients with AF died from a fatal recurrent stroke of ischemic or unknown type (1.3% vs. 0.9%; Odds Ratio 1.7, 95% Confidence Interval 1.2-2.4) (unpublished data). Three quarters of IST patients received heparin and/or aspirin and one quarter no antithrombotic therapy. The rate of early recurrent stroke was equally frequent in both groups (3.9% in AF against 3.3% in SR; Odds Ratio 1.2, 95% Confidence Interval 1.0-1.5) when all treatment groups were combined. The IST data are potentially weakened by the possibility of selection bias, since patients with AF and a high risk may have been treated with anticoagulants and not have been entered into the study. On the other hand, the frequency of AF of 18%, which is very similar or even higher than that found in other series of stroke patients, does not point in that direction. The data for the patients who did not receive heparin are given in table 3. In these patients (half received no

antithrombotic treatment and the others received aspirin) the risk of stroke recurrence was somewhat higher than in patients without AF.

In the TOAST trial no difference between patients with and without CES was found.[13]

The HAEST and CESG studies are not mentioned in table 3, as all patients had a cardioembolic stroke. In the HAEST study all patients had AF and the recurrence rate was high (7.5%/14 days).[10] Furthermore, recurrent stroke was a primary outcome event in this trial and patients were rigorously checked for recurrences. In CESG, 2 of 21 patients (9.5%) had a recurrence.[5] In CAST,[12] 7% of patients had AF and in FISS,[14] 13% had a CES , but recurrence rates were not reported separately for these groups.

Epidemiological studies have also focused on differences in the risk of early recurrence between patients with presumed cardioembolic stroke and those with a likely vascular cause (see table 3). Two population studies discussed earlier, OCSP and REP, showed no clear evidence of a higher risk in patients with a cardiac cause.[7,17] In the Oxfordshire Community Stroke Project, the prevalence of AF was 98 (18%) among 545 patients with first ever stroke.[8] Twenty-one patients with AF received anticoagulants at some time after the stroke. The risk of early recurrence within 30 days was 1% in AF patients against 4% in patients with sinus rhythm. A potential weakness of the study is that cardiac screening was very rarely performed which may have led to an underestimation of the prevalence of cardiac disease. Furthermore, some recurrences might have been missed as many patients stayed at home and were not carefully studied in the acute phase. In the first study from the Rochester Epidemiology Project, 318 (23%) of 1,382 patients with first ever cerebral infarction had at least one major potential cardiac source of emboli.[16] AF was present in 19% of all cases. The recurrence risk within 30 days was 2%, both in patients with or without a cardiac abnormality. Details on antithrombotic treatment were known only in the subgroup of patients who survived the first week. Slightly less than half of them were treated with heparin, warfarin or both within the first 7 days. The 30 day stroke recurrence rate was 3% for treated vs. 2% in untreated patients. Gustafsson also did not find a significant difference in the 14 day recurrence rate: 4/88 in AF patients versus 4/188 in patients with sinus rhythm.[15]

Table 3 Risk of early stroke recurrence in patients with or without cardio-embolic stroke (CES) in untreated patients or aspirin-treated patients from randomized, controlled trials and patients from stroke data banks and community studies, with unspecified treatment

Study	Cardio-embolic stroke	Non-cardio-embolic stroke	Observation Period (days)
Clinical trials			
IST (1997)[11]	1612 (all AF)	8105#	14
TOAST (1998)[13]	123 (52 AF)	508	7
Stroke data banks and community studies†			
NINDS-SDB (1989)[7]	246	958	30
Gustafsson (1991)	88 (all AF)	188	30
REP '60-'84 (1992)[16]	318	1064	30
OCSP (1992)[8]	76 (all AF)	448	30
REP'85-'89 (2000)[17]	132	310	30

Half were treated with aspirin
† Procedure related strokes were exclude

Table 3 Risk of early stroke recurrence in patients with or without cardio-embolic stroke (CES) in untreated patients or aspirin-treated patients from randomized, controlled trials and patients from stroke data banks and community studies, with unspecified treatment, *continued*

Recurrence rate CES (%)		Recurrence rate non-CES (%)		
During observation	**Per day**	**During observation**	**Per day**	**OR (95% CI)***
4.9	0.35	3.6	0.26	1.38 (1.06-1.79)
1.6	0.23	1.4	0.20	1.18 (0.26-6.25)
4.1	0.14	3.1	0.10	1.31 (0.59-2.84)
4.5	0.15	2.1	0.07	2.2 (0.46-10.94)
2.0	0.07	2.0	0.07	0.96 (0.34-2.5)
1.0	0.03	4.0	0.13	0.25 (0.02-1.7)
4.85†	0.16†	5.2†	0.17†	0.91 (0.34-2.56)

* Cardio-embolic stroke (CES) vs. non-cardio-embolic stroke

There may also be differences in the recurrence rate between various types of underlying cardiac disease, but the available data are insufficient to determine major differences with the possible exception of rheumatic heart disease, which carries a higher risk, and atrial fibrillation, which is more benign.[18] Yasaka and colleagues studied risk factors for early recurrence in 227 patients with cardioembolic stroke, none of whom were treated with anticoagulants.[19]

A quarter of the study population had rheumatic heart disease. Recurrent brain or systemic embolization within 14 days occurred in 31 (13.7%) and 19 (8.4%) patients, respectively, and was associated with a higher mortality. The presence of rheumatic heart disease, prosthetic valves and intracardiac thrombi were associated with recurrent emboli. The incidence of rheumatic heart disease, however, has strongly declined in the western world. In the Rochester Epidemiology Project '60-'84 cardiac valve disease and congestive heart failure (but not AF) were independent predictors of recurrent stroke.[16]

In conclusion, there is contradictory evidence whether a cardiac source of embolism, especially AF, is associated with a higher risk of early stroke recurrence. The main disadvantage of clinical trials is that they represent a selected group, whereas the potential impact of antithrombotic treatment is an uncertain factor in epidemiological studies.

Some other factors have been found to be associated with the risk of early recurrence. Sacco found that a history of hypertension and diabetes mellitus as well as diastolic hypertension and an elevated blood sugar on admission and increased weakness scores were associated with early recurrence in a logistic regression analysis.[7] Yasaka also reported low plasma levels of antithrombin III, dehydration to be associated with recurrent stroke.[19] Studies concentrating on late stroke recurrence have found that a history of hypertension, ischemic heart disease, diabetes and previous thromboembolism were associated with a higher risk.[20-22] These data suggest that risk factors for early and late recurrence are strikingly similar.

Prevention of early recurrence

Immediate use of antithrombotic therapy might reduce the risk of early recurrent stroke, but may also increase the risk of intra- and extracranial hemorrhage. Older clinical trials, mostly non-randomized and retrospective, have suggested a reduction of the absolute risk of early recurrence by anticoagulants from 15% to 5% during the first month.[18] We shall concentrate

on prospective, randomized, controlled trials addressing the safety and efficacy of antithrombotic treatment in acute stroke, in which recurrent stroke and intracranial hemorrhage were predefined endpoints and patients were randomized within three days of stroke onset. The results are summarized in figures 1-3.

In 1960, Marshall and Shaw published a clinical trial of 51 patients with a clinical diagnosis of ischemic stroke of less than 72 hours duration and without a potential source of embolism in the heart.[23] Patients were randomized to open label treatment with 3 doses of 12,500 units unfractionated heparin plus the oral anticoagulant phenindione twice daily aiming at PT ratio between 2 and 3, or to no treatment. Two patients in the control group and none in the anticoagulants group had an early recurrent event within 6 weeks after randomization. Three treated patients died of cerebral hemorrhage vs. 1 untreated patient.

Low-molecular-weight heparins (LMWH) were tested in the FISS[14], TOAST[13] and FISS-bis study.[24] In1995, Kay et al published the double-blind, placebo-controlled FISS trial in which 312 Chinese patients with acute ischemic stroke were treated with subcutaneous LMWH, either low-dose or high-dose, or placebo within 48 hours of stroke onset.[14] Patients were younger than 80 years and did not have valvular heart disease necessitating anticoagulant therapy. Treatment lasted 10 days. The main measure of outcome, clinical condition at 6 months, was significantly improved in both actively treated groups. Early recurrent stroke during the treatment period occurred in 1 patient receiving the high-dose, 2 in the low-dose and 5 in the placebo group. Early hemorrhagic transformation was found in 5 patients in the high-dose group, 7 in the low-dose, and 9 of the placebo patients. Symptomatic hemorrhagic transformation occurred in respectively 0,2 and 1 patients.

In an attempt to reproduce the results of FISS, a replication study with a larger number of patients (placebo: n=250; low-dose LMWH: n=271; high-dose LMWH: n=245) was undertaken.[24] In 1998 FISS-bis was published and there was no overall benefit of either heparin dose on clinical condition at 6 months. Recurrent stroke was not a specified outcome event and was not reported in the publication.

Figure 1 The efficacy of anticoagulant treatment in the prevention of early recurrent stroke in patients with acute ischemic stroke

Study	Events/patients Treatment	Control	OR (95% CI)
CESG (1983)	0/24	2/21	0.11 (0.01-1.85)
Marshall (1960)	0/26	2/25	0.12 (0.01-2.05)
FISS (1995)	3/203	5/105	0.27 (0.06-1.20)
HAEST (2000)	19/224	17/225	1.13 (0.55-2.36)
TOAST (1998)	10/638	11/628	0.89 (0.35-0.28)
IST (1997)	283/9716	370/9717	0.76 (0.65-0.89)
Total	315/10831	407/10721	0.76 (0.65-0.88)

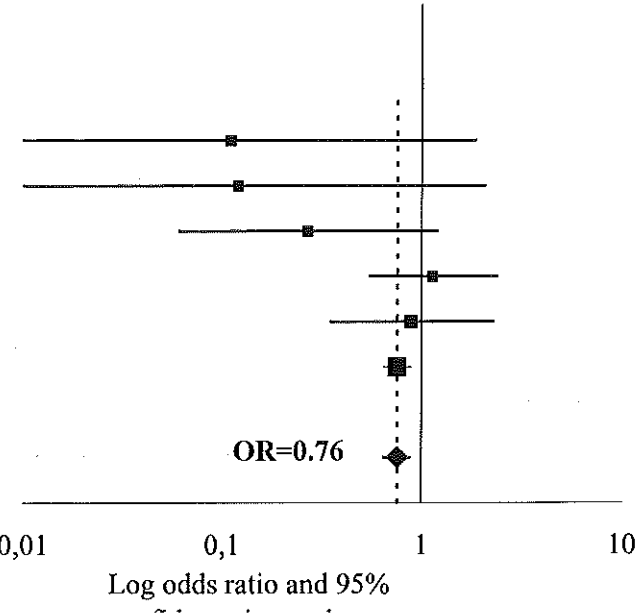


Figure 2 Number of symptomatic intracranial hemorrhages in patients with acute ischemic stroke and anti-coagulant treatment

Study	Events/patients Treatment	Control	OR (95% CI)
CESG (1983)	0/24	0/21	not applicable
Marshall (1960)	3/26	1/25	3.13 (0.26-330)
FISS (1995)	2/203	1/105	1.04 (0.92-1.16)
HAEST (2000)	6/224	4/225	1.52 (0.42-5.46)
TOAST (1998)	14/638	4/628	3.5 (1.07-12.64)
IST (1997)	120/9716	41/9717	2.95 (2.04-4.16)
Total	145/10831	51/10721	2.83 (2.03-3.96)

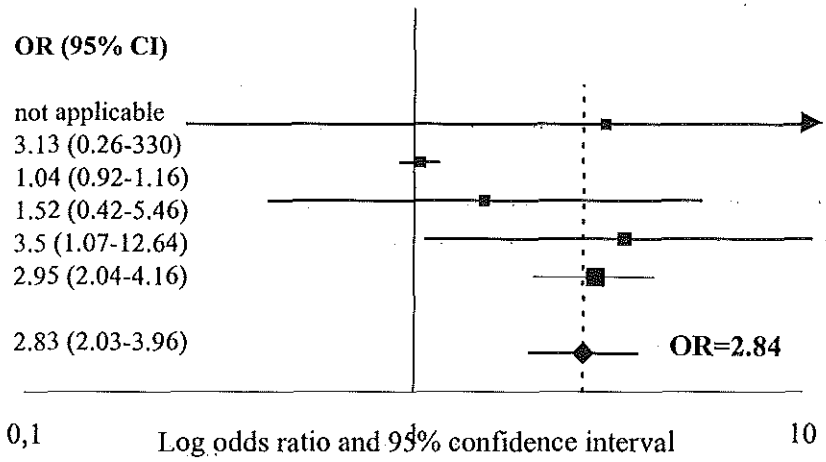
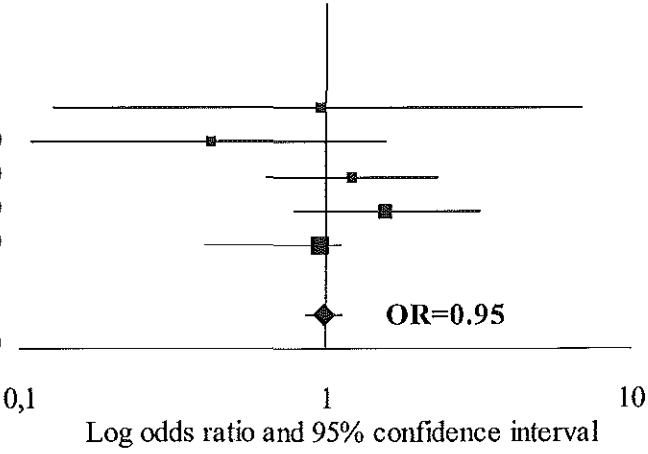


Figure 3 Number of recurrent ischemic strokes and symptomatic intracranial hemorrhages in patients with acute ischemic stroke and anticoagulant treatment

	Events/patients		
Study	**Treatment**	**Control**	**OR (95% CI)**
CESG (1983)	0/24	2/21	not applicable
Marshall (1960)	3/26	3/25	0.96 (0.13-6.86)
FISS (1995)	5/203	6/105	0.42 (0.11-1.59)
HAEST (2000)	25/224	21/225	1.22 (0.64-2.35)
TOAST (1998)	24/638	25/628	1.59 (0.79-3.23)
IST (1997)	403/9716	411/9717	0.97 (0.4-1.13)
Total	460/10831	458/10721	0.95 (0.83-1.09)



The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) evaluated the LMWH danaparoid among 1,281 patients with ischemic stroke treated within 24 hours of stroke onset.[13] Danaparoid was given intravenously and treatment was continued for 7 days. The primary measures of were the Glasgow Outcome Scale and the Barthel Index at 3 months. Within 10 days of onset of treatment, the risk of recurrent ischemic stroke was similar in both groups (10/638 vs.11/628 in controls). Serious intracranial bleeding events occurred in 14 patients given ORG 10172 (15 events) and in 4 placebo-treated patients (5 events, p=.05). Treatment with ORG 10172 was not associated with an improvement in favorable outcome at 3 months.

The efficacy of antithrombotic treatment in the long-term prevention of stroke recurrence has been firmly established in patients with AF by the European Atrial Fibrillation Trial.[25] Only two trials have studied the value of heparin in the acute treatment of CES. In 1983, the Cerebral Embolism Study Group published the results of a small open randomized trial in 45 patients with acute cardioembolic stroke of less than 48 hours duration.[5] The trial was primarily designed to assess the safety, and not efficacy, of early treatment with continuous intravenous heparin (APTT 1.5-2.5) compared with no treatment during 10 days. Early recurrent stroke occurred in 2/21 control patients and none of the 24 treated patients. Symptomatic intracranial hemorrhage did not occur, but intracranial bleeding on a control CT-scan was found in 2 control patients. The recently published HAEST trial randomized 449 patients with acute ischemic stroke (within 30 hours after onset) and AF to treatment with aspirin (160mg/day) or a high dose of the LMWH dalteparin (100IU/kg sc bid). No difference in the frequency of early recurrent ischemic stroke or cerebral hemorrhage was detected.

By far the largest trial to date is the IST which involved 19,435 patients with acute stroke, randomized in 467 hospitals.[11] Three quarters of the patients were randomized to antithrombotic therapy, i.e. to one of the three regimens: aspirin, subcutaneous unfractionated heparin (low dose or medium dose) or the combination, and one quarter to control. Patients with mild, moderate and severe deficits, presenting within 48 hours of the onset of suspected acute ischemic stroke were eligible for the study. Duration of treatment was 14 days. Nearly all patients had a CT-scan either before or after randomization. The primary outcomes were death within 14 days and death or dependency at 6 months. Symptomatic intracranial hemorrhage and recurrent ischemic stroke within 14 days were protocol-specified secondary outcome events. Patients

allocated to heparin had significantly fewer recurrent ischemic strokes within 14 days (2.9% vs. 3.8%) but this was completely offset by an increase in hemorrhagic strokes (1.2% vs. 0.4%). A total of 3,169 (17%) patients were in AF, 784 of whom were allocated to 12,500 IU heparin sc bid, 773 to 5,000 IU heparin sc bid, and 1612 to avoid heparin. The proportion of AF patients with further fatal or non-fatal events within 14 days allocated to 12,500 IU heparin, 5,000 IU heparin and to avoid heparin were respectively: ischemic stroke 2.3%, 3.4%, 4.9% (p for trend=0.001); hemorrhagic stroke 2.8%, 1.3%, 0.4% (p<0.0001); and any stroke or death 18.8%, 19.4% and 20.7% (p=0.3). No effect of heparin dose on the proportion of patients who were dead or dependent at 6 months was found.[26]

In acute ischemic stroke, a majority of patients show evidence of *in vivo* platelet activation, which can be inhibited by aspirin.[27,28] The only antiplatelet agent that has been evaluated for the treatment of acute ischemic stroke is aspirin. In CAST, aspirin treatment (160 mg/day) was started within 48 hours of the onset of suspected acute ischemic stroke and continued in hospital for up to 4 weeks.[12] In total, 20,000 patients were randomized. The primary outcome events were death from any cause during the 4-week treatment period and death or dependence at discharge, the secondary fatal or non-fatal recurrent hemorrhagic or ischemic stroke. There was a significant 14% (SD 7) proportional reduction in mortality during the scheduled treatment period (343 [3.3%] deaths among aspirin-allocated patients vs. 398 [3.9%] deaths among placebo-allocated patients; 2p = 0.04). Significantly fewer recurrent ischemic strokes occurred in the aspirin-allocated than in the placebo-allocated group (167 [1.6%] vs. 215 [2.1%]; 2p = 0.01) but slightly more hemorrhagic strokes (115 [1.1%] vs. 93 [0.9%]; 2p > 0.1). In IST, aspirin-allocated patients had significantly fewer recurrent ischemic strokes within 14 days (2.8% vs. 3.9%) with no significant excess of hemorrhagic strokes (0.9% vs. 0.8%), resulting in a significant reduction in death or non-fatal recurrent stroke with aspirin (11.3% vs. 12.4%).[11]

With the results of IST and CAST combined, aspirin produces a small reduction of about 10 early deaths or recurrent strokes per 1000 patients treated. Both trials suggest that aspirin should be started as soon as possible after the onset of all subtypes of ischemic stroke.

Summary

The diagnosis of early recurrent stroke is not always easy in patients with acute ischemic stroke, since up to 20% of patients show spontaneous fluctuations, some of which may be caused by repeated embolism, but many have other causes, including systemic disorders. The risk of recurrent stroke seems to be higher in patients entered in clinical trials than in population based studies or stroke data banks. The aggregate results of randomized clinical trials of heparin in acute ischemic stroke suggest that this treatment reduces the risk of early recurrence by about one quarter, but also that this benefit is completely offset by an equal risk of hemorrhage. Present data neither suggest a clear subgroup, in particular those with cardioembolic stroke, with a higher risk of early recurrence, nor one that specifically benefits from early treatment with anticoagulants. Findings from two large trials show that aspirin is effective in reducing death and early recurrence (10 events prevented per 1000 treated) and safe.

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Chapter 8

Detection of paroxysmal atrial fibrillation in the acute phase of stroke by means of 84-hour Holter-ECG

Saxena R, Koudstaal PJ, on behalf of the DCLHb in Acute Stroke study investigators. Detection of paroxysmal atrial fibrillation in the acute phase of stroke by means of 84-hour Holter-ECG. *(Submitted)*

Abstract

Atrial fibrillation (AF), both continuous and paroxysmal (PAF), constitute a major risk factor for stroke. The most effective way to diagnose PAF in acute stroke patients without a history of AF is still uncertain. We compared the yield of 84-hours (h) Holter-ECG recording with that of a single Holter-ECG during the first 24 hours and of repeated ECG's during the first 7 days.

We prospectively studied 85 patients with ischemic stroke in the anterior circulation of less than 18 hours duration by means of continuous ECG monitoring during 84 hours within the setting of a multi-center stroke trial. Medical history, including AF, was assessed from patients, general practitioners and available hospital information. Standard 12 lead ECG's were made at entry, after 24 hours and once between day 3 and 7.

Twenty-one patients had AF, including 11 patients with a history of AF. Eight of 21 patients had PAF. Five of these 8 patients did not have a history of AF. Seven of eight were identified by 84-h Holter-ECG recording, 5 by repeated ECG's, and none by Holter-ECG recording during the first 24 hours only.

PAF is commonly present in patients with acute ischemic stroke, and often newly discovered. It can be most effectively identified by 84-h Holter-ECG recording and only slightly less effectively by repeated ECG's. The yield of Holter-ECG monitoring during the first 24 hours only is extremely low.

Introduction

Atrial fibrillation (AF), both continuous and paroxysmal (PAF), are a major cause of cardiogenic embolism to the brain.[1-3] An accurate identification of AF is important since several large studies have demonstrated the superiority of warfarin compared to anti-platelet treatment in primary and secondary stroke prevention studies.[4-10] The best way to identify PAF is still uncertain.

Common practice in the Netherlands, and probably also in other countries, is to perform a single 24-h Holter-ECG monitoring in stroke patients with a history of cardiac disease, abnormal standard ECG or palpitations or loss of consciousness preceding or during the stroke.[11] It is not known how many patients with PAF are missed with this practice.

The Framingham study and retrospective reviews have found that PAF accounts for 14-24% of strokes associated with AF and that the PAF likely precedes the event.[12-15] Studies in consecutive stroke patients have found that if PAF is not detected on the initial ECG, 24-h Holter-ECG recording can identify PAF in 1-5% of patients.[16-22] Schuchert et al. evaluated the additional yield of 72-h ambulatory ECG monitoring in 82 consecutive stroke patients without a history of AF and with sinus rhythm (SR) on initial ECG. Five patients with AF were detected, but only 1 within 24h.[23] Another study found that in patients whose initial ECG showed normal sinus rhythm (SR) follow-up 12-lead ECG on 2 successive days detected AF in an additional 9%.[24]

We wanted to determine whether ECG-monitoring during 84 hours instead of 24 hours or repeated ECG's, 3 in 7 days, increases the likelihood of identifying PAF in patients with acute ischemic stroke, in particular in those without a history of AF. We used the setting of an acute stroke trial in which the safety of administration of diaspirin cross-linked hemoglobin (DCLHb) was studied.[25] DCLHb did not cause any kind of arrhythmia, or other adverse effects on the heart (see chapter 6), so the data for the control and treatment groups were combined.

Subjects and methods

Patients

Details regarding the trial are provided elsewhere (see chapter 3). In short, it was a single-blind, randomized controlled trial in which the safety and efficacy of DCLHb administered within 18 hours of symptom onset was studied in acute ischemic stroke patients. Posterior circulation strokes, congestive heart failure, acute myocardial infarction and systolic blood pressure (BP) > 230 mmHg or diastolic BP >130 mmHg were exclusion criteria. All patients received standard care and treatment, including prophylactic medication such as acetylsalicylic acid and heparin. If patients were already on anti-hypertensive therapy, this treatment was continued throughout the study. New anti-hypertensive therapy was not initiated during the first week unless BP exceeded 220/135 mmHg for more than an hour.

Assessments

For the purpose of this study PAF was defined as one or more episodes of AF documented on the 12-lead ECG's or Holter-ECG made during the study period or AF discovered after sinus rhythm on the initial ECG.

Medical history was assessed from patients, referring general practitioners and available hospital computerized information.

Three ECG's were performed in each patient: the first on admission, the second on the next day and the third between the 3rd and 7th day. Holter-ECG monitoring was started at study entry and continued for 84 hours. The ECG's were assessed by different cardiologists than those who assessed the Holter-ECG recordings and all were blinded to treatment.

A general physical examination was performed on admission, and on day 3, 7, 14. Pulse was checked for irregularity on these occasions.

Statistics

For statistical analysis we used SPSS, version 9.0. For comparison between two groups Student's unpaired t-test or chi-squared analysis or Fisher's exact test were used as appropriate. A p-value < 0.05 was considered to indicate a statistically significant difference.

Results

A total of 85 patients were randomized in the study. There were no differences in any of the cardiac parameters, in particular history of AF and cardiovascular history, between patients treated with DCLHb or in controls (see chapter 6), so the data for these 2 treatment groups were combined.[25] A total of 21 patients had AF. Eleven patients (13%) were already known to have AF, and of the remaining 74 patients without a history of AF 10 patients (14%) had newly discovered AF. Demographic data and selected cardiovascular risk factors are shown in table 1.

Of the 11 patients with a history of AF, 5 had continuous AF, 3 had PAF and 3 did not show AF during the study. Of the 10 patients with recent AF, it was continuous in 5 and firstly recognized on the admission ECG, and paroxysmal in the other 5 (see table 2). One patient (with recent AF) had just one single episode of PAF lasting one hour, all others had episodes lasting from more than 7 hours till days.

Table 1 Characteristics of patients with acute ischemic stroke with recent AF, known AF or without AF

	No AF	Known AF	Recent AF
Number of patients	64	11	10
Age	63±14	80±6 *	74±5 * †
Male	31 (48%)	4 (36%)	4 (40%)
Cortical stroke	40 (63%)	9 (82%)	9 (90%)
Treatment with DCLHb	28 (42%)	6 (55%)	6 (60%)
Medical history			
Hypertension	27 (42%)	6 (55%)	5 (50%)
Myocardial infarction	8 (13%)	0 (0%)	1 (10%)
Angina pectoris	9 (14%)	1 (9%)	2 (20%)
Hypercholesterolemia	2 (3%)	0 (0%)	0 (0%)
Stroke	16 (25%)	5 (45%)	4 (40%)

* $p<0.001$ compared to controls

† $p<0.02$compared to controls

The diagnostic yield of the repeated ECG's and of prolonged Holter-ECG monitoring of the patients with PAF was as follows. None of the 8 patients with PAF had the arrhythmia on their admission ECG, 5 had AF on the subsequent ECG's as well as on 84-h Holter-ECG monitoring, and in 2 patients AF was discovered by Holter-ECG-monitoring only (after 72 hours) and not seen on ECG's. In 1 patient with recent AF, the arrhythmia was not detected by the protocol-specified tests, but by finding an irregular pulse on day 7 and subsequent confirmation by an extra ECG. In none of the 7 patients with AF on Holter-ECG monitoring PAF appeared within the first 24 hours. These data are summarized in table 2.

Table 2 Yield of the protocol specified ECG's and 84-h Holter-ECG monitoring

	Known AF	**Recent AF**
Paroxysmal AF	3	5
On admission ECG	0	0
Only on repeated ECG's	0	0
Only on Holter-ECG monitoring	1	1
On repeated ECG's	2	3
On Holter-ECG within 24 h	0	0
On Holter-ECG between 24-48 h	1	1
On Holter-ECG between 48-72 h	0	2
On Holter ECG after 72 h	2	1
Not detected by protocol specified testing	0	1
Continuous AF	5	5
On all ECG's and on Holter-ECG	5	5
No AF during study period	3	not applicable
Total number of patients with AF	11	10

Discussion

The objective of this study was to evaluate whether Holter-ECG recording of 84 hours detects more patients with paroxysmal AF after an acute ischemic stroke than Holter-ECG recording only during the first 24 hours or than repeated ECG's (3 ECG's during the first 7 days). Of the 8 patients with PAF, 5 did not have a history of AF. The 84-h Holter-ECG recording had the highest yield by identifying 7 of 8 patients. By means of repeated ECG's 5 of 8 patients were identified. The results of ECG monitoring during the first 24 hours only were disappointing as none of the patients had their paroxysms of AF within that period.

The results of the single 24-h ECG recording is in agreement with those in several other studies:[16-22] the identification of patients with PAF is inadequate, and even nil in our study, whereas the yield of the 84-h recordings is much better. This confirms the findings of Schuchert et al. who found that during 24-h recording only 1 of 82 patients showed PAF, whereas 4 more were discovered with the longer (72-h) recording.[23] None of these patients had a history of AF. The results of these studies are provided in table 3. Most of these studies also included patients with known AF, so the efficacy is even lower. Repeated ECG's proved to be better than 24-h Holter ECG since 3 of 5 patients with previously unknown AF were identified. Lindgren and colleagues also discovered a substantial number of stroke patients with PAF: 12 of 131 patients who's initial ECG showed SR. However, a large proportion of these patients had a history of AF.[24]

It has been suggested that when AF is recognized for the first time during the acute phase of ischemic stroke, so-called recent AF, the arrhythmia may have been the consequence rather than the cause of stroke.[26] Others have shown, however, that in nearly all such patients AF ultimately becomes persistent.[13] Whether AF is caused by the stroke or vice-versa was beyond the scope of our study.

In conclusion, recent AF was found in 14% of patients with acute ischemic stroke and no history of AF. PAF was effectively discovered by 84-h Holter-ECG recording and only slightly less effectively by repeated ECG's. The yield of Holter-ECG monitoring during the first 24 hours only was nil.

Table 3 Patients with stroke or TIA undergoing Holter-ECG recording and 12-lead ECG*

Study	Patients (n)	AF (n)	PAF not detected on initial ECG (n/%)	Comments
Consecutive stroke or TIA patients, some of whom had a history of AF				
Koudstaal[15]	100	4	1 (1%)	2 did not have admission ECG
Britton[16]	100	23	2 (2.6%)	
Richardt[17]	135	16†	7 (5.6%)†	
Norris[18]	312	36	15 (5.4%)	
Mikolich[19]	30	2	1 (3.6%)	
Hornig[20]	300	33	10 (3.6%)	Only 87% received Holter testing, 4 of 10 had a history of AF
Rem[21]	184	13	4 (2.3)	2 did not have admission ECG
Consecutive stroke patients without a history of AF and SR on initial ECG				
Shuchert[22]	82	5	5 (6.1%)	72-hour recording, 4 of 5 discovered *after* 24 hours

* Table adapted from Bell et al (2000)[27]

† Arrythmias, not only AF

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Chapter 9

Risk of early death and recurrent stroke and effect of heparin in 3169 patients with acute ischemic stroke and atrial fibrillation in the international stroke trial

Saxena R, Koudstaal PJ, Lewis S, Berge E, Sandercock PAG for the International Stroke Trial Collaborative Group. Risk of early death and recurrent stroke and effect of heparin in 3169 patients with acute ischemic stroke and atrial fibrillation in international stroke trial. *Stroke* 2001;32:2333-2337.

Abstract

We wished to investigate the apparently high risk of early death after an ischemic stroke among patients with atrial fibrillation (AF), identify the main factors associated with early death, and to assess the effect of treatment with different doses of subcutaneous unfractionated heparin (UFH) given within 48 hours.

We studied the occurrence of major clinical events within 14 days among 18,451 patients from the International Stroke Trial, firstly for all treatment groups combined and then, among patients with AF, we examined the effects of treatment with subcutaneous unfractionated heparin (UFH) started within 48 hours and continued till 14 days after stroke onset.

3,169 (17%) patients were in AF. A total of 784 patients were allocated to UFH 12,500 IU sc bid, 773 to UFH 5,000 IU sc bid, and 1612 to "no heparin". Within each of these groups, half of the patients were randomly assigned to aspirin 300 mg once daily. Compared with patients without AF, patients with AF were more likely to be: female (56% vs. 45%), old (mean 78 vs. 71 yrs), have an infarct on the pre-randomization CT (57% vs. 47%), and impaired consciousness (37% vs. 21%). The initial ischemic stroke type was more often a large artery infarct (36% vs. 21%). A lacunar stroke syndrome was less common (13% vs. 26%). Death within 14 days was commoner in patients with AF (17% vs. 8%) and more often attributed to neurological damage from the initial stroke (10% vs. 4%). The frequency of recurrent ischemic or undefined stroke was not significantly different (3.9% vs. 3.3%). The proportion of AF patients with further events within 14 days allocated to UFH 12,500 IU (n=784), UFH 5,000 IU (n=773) and to "avoid heparin" (n=1612) respectively, were: ischemic stroke 2.3%, 3.4%, 4.9% (p=0.001); hemorrhagic stroke 2.8%, 1.3%, 0.4% (p<0.0001); and any stroke or death 18.8%, 19.4% and 20.7% (p=0.3). No effect of heparin on the proportion of patients dead or dependent at 6 months was apparent.

Acute ischemic stroke patients with AF have a higher risk of early death, which can be explained by older age and larger infarcts, but not by a higher risk of early recurrent ischemic stroke, although slightly more patients with AF died from a fatal recurrent stroke of ischemic or unknown type (1.3% vs. 0.9%). In patients with AF the absolute risk of early recurrent stroke is low, and there is no net advantage to treatment with heparin. These data do not support the widespread use of intensive heparin regimens in the acute phase of ischemic stroke associated with AF.

Introduction

Atrial fibrillation (AF) is found in 6 to 20% of patients with acute stroke.[1-8] The reported risk of recurrent stroke varies between 10 and 20% during the first year.[9-13] The risk of very early recurrence has been investigated in several studies[4-6,10,11,14-17] and varies between 0.1% and 1.3% per day during the first two weeks after the initial event.[6-10,14,15] The striking variation between studies can be explained partly by differences in study design (some studies were retrospective and others prospective), and by differences in the study populations (some studies included AF patients with co-existent cardiac abnormalities and others only patients with "lone" AF). Several studies have reported higher case fatality and morbidity after an ischemic stroke among AF patients compared with patients in sinus rhythm.[5,6,11,18-20] The underlying cause of this high case fatality is unclear. Possible factors include a higher frequency of large, especially cortical, infarcts,[11,17,19,21] the presence of concomitant ischemic heart disease,[15] and a high frequency of early recurrent stroke.

Analysis of primary prevention studies in patients with AF have shown that oral anticoagulant therapy reduces the risk of stroke by 60-70%.[22,23] In the European Atrial Fibrillation trial (EAFT)[13], a secondary prevention study, the relative risk reduction with oral anticoagulation was of similar magnitude, but the best time to start anticoagulant therapy was not clear. In the EAFT, half of the patients were randomized more than two weeks after stroke onset, and effective anticoagulation was not obtained within the first weeks, when the risks (hemorrhagic transformation of the infarct) and benefits (prevention of recurrent ischemic stroke) are probably highest. Only two studies have focused on treatment with anticoagulants in the acute phase of stroke, but they showed no evidence of benefit of heparin compared with aspirin[24] or no antithrombotic treatment.[25]

The data from the International Stroke Trial,[26] a large randomized controlled trial of heparin, aspirin, both, or neither in patients with acute ischemic stroke provided an opportunity to study these questions further. In the main phase of the trial, data on the presence of absence of AF at baseline were recorded, and over 3000 patients with this arrhythmia were included. The aim of this paper was to compare the early case fatality in patients with AF and those without AF, and to examine whether any difference in case fatality was chiefly related to

clinical features at baseline, or to the difference in the frequency of recurrent stroke in the acute phase. We also wanted to study the effect of different heparin doses on the risk of recurrent ischemic stroke and intracranial hemorrhage in patients with AF.

Subjects and Methods

Patients and treatment

We studied data available for the 18,451 patients entered during the main phase of the International Stroke Trial (IST) between March 1993 and May 1996. (AF status at baseline was not recorded in the 984 patients entered during the pilot phase.) The IST was a multicenter, multinational randomized open trial which aimed to assess the effectiveness of early antithrombotic treatment in patients with acute stroke.[1] Patients with mild, moderate and severe deficits, presenting within 48 hours of the onset of suspected acute ischemic stroke were eligible for the study, provided that the responsible physician did not initially consider there to be any clear indications for, or clear contraindications to, any one of the trial treatments (aspirin or heparin). Patients were randomized in a 3 x 2 factorial design, i.e. to subcutaneous UFH (5000 IU bid or 12,500 IU bid), aspirin 300 mg, both, or neither. Coagulation times were monitored at the discretion of the treating physician. Treatment was given for 14 days or until prior hospital discharge. CT scan to exclude intracranial hemorrhage (ICH) was to be performed before randomization where possible, and was mandatory in comatose patients. A non-comatose patient could be randomized before CT scan only if there was likely to be a long delay in getting the CT scan and the physician considered the stroke most likely to be ischemic. For those allocated active treatment, the initial doses could be given while CT was being arranged, but treatment was stopped if ICH was found.

Classification

On the basis of the neurological symptoms at study entry, patients were categorized by means of a computer algorithm according to the criteria of Bamford et al into one of the following clinical syndromes: total anterior circulation stroke (TACS), partial anterior circulation stroke (PACS), posterior circulation stroke (POCS), lacunar anterior circulation stroke (LACS), or other.[27]

Outcome measures

Protocol-specified primary outcomes were death from any cause within 14 days and death or dependency at 6 months. Protocol-specified secondary outcomes were: symptomatic intracranial hemorrhage within 14 days (including symptomatic hemorrhagic transformation of the original infarct) as confirmed by CT, magnetic resonance imaging or necropsy; ischemic stroke (including any recurrent stroke of ischemic or unknown type), major extracranial hemorrhage, and other major clinical event such as pulmonary embolism within 14 days. There were no specific criteria to define a recurrent ischemic stroke, so that the decision on whether or not recurrent stroke had occurred was left to the judgment of the responsible physician.

Analysis and statistical methods

The protocol specified two main analyses for the primary outcomes, namely "immediate heparin" (low or medium dose) vs. "avoid heparin" and "immediate aspirin" vs. "avoid aspirin". In these analyses we have also compared medium vs. low dose heparin vs. "avoid heparin". In the factorial design, half of the heparin and control patients were allocated aspirin, and since there was no interaction between aspirin and heparin,[26] we combined the aspirin and no aspirin groups for the purpose of these analyses. All analyses were "intention-to-treat" and thus patients were included in the analysis in the group to which they were allocated, irrespective of their compliance with trial treatment.

Analysis of total numbers of patients affected was done by chi-square tests and chi-square tests for trend.

Results

At study entry, a total of 3,169 (17%) patients were in AF and 15,282 were not in AF. The mean delay between the stroke and randomization was 18.7 hours in patients with AF, versus 20.4 hours in those without (difference in means 1.7 hours, 95% CI 1.2 to 2.2).

Compliance with the allocated treatment was good; only 6% of patients with AF and 8% of patients without AF received heparin despite being allocated to the "avoid heparin" group. Compliance with aspirin allocation was equally good across all treatment groups.

Table 1 Baseline characteristics in stroke patients with and without atrial fibrillation (AF), recorded at the time of randomization

	AF	No AF	OR (95% CI)*
Number of patients	3169	15282	
Age (mean, years)	77.8	70.6	7.2 (6.9-7.6) †
Female sex	1779 (56%)	6823 (45%)	1.6 (1.5-1.7)
Symptoms noticed on waking	854 (27%)	4552 (30%)	0.9 (0.8-1.0)
Impaired consciousness	1166 (37%)	3039 (21%)	2.4 (2.2-2.6)
Subtype of stroke			
TACS	1155 (36%)	3252 (21%)	2.1 (2.0-2.3)
PACS	1310 (41%)	6166 (40%)	1.0 (1.0-1.1)
LACS	409 (13%)	4014 (26%)	0.4 (0.4-0.5)
POCS	289 (9%)	1809 (12%)	0.8 (0.7-0.9)
Other	6 (0.2%)	41 (0.3%)	0.7 (0.3-1.7)
Had pre-randomization CT scan	1968 (62%)	10434 (68%)	0.8 (0.7-0.8)
Infarct on pre-randomization CT scan ‡	1127 (57%)	4915 (47%)	1.5 (1.4-1.7)

* Odds ratio (OR) and 95% confidence interval (CI). An OR greater than one indicates that the characteristic is more common in patients with AF; If the 95% CI does not include unity, the result is significant at the 5% level or greater

† This is the difference between the two means with 95% confidence intervals, not an odds ratio

‡ Among those who had a pre-randomization CT-scan

Table 1 Baseline characteristics in stroke patients with and without atrial fibrillation (AF), recorded at the time of randomization, *continued*

	AF	No AF	OR (95% CI)*
Final diagnosis of initial event leading to randomization			
Ischemic stroke	2865(90%)	13813 (90%)	1.0 (0.9-1.1)
Hemorrhagic stroke	79(2%)	490 (3%)	0.8 (0.6-1.0)
Stroke of unknown type	172(5%)	592 (4%)	1.4 (1.2-1.7)
Not a stroke/unknown	53(2%)	387 (3%)	0.7 (0.5-0.9)

‡ Among those who had a pre-randomization CT scan

Table 1 summarizes the principal baseline characteristics of the patients. Compared to patients without AF, patients with AF were more likely to: be female (56% vs. 45%), old (mean age 78 years vs. 71 years), and have impaired consciousness (37% vs. 21%). The initial stroke was more often a large infarct with the clinical deficits suggesting involvement of the entire territory of the middle cerebral artery (TACS) (36% vs. 21%). Lacunar stroke was less common among patients with AF (13% vs. 26%). Correspondingly, infarction on the pre-randomization CT scan in was more often seen in patients with AF (57% vs. 47%). Since the study design permitted randomization while CT was being arranged, inevitably, in a few patients, it was discovered that the event leading to randomization was a hemorrhagic stroke (AF 2%, not AF 3%). Treatment was stopped after the CT scan in such cases.

The risk of recurrent ischemic stroke within 14 days was low and not significantly altered by the presence or absence of AF (Table 2): 123 (3.9%) and 500 (3.3 %) in patients with and without AF, respectively. Symptomatic intracranial hemorrhage within 14 days occurred significantly more often in AF patients: 39 (1.2%) vs. 109 (0.7%). A total of 534 (17%) AF patients died within 14 days compared with 1149 (8%) patients without AF (OR 2.5, 95% CI 2.2 to 2.8). Table 3 shows that AF patients were more likely to die from neurological damage from the initial stroke or from pneumonia, coronary heart disease or pulmonary embolism, and from early recurrent stroke.

Table 2 Events within 14 days in patients with and without atrial fibrillation (AF), all treatment groups combined

	AF	No AF	OR (95% CI)*
Number of patients	3169	15282	
Fatal or non-fatal recurrent ischemic stroke	123 (3.9%)	500 (3.3%)	1.2 (1.0-1.5)
Fatal or non-fatal symptomatic intracranial hemorrhage	39 (1.2%)	109 (0.7%)	1.7 (1.2-2.5)
Fatal or non-fatal recurrent ischemic stroke or symptomatic intracranial hemorrhage	161 (5.1%)	603 (4.0%)	1.3 (1.1-1.6)
Fatal or non-fatal recurrent ischemic stroke or symptomatic intracranial hemorrhage or death	630 (19,9%)	1548 (10.1%)	2.2 (2.0-2.4)
Death from any cause	534 (16.9%)	1149 (7.5%)	2.5 (2.2-2.8)

* Odds ratio (OR) and 95% confidence interval (CI)

Table 3 Causes of death within 14 days in stroke patients with and without atrial fibrillation (AF), all treatment groups combined

	AF	No AF	OR (95% CI)*
Number of patients	3169	15282	
Neurological damage from initial stroke	305 (9.6%)	557 (3.6%)	2.9 (2.5-3.4)
Recurrent stroke, ischemic or unknown	42 (1.3%)	131 (0.9%)	1.7 (1.2-2.4)
Recurrent stroke, hemorrhagic	6 (0.2%)	32 (0.2%)	1.0 (0.4-2.4)
Pneumonia	85 (2.7%)	180 (1.2%)	2.5 (2.0-3.3)
Coronary heart disease	44 (1.4%)	92 (0.6%)	2.6 (1.8-3.7)
Pulmonary embolism	21 (0.7%)	50 (0.3%)	2.3 (1.4-3.8)
Other vascular or unknown	26 (0.8%)	84 (0.5%)	1.7 (1.1-2.6)
Non vascular	5 (0.2%)	23 (0.2%)	1.2 (0.4-3.1)
Total	534 (16.9%)	1149 (7.5%)	

* Odds ratio (OR) and 95% confidence interval (CI), using those 'not dying' as a comparator

Table 4 Effect of heparin in different doses on events within 14 days and outcome at six months in patients with atrial fibrillation (AF)*

	Heparin 12,500 IU	**Heparin 5,000 IU**	**No heparin**	**P-value overall χ2**	**P-value χ2 trend**
Number randomized	784	773	1612		
Events within 14 days					
Recurrent stroke of ischemic or unknown type	18 (2.3%)	26 (3.4%)	79 (4.9%)	0.006	0.001
Symptomatic ICH	22 (2.8%)	10 (1.3%)	7 (0.4%)	<0.0001	<0.0001
Recurrent stroke or symptomatic ICH	39 (5.0%)	36 (4.7%)	86 (5.3%)	0.8	0.6
Recurrent stroke, symptomatic ICH, or death	147 (18.8%)	150 (19.4%)	333 (20.7%)	0.5	0.3
Outcome at six months					
Dead from any cause	305 (38.9%)	292 (37.8%)	630 (39.1%)	0.8	0.8
Dead or dependent	612 (78.1%)	609 (78.8%)	1266 (78.5%)	0.9	0.8

ICH= intracranial hemorrhage

* Within each of these groups, half of the patients were randomly assigned to aspirin 300 mg once daily

The frequency of various events, subdivided by heparin allocation, are shown in table 4. The three treatment groups were comparable with respect to baseline characteristics (data not shown) and, within each group, half of the patients were randomly allocated to aspirin. However, there was no evidence of an interaction effect between aspirin and heparin, and patients receiving aspirin are therefore not excluded from the analysis. The proportion of AF patients with fatal or non-fatal events within 14 days allocated to UFH12,500 IU, 5,000 IU and to "avoid heparin", respectively, were: stroke of ischemic or unknown type 2.3%, 3.4%, 4.9% (p=0.001); symptomatic intracranial hemorrhage 2.8%, 1.3%, 0.4% (p<0.001); and any recurrent stroke or death 18.8%, 19.4%, and 20.7% (p=0.3). So, despite a clear and dose-dependent reduction in ischemic strokes among patients allocated heparin, this advantage was offset by a similar sized increase in hemorrhagic strokes in the heparin allocated groups. Consequently, there was no net difference in death or the event "death or dependency six months after the stroke".

A small proportion (3%) of patients included (among those first scanned after randomization) turned out to have an intracranial hemorrhage. We therefore repeated the analysis restricted to patients known to have non-hemorrhagic stroke. The results of this analysis were remarkably similar to those in table 4.

Discussion

These data from the International Stroke Trial confirm the findings from other studies that patients with AF have a case fatality that is about twice as high as the case fatality in patients without AF (17% versus 8% after two weeks). In IST, patients with AF were, compared with those not in AF, on average older, had more co-morbidity and more severe strokes, and these factors are likely to have contributed to the higher case fatality in AF. On the other hand, recurrent stroke (ischemic/unknown or hemorrhagic) occurred equally often in patients with and without AF, and although slightly more patients with AF died from a fatal recurrent stroke of ischemic or unknown type, early recurrences could not explain the worse outcome in patients with AF. The most common cause of early death in patients with AF was neurological damage from the initial stroke, as might be anticipated, since they had more severe strokes, with a predominantly cortical localization.[11,17,19,21,28]

Absolute risk of early recurrent stroke in AF

The overall risk of early recurrent stroke of ischemic or unknown type in patients with AF was lower than reported in earlier studies, and not significantly different from the group without AF, which is in agreement with some previous studies, [3-5,16,17,24] but disagrees with several others. [6,10,11,14] One explanation for the different estimates might be different selection of patients. However, about one sixth of the patients in IST were in AF, which is very similar to that found in community-based incidence studies of stroke. This suggests that our patients are likely to be reasonably representative of patients with acute ischemic stroke and AF, so factors related to patient selection may not be the explanation. Other explanations for the low estimate of the incidence of recurrent ischemic stroke among AF patients could be methodological: recurrent stroke was not a primary measure of outcome in the trial; the trial did not apply a rigid definition of recurrent ischemic stroke, but relied on the clinical judgment of local investigators. The effect of this may have been to count only severe recurrent strokes and hence we may have under-ascertained mild recurrent strokes. The high early case fatality (about 30%) after recurrent stroke tends to confirm the hypothesis that only severe recurrences were reported. Furthermore, since patients could be randomized up until 48 hours after onset of symptoms, some very early recurrences may have been missed.

Effect of different heparin doses on risk of recurrence

We found that, although the higher heparin dose was associated with the fewest recurrent ischemic strokes, the clear dose-related trend to benefit was offset by a significant and dose-dependent increase in the risk of hemorrhagic stroke. These trends were also seen after exclusion of the small number of patients with intracranial hemorrhage at randomization (who were first scanned after randomization), and there was no evidence of benefit from heparin on death or disability at six months.

Part of the apparent effects of heparin on recurrent ischemic stroke and symptomatic hemorrhage could be due to an observer bias. Since the trial was open, there may have been systematic bias operating against heparin by an increased attention to bleeding in patients allocated heparin, especially the higher dose. However, this does not apply to outcome at 6 months, as this was effectively blinded. Also, the short-term effects of heparin were similar to those in other placebo-controlled trials, such as the TOAST study, a placebo-controlled trial of intravenous heparinoid in acute ischemic stroke, in which the subgroup of patients with cardioembolic stroke also showed no evidence of benefit from anticoagulation.[29]

Implications for practice

What then are the consequences of these analyses for the treatment of patients with AF in the acute phase of ischemic stroke? Firstly, the risk of major recurrent stroke is lower than many clinicians fear. Secondly, the risk of bleeding complications with the higher heparin dose is a concern. A recent combined analysis of the 40,000 randomized patients in the IST and Chinese acute stroke trial (CAST) showed that treatment with aspirin reduces the risk of recurrent ischemic stroke within 14 days by 30%, and the effect is similar in the presence or absence of AF.[30] For many patients presenting with acute ischemic stroke and atrial fibrillation, aspirin offers a safe and effective option for preventing early recurrent stroke in the first week or two after stroke onset. For patients who remain in AF and have no contraindications, oral anticoagulation with target INR 2.0-3.0 is likely to be the most effective secondary prevention.[13]

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Chapter 10

General discussion

The findings of this thesis

Two strategies for treatment of acute ischemic stroke were explored in this thesis:

1. The safety and efficacy of diaspirin cross-linked hemoglobin (DCLHb)
2. The safety and efficacy of heparin in patients with atrial fibrillation (AF)

The following are the main findings:

- DCLHb was neither safe nor efficacious for the treatment of acute stroke. More serious adverse events and deaths occurred in DCLHb-treated patients than in controls, and outcome scale scores were worse.
- DCLHb prevented the decrease in blood pressure (BP) that normally occurs in the first days after stroke. Although clinical outcome was worse in patients treated with DCLHb, this was not caused through adverse effect on the heart and the pressor effect by itself was well tolerated.
- Infusion of DCLHb was associated with a dose-dependent increase in plasma ET-1 concentration, which was correlated with the rise in blood pressure. We suggest that the pressor effect was caused, at least in part, by ET-1.

- Acute ischemic stroke patients with AF have a higher risk of early death, which can be explained by older age and larger infarcts, but not by a higher risk of early recurrent ischemic stroke, although slightly more patients with AF died from a fatal recurrent stroke.
- Paroxysmal AF was commonly found in patients with acute ischemic stroke and was effectively discovered by 84-h Holter-ECG recording and slightly less effectively by repeated ECG's. The result of a single Holter-ECG during the first 24 hours was nil.
- In patients with AF the absolute risk of early recurrent stroke is low, and there is no net advantage to treatment with heparin in the acute phase.

Treatment of acute ischemic stroke with DCLHb

In our phase II study, treatment with DCLHb was an independent predictor of worse outcome. Our study showed no obvious reason for the failure of this drug. The pressor effect of DCLHb on itself was well tolerated. None of the patients showed signs of hypertensive encephalopathy or hemorrhagic transformation of the infarct. Neither did DCLHb cause myocardial ischemia or congestive heart failure. Two patients had an adverse event, which was possibly drug-related: one of fatal brain and pulmonary edema, the other of transient renal and pancreatic insufficiency. Levels of endothelin-1 (ET-1, a potent vasoconstrictor) were dose-dependently increased in response to DCLHb and correlated with increments in blood pressure (BP).

At the time we were evaluating DCLHb as a neuroprotective agent in stroke, it was also tested as a resuscitative fluid in patients with traumatic hemorrhagic shock in an American multicenter, phase III, randomized, controlled, single-blinded efficacy trial.[1] At 28 days, 24 of the 52 (46%) patients infused with DCLHb died, versus 8 of the 46 (17%) patients infused with saline ($p = .003$). The 28-day morbidity rate, as measured by the multiple organ dysfunction score, was 72% higher in the DCLHb group ($p = .03$). Why DCLHb adversely affected outcome was not clear. When these results became known, the European trauma trial, which by then had enrolled 117 patients, was suspended and these results have not been published. Soon hereafter, the manufacturer, Baxter Healthcare Corporation, announced that it had decided to focus its research-and-development efforts in oxygen-carrying therapeutics, on genetically engineered hemoglobin molecules. This decision ended the company's clinical development of DCLHb.

Unfortunately, there is no obvious reason for the failure of DCLHb. In experimental studies addressing infarct size, animals were given various doses of DCLHb to achieve hemodilution up to 9%. After this treatment, temporary ligation of the middle cerebral artery resulted in a smaller infarct size[2,3] and increased cerebral blood flow[4] compared with animals receiving albumin. The beneficial effects were most profound when DCLHb was given in a manner that induced its hypertensive response.[5] Further studies showed that the mechanism of infarct reduction might be through scavenging of NO in brain tissue.[6] However, the relevance of these pre-clinical models to clinical efficacy can be

questioned. Apart from the obvious dissimilarities between man and rat, efficacy was judged by reduced infarct size in animal studies as opposed to reduced death and dependency/disability in human stroke trials. Also the timing and dosing schedule was quite different. In most animal experiments, a highly favorable response was found after a single high dose exchange transfusion, either before or within one hour after initiation of ischemia.[2,3] After our study had finished, such high doses were found to be safe in patients undergoing coronary by-pass surgery.[7] Another problem may have been failure of the drug to reach the ischemic area because of arterial occlusion. The cerebral artery occlusion was temporary in most animal stroke models,[2,3,5] but this is not necessarily the case in humans.

The effect on BP, although seemingly beneficial in animal stroke studies, may have contributed to study failure. Elevation of BP is not necessarily bad. However, it is only useful if it is accompanied by increased blood flow. For example, tissue perfusion can be improved by chronotropic or inotropic agents. Contrary to this mechanism, agents that elevate BP by peripheral vasoconstriction do so at the expense of raising peripheral resistance, so that capillary blood flow may decrease. The additional oxygen-carrying capacity provided by administering the hemoglobin solution could then be completely offset by the increased resistance to flow.

Many studies were carried out to determine which of the abovementioned mechanisms to raise BP predominated. Invariably, the increase in BP was not a result of volume changes, but a direct pharmacological effect.[8-11] Using microspheres to estimate regional blood flow in anesthetized rats, infusion of DCLHb caused an increase in MAP, cardiac output, stroke volume and peripheral resistance, while blood flow to the heart, gastrointestinal tract, mesentery, pancreas and skin increased, apparently at the expense of increased resistance and lower flow to the musculoskeletal system and liver. Blood flow to the brain was not affected.[12-14] In hemorrhaged rats, resuscitation experiments showed that when the volume of DCLHb was 50% of the volume of shed blood, perfusion to vital organs such as the heart and brain exceeded pre-hemorrhage levels.[15,16] In a swine model of non-lethal hemorrhagic shock, infusion of DCLHb caused dose-dependent increase of cardiac output and improved base excess and lactate concentrations, two indices of global perfusion, more rapidly and to a greater extent than Ringer's Lactate.[17] In brief, DCLHb elevated pressure as well as cardiac output and tissue perfusion.

In the phase I study, a single infusion of DCLHb (25, 50, or 100 mg/kg) in healthy volunteers was safe and well tolerated.[18] A dose-related increase in blood pressure occurred without evidence of decreased peripheral perfusion as measured by laser Doppler flowmetry, pulse oximetry, or toe temperature. DCLHb was tested as a hemoglobin substitute in several phase II and III trials. 34 patients undergoing elective repair of an abdominal aortic received DCLHb: 50, 100 and 200 mg kg/kg i.v. DCLHb produced an increase in MAP of approximately 25%, with small decreases in cardiac index and calculated oxygen delivery.[19] The effects on oxygen consumption were minimal. In another phase II randomized, placebo-controlled, single-blind, cross-over trial, a 30-minute infusion of 25, 50, or 100 mg/kg DCLHb or placebo was evaluated in 18 subjects receiving chronic hemodialytic therapy.[20] The maximum increase in systolic blood pressure from baseline increased significantly with DCLHb dose compared with placebo. There were no adverse events. In a phase II, prospective, observational study 14 critically ill patients requiring vasopressor therapy to maintain MAP were administered 100 ml boluses of 10% diaspirin cross-linked hemoglobin, up to a maximum of 500 ml. Reduction in norepinephrine requirements was used as the main end point to assess the efficacy of DCLHb as a vasopressor. Again DCLHb demonstrated a marked vasopressor action, allowing norepinephrine requirements to be reduced. Heart rate, central venous pressure, mean pulmonary arterial pressure, systemic vascular resistance index, and urine output did not demonstrate any significant changes from preinfusion values. However, pulmonary vascular resistance index increased and cardiac index and oxygen delivery index decreased significantly.[21] In a phase III randomized, controlled trial DCLHb was tested as an alternative to blood transfusion after cardiac bypass surgery.[7] 209 were determined to require a blood transfusion and were randomized to receive up to three 250-ml infusions of DCLHb (n = 104) or three units of packed erythrocytes (n = 105). Primary efficacy end points were the avoidance of blood transfusion through hospital discharge. Administration of DCLHb allowed a significant number (19%) of cardiac surgery patients to avoid exposure to erythrocytes postoperatively. Mortality was similar between the DCLHb (6 of 104 patients) and the control (8 of 105 patients) groups. The hemodynamic effects of DCLHb included slightly greater increase in systemic and pulmonary vascular resistance with associated increases in systemic and pulmonary arterial pressures compared with packed red blood cells. Cardiac output values decreased more in the DCLHb group patients. To summarize, DCLHb produced an increase in MAP. Contrary to most animal studies, this was however, generally accompanied by decreased cardiac index and calculated

oxygen delivery,[19,21] and increased systemic and pulmonary vascular resistance,[7] but this did not appear to affect the patients' clinical condition.

Acute ischemic stroke and hemorrhagic shock patients are in dire need of oxygen, either locally or systemically. As there was no specific adverse effect that appeared to cause damage and considering the information we now have on the vasoconstrictor effects of DCLHb it is tempting to hypothesize that both populations were adversely affected by vasoconstriction and subsequent lack of oxygen.

In our population, DCLHb caused a rise in BP that was accompanied by a decrease in heart rate and increase in ET-1. This would suggest that the pressor effect is not caused by a positive chronotropic effect on the heart, but possibly through vasoconstriction by ET-1. Since this was a study testing the pharmacological effects of DCLHb in stroke patients, we could not administer ET-1 antagonists to prove that the pressor effect is caused by ET-1. The circumstantial evidence from many other studies, however, is compelling.[15,22-25] The increased ET-1 levels may have contributed to the ischemic damage through the potent vasoconstrictor effect of ET-1.[26] On the other hand, there is also evidence that a systemic increase in endothelin causes a vasodilator effect in the brain.[27] The NO binding effect of DCLHb could also have caused vasoconstriction in the cerebral vessels. Inhibition of NO has been shown to cause vasoconstriction and decreased blood flow in the middle cerebral artery.[28]

Suggestion for future research

The hemodynamic response caused by DCLHb increases vascular resistance, and probably eliminates its potential as a neuroprotective agent in acute ischemic stroke. Newer, possibly genetically engineered, hemoglobin solutions that overcome this limitation should fare better in clinical development.

Treatment of acute ischemic stroke in the presence of AF with heparin

The International Stroke Trial (IST) showed no benefit of treatment with subcutaneous unfractionated heparin (UFH, 12.500 or 5000 IU twice daily) on

death or dependency at 6 months nor on the other primary outcome measure of death within 14 days.[29] Patients allocated to heparin had significantly fewer recurrent ischemic strokes within 14 days but this was offset by a similar-sized increase in hemorrhagic strokes. It is generally assumed that the overall results of a clinical trial are applicable to all patients in the trial, or in other words, that the relative treatment effect in individual patients is similar to the overall trial result. We aimed to investigate whether this was true for the subset of 3169 patients with atrial fibrillation (AF) randomized in IST.

Methodological issues concerning subgroup analyses

Caution is required when performing subgroup analysis, but in this case there were sound clinical arguments to consider the subgroup of patients with AF more closely. Five primary prevention studies in patients with AF[30-34] and one on secondary prevention[35] showed that the long-term benefit of anticoagulants for secondary prevention of stroke greatly outweighs that of aspirin.[35] The European Atrial Fibrillation trial (EAFT), however, did not provide information on risk/benefit ratio of anticoagulation in the early period after onset of symptoms. There has been only one trial that focused on treatment with anticoagulants in the acute phase of ischemic stroke associated with AF.[36] A total of 449 patients were randomized. The authors concluded that while the data could not provide any evidence that dalteparin is superior to aspirin, the study could not exclude the possibility of smaller, but still worthwhile effects of either of the trial drugs. The trial was also not large enough to reliably estimate a significant increase in cerebral hemorrhage on dalteparin. Our IST subgroup represents by far the largest group of acute ischemic stroke patients with AF treated with anticoagulants. The variable AF was recorded at baseline. Clinicians might have excluded some patients with AF from randomization, because they believed them to require anticoagulation. However, about one sixth of the patients in IST were in AF, which is very similar to that found in community-based incidence studies of stroke. This suggests that our patients are likely to be reasonably representative of patients with acute ischemic stroke and AF.

The effect of heparin on recurrent stroke in patients with AF or SR during the first 14 days

Among stroke patients with AF, heparin during the first 14 days stroke reduced the absolute risk for recurrent stroke by 2.1% (medium-dose heparin: 2.6%; low-dose heparin:1.5% vs. aspirin:1.2%). Heparin increased the risk of

hemorrhagic stroke by 1.6% (2.4% in the medium-dose heparin:2.4%; low-dose heparin 0.7%) vs. 0.3% by aspirin. Among patients in sinus rhythm (SR) the absolute risk reduction was only 0.7% (without dose-dependency) and the increase in symptomatic ICH was also 0.7% and higher in the medium-dose (1.4%) than the low-dose regimen (0.3%). Aspirin prevented 1.1% more ischemic strokes at the cost of only 0.1% more intracranial bleeds than treatment without aspirin. To summarize, there was no net advantage of heparin among patients with AF, because the higher benefit on recurrent ischemic stroke was counterbalanced by an increase in intracranial hemorrhages. The low-dose heparin regimen had a better risk/benefit ratio than the medium-dose regimen.

Comparison with other studies

In EAFT, the secondary prevention trial, treatment with anticoagulants reduced the risk of recurrent stroke from 12%/year to 4%/year, while none of the patients on anticoagulant treatment suffered an intracranial bleed.[35] The striking difference in the incidence of intracerebral hemorrhage may be explained by the timing of the treatment and the size of the infarcts. In EAFT, patients were recruited in the first 3 months after stroke and not many were randomized within the first 14 days, when the risk of intracerebral hemorrhage is the highest. The larger size of the infarcts in IST could also very well have contributed to the high number of bleedings. In EAFT, only TIA's and minor disabling infarcts were randomized. Treatment was unblinded in both trials, but coagulation times were monitored in EAFT, which could also have contributed to the very low risk of bleeding.

In HAEST, the only acute stroke trial concentrating on patients with AF, double-blind treatment with low-molecular-weight heparin (LMWH, dalteparin 100 IU/kg s.c. twice daily) was tested against aspirin in 449 patients.[36] Coagulation times were not monitored. The primary measure of outcome, the frequency of recurrent ischemic stroke during the first 14 days, was 8.5% (19/224) in dalteparin-allocated patients vs. 7.5% (17/225) in aspirin-allocated patients. There was no increased risk of symptomatic cerebral hemorrhage (6/224 vs. 4/225) or symptomatic and asymptomatic cerebral hemorrhage (26/224 vs. 32/225). Neither did dalteparin have an effect on progression of symptoms within the first 48 hours.

The 14 day event rate of ischemic stroke was much higher in HAEST than in IST: 7.5% vs. 3.3%. Again, patient selection in IST may have played a role. Clinicians may not have randomized patients, whom they believed to have a

high risk of stroke recurrence or a clear indication for anticoagulation. Other explanations for the low estimate of the incidence of recurrent ischemic stroke could be methodological: in IST recurrent stroke was not a primary measure of outcome in the trial; the trial did not apply a rigid definition of recurrent ischemic stroke, but relied on the clinical judgment of local investigators, and patients were not rigidly monitored which may have led to underreporting of mild recurrent strokes. The high early case fatality (about 30%) after recurrent stroke tends to confirm the hypothesis that only severe recurrences were reported. Furthermore, since patients could be randomized up until 48 hours after onset of symptoms vs. 30 hours in HAEST, some very early recurrences may have been missed.

Although the net result of HAEST was the same as in our subgroup of AF patients, dalteparin did not seem to have a stronger effect on the prevention of ischemic strokes or the occurrence of intracranial hemorrhage as compared to aspirin. There are several factors that could have contributed to this difference. Firstly, very severe (thus large) strokes were excluded from HAEST, which could have lessened the risk of bleeding. Furthermore, the specific types of heparin used (LMWH vs. UFH) may have influenced the bleeding risk. Indeed, several trials in other areas of vascular medicine have shown that LMWH is superior to UFH in their risk/benefit ratio, in that it is associated with lower risk of hemorrhage and more powerful antithrombotic effects than standard unfractionated heparin (UHF).[37-39] Nevertheless, this has not been definitely confirmed in several medium-sized stroke studies.[40-42] Finally, the unblinded nature of treatment in IST raises the question of a systematic bias against heparin, namely, an increased attention to bleeding when patients were on heparin.

Conclusions

In the 3169 patients with AF from IST, the medium-dose heparin regimen was clearly more effective in preventing early recurrent stroke than the low-dose heparin regimen or than aspirin, but it was also the most hazardous, considering the steep increase in intracerebral hemorrhage. UFH (12.500IU) can produce a highly variable anticoagulant response, both inter- and intraindividually.[43] Compared with UFH, LMWH's produce a more predictable anticoagulant response such that they can be given subcutaneously once or twice daily without monitoring.[44] However, both UFH and LMWH [36] have not been found to be useful in improving outcome in AF patients with (major) ischemic stroke. Further trials with anticoagulation are not warranted in this group of patients.

AF patients with a TIA or rapidly resolving minor ischemic stroke should be treated with anticoagulants immediately after the event on the basis of the EAFT results.[35]

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Summary

Stroke is a major cause of death and an important cause of hospital admission and long-term disability in Western countries. The only known effective treatment, thrombolysis with recombinant tissue plasminogen activator, can be applied to only a small percentage of patients.

In this thesis, two types of treatment for acute ischemic stroke, both studied in randomized controlled trials, are described. *Firstly,* we evaluated the safety, efficacy and pharmacodynamics of the use of a hemoglobin solution, diaspirin cross-linked hemoglobin (DCLHb, HemAssist®) in patients with acute ischemic stroke. *Secondly,* we studied the effect of heparin in patients with acute ischemic stroke in combination with atrial fibrillation (AF) entered in a large clinical study, the International Stroke Trial (IST). We had specific interest in the prevention of early stroke recurrence.

Chapter 1 is the introduction to this thesis and describes the contents of the other chapters.

Chapter 2 describes the rationale for using DCLHb and heparin in the treatment of acute stroke.

DCLHb is a purified cell-free human hemoglobin solution, and was originally developed as a blood substitute. After DCLHb was discovered to enhance oxygen delivery while increasing blood pressure and organ perfusion, animal stroke studies were undertaken and showed favorable results. This eventually led to the start of our safety study: DCLHb in Acute Ischemic Stroke (DIAS). The pressor response observed after infusion of DCLHb is not a unique property of DCLHb, but rather a general property of hemoglobin solutions. Several studies performed to elucidate the mechanism of the pressor effect of DCLHb are described. The main conclusions are that DCLHb primarily interacts with the peripheral vascular autoregulatory system, rather than the central nervous system. DCLHb has been shown to interact with nitric oxide NO and to induce contraction of vascular smooth muscle cells. Studies in rats have shown that infusion of DCLHb leads to an increase of ET-1 levels along with an increase in BP. This pressor effect was significantly attenuated by pretreatment with a ET-receptor antagonist, thus providing evidence for the hypothesis that the pressor effect is mediated, at least in part, through the increase of ET-1 levels.

Heparin is regularly administered to stroke patients, regardless of the lack of convincing evidence of safety or efficacy. Heparin is given to promote early

clot lysis and to prevent thromboembolic events such as early stroke recurrence. Since the recurrence rate is presumed by some to be even larger in patients with atrial fibrillation (AF), clinicians are even more inclined to treatment with heparin in ischemic stroke in the presence of AF. In this part of chapter 2 the evidence on the safety and efficacy of heparin in acute stroke is reviewed, and the conclusion is that there is only one group of stroke patients in which the beneficial effects of heparin are uncontroversial. In the EAFT patients with non-rheumatic AF and recent TIA or minor stroke, anticoagulant treatment reduces the risk of recurrent stroke by two thirds. However, the EAFT does not provide information on the balance between the risk and benefit of anticoagulant therapy in the early period after onset of symptoms.

To our knowledge there has been only one study that focused solely on treatment with anticoagulants in the acute phase of an ischemic stroke in the presence of AF. In the HAEST study Berge et al investigated whether treatment with low-molecular weight heparin (LMWH) is superior to aspirin for the prevention of recurrent stroke during the first 14 days in patients with AF. Treatment was started within 30 hours of stroke onset. The authors concluded that while the data could not provide any evidence that LMWH is superior to aspirin in this setting, the study could not exclude the possibility of smaller, but still worthwhile effects of either of the trial drugs. The trial was also not large enough to reliably estimate a significant increase in cerebral hemorrhage on dalteparin.

Since 3169 patients with AF were randomized in the IST, we decided to study this subgroup in more detail. Even though this is a subgroup analysis, we considered it worthwhile still since many questions regarding AF in association with acute ischemic stroke are unanswered.

Chapter 3 describes the design, conduct and main results of the DIAS trial. This was a multi-center, randomized, single-blind, controlled, dose-finding, safety trial consisting of 3 parts. Twelve doses of 25, 50 and 100mg/kg DCLHb or equal volumes normal saline were administered over 72 hours to 85 patients with acute ischemic stroke in the anterior circulation, within 18 hours of symptom onset. The primary objective of this study was to evaluate the safety of DCLHb in patients with acute ischemic stroke. DCLHb caused a rapid rise in mean arterial blood pressure. The pressor effect was not accompanied by complications. Dose-dependent increases of lactate dehydrogenase (LDH), creatine kinase (CK), bilirubin and aspartate aminotransferase (AST) were found. These laboratory abnormalities were clinically asymptomatic and

disappeared within a week. Two patients, in the 100 mg/kg group, had an adverse event which was possibly drug-related: one of fatal brain and pulmonary edema, the other of transient renal and pancreatic insufficiency. Jaundice (yellow discoloration of skin and sclerae without evidence of liver insufficiency) and hemoglobinuria were minor adverse drug reactions that predominantly occurred in the 100 mg/kg group. Although patients in the DCLHb group tended to have more severe strokes at baseline than the control group, multivariate logistic regression analysis showed that a severe stroke at baseline (odds ratio (OR) 20.9;confidence interval (CI) 4.1-102.4) and treatment with DCLHb (OR 3.9; CI 1.4-12.0) were independent predictors of a worse outcome (Rankin 3-6) at 3 months. In conclusion, outcome scale scores were worse in the DCLHb group and more serious adverse events and deaths occurred in DCLHb-treated patients than in controls.

BP is often elevated in the acute phase of stroke. Lowering BP is commonly discouraged as this may adversely affect outcome. As the DIAS trial was to our knowledge the first to elevate BP in a randomized controlled fashion, we took a more detailed look at the pressor effect of DCLHb, in **chapter 4**. DCLHb (25, 50 and 100 mg/kg, n=10, 10 and 20, respectively) or placebo (n=45) was infused intravenously every 6 hours for 72 hours. We measured BP and heart rate every 15 minutes. Safety was further monitored by repeated physical and neurological examinations and by CT and MRI scans.

DCLHb caused a rapid rise in mean BP from 113±14 at baseline to 129±21, 135±11 and 135±24 for the 25, 50 and 100mg/kg respectively, versus. 109±16mmHg in controls. The duration of this effect on BP was dose-dependent. The pressor effect was not accompanied by complications such as hemorrhagic transformation of the infarct, brain edema or hypertensive encephalopathy and there was no excess need for anti-hypertensive treatment. However, clinical outcome at 3 months was worse in the treatment group.

Even though the pressor effect by itself was well tolerated, clinical outcome, described in chapter 3, was worse in patients treated with DCLHb. Further studies should therefore try and evaluate other methods of induced hypertension and explore their clinical efficacy.

For more than 50 years it has been known that hemolyzed blood can increase BP. Although pre-clinical studies suggest that this pressor response is due to

interaction of hemoglobin with endothelium-derived vasoactive substances, its mechanism in humans is unknown. We investigated the involvement of endothelin-1 (ET-1) in the BP response to DCLHb. This is described in **chapter 5**. These data were only collected for the patients randomized in Rotterdam.

DCLHb in a dose increasing fashion (25, 50 and 100 mg/kg, n=8, 8 and 11, respectively) or placebo (n=26) was infused intravenously every 6 hours for 72 hours to patients with an acute ischemic stroke. BP and heart rate (every 15 minutes) and plasma concentrations of ET-1, catecholamines, renin, vasopressin and atrial natriuretic peptide (before and 24 and 66 hours after the start of infusions) were measured.

In the placebo group, mean arterial pressure (MAP) of 112 (109-115) mmHg (mean and 95%CI) at baseline decreased spontaneously by 11.4 (5.4 -17.5) and 12.5 (5.4-19.5) mmHg after 24 and 66 hours, respectively. This decrease in MAP was attenuated in patients treated with DCLHb, reaching statistical significance in the highest dose group. Plasma ET-1 concentration decreased slightly in the placebo group from 4.2 (3.1-5.3) pg/ml (median and range) at baseline to 2.4(1.9-3.7) pg/ml after 24 hours ($p=0.0044$) and 2.8 (1.9-3.7) pg/ml after 66 hours ($p=0.0042$), but increased dose-dependently in response to DCLHb infusion. With the highest dose of DCLHb, plasma ET-1 concentration rose from 4.8 (0.1-7.8) pg/ml at baseline to 21.2 (13.4-53.2) pg/ml after 24 hours ($p<0.001$) and to 27.6 (11.9-47.8) pg/ml after 66 hours ($p<0.001$). Increments in plasma ET-1 concentration and in MAP were correlated ($r=0.30$, $p=0.02$). Other vasoactive hormones were not affected by DCLHb infusion.

Infusion of DCLHb in patients with acute ischemic stroke is associated with a dose-dependent increase in plasma ET-1 concentration. We suggest that this increase prevents the natural decrease in BP in these patients.

DCLHb was not beneficial when given to patients with acute ischemic stroke, whilst it was clearly hemodynamically active. In **chapter 6**, we wanted to determine whether the adverse effect of DCLHb on outcome was mediated through adverse effects on the heart. Blood pressure and heart rate were measured every 15 minutes. Continuous ECG monitoring was performed during 84 hours. Standard 12 lead ECG's were made at entry, after 24 hours and once between day 3 and 7. Patients were checked for adverse events on a daily basis. Plasma levels of creatine kinase (CK), lactate dehydrogenase (LDH) including sub-fractions were determined regularly.

DCLHb caused a rapid rise in mean arterial blood pressure that was not accompanied by complications or excess need for anti-hypertensive treatment. Cardiac functions as assessed by ECG's, Holter registration, and physical examination were not affected. Cardiac adverse events, such as heart failure, dysrhythmias occurred equally in both groups. Dose-dependent increases of LDH and CPK were found, but without an increase in CK-MB or LDH-1 sub-fractions. We conclude that the adverse effect of DCLHb on clinical outcome was not caused through adverse effect on the heart.

Chapter 7 provides the introduction to the second part of this thesis and discusses early recurrence of stroke, one of the feared complications in the acute phase of ischemic stroke. The frequency of early recurrent stroke varies in different studies and is often believed to be higher in patients with a potential cardiac source of embolism. This chapter reviews the definition and epidemiology of early recurrent stroke, tries to identify risk factors for early stroke recurrence and focuses on the benefits and risks of antithrombotic therapy in the prevention of this complication.

The diagnosis of early recurrent stroke is not always easy in patients with acute ischemic stroke, since up to 20% of patients show spontaneous fluctuations, some of which may be caused by repeated embolism, but many have other causes, including systemic disorders. The risk of recurrent stroke seems to be higher in patients entered in clinical trials than in population based studies or stroke data banks. The aggregate results of randomized clinical trials of heparin in acute ischemic stroke suggest that this treatment reduces the risk of early recurrence by about one quarter, but also that this benefit is completely offset by an equal risk of hemorrhage. Present data neither suggest a clear subgroup, in particular those with cardioembolic stroke, with a higher risk of early recurrence, nor one that specifically benefits from early treatment with anticoagulants. Findings from two large trials show that aspirin is safe and effective in reducing death and early recurrence, preventing 10 events per 1000 patients treated.

Atrial fibrillation (AF), both continuous and paroxysmal (PAF), constitute a major risk factor for stroke. The most effective way to diagnose PAF in acute stroke patients without a history of AF is still uncertain. In **chapter 8** we

compare the yield of 84-hour Holter-ECG recording with that of a single Holter-ECG during the first 24 hours and of repeated ECG's during the first 7 days.

We prospectively studied 85 patients with ischemic stroke in the anterior circulation of less than 18 hours duration by means of continuous ECG monitoring during 84 hours within the setting of the DIAS-trial. Medical history, including AF, was assessed from patients, general practitioners and available hospital information. Standard 12 lead ECG's were made at entry, after 24 hours and once between day 3 and 7. The patients with AF were divided in 2 groups: with or without a history of AF.

Twenty-one patients had AF, including 11 with a history of AF. Eight of 21 patients had PAF. Five of these 8 patients did not have a history of AF. Seven of 8 patients with PAF were identified by 84-hour Holter-ECG recording, 5 by repeated ECG's, and none by Holter-ECG recording during the first 24 hours only. It is concluded that PAF is commonly present in patients with acute ischemic stroke, and often newly discovered. It can be most effectively identified by 84-h Holter-ECG recording and only slightly less effectively by repeated ECG's. The yield of Holter-ECG monitoring during the first 24 hours only is extremely low.

In **chapter 9** we investigated the apparently high risk of early death after an ischemic stroke among patients with atrial fibrillation (AF), identified the main factors associated with early death, and assessed the effect of treatment with different doses of subcutaneous unfractionated heparin (UFH) given within 48 hours. We studied the occurrence of major clinical events within 14 days among 18,451 patients from IST, firstly for all treatment groups combined and then, among patients with AF, we examined the effects of treatment with subcutaneous UFH started within 48 hours and continued till 14 days after stroke onset.

3,169 (17%) patients were in AF. A total of 784 patients were allocated to UFH 12,500 IU sc bid, 773 to UFH 5,000 IU sc bid, and 1612 to "no heparin". Within each of these groups, half of the patients were randomly assigned to aspirin 300 mg once daily. Compared with patients without AF, patients with AF were more likely to be: female (56% vs. 45%), older (mean 78 vs. 71 yrs), have an infarct on the pre-randomization CT (57% vs. 47%), and have impaired consciousness (37% vs. 21%). The initial ischemic stroke type was more often a large artery infarct (36% vs. 21%). A lacunar stroke syndrome was less common (13% vs. 26%). Death within 14 days was commoner in patients with

AF (17% vs. 8%) and more often attributed to neurological damage from the initial stroke (10% vs. 4%). The frequency of recurrent ischemic or undefined stroke was not significantly different (3.9% vs. 3.3%). The proportion of AF patients with further events within 14 days allocated to UFH 12,500 IU (n=784), UFH 5,000 IU (n=773) and to "avoid heparin" (n=1612) respectively, were: ischemic stroke 2.3%, 3.4%, 4.9% (p=0.001); hemorrhagic stroke 2.8%, 1.3%, 0.4% (p<0.0001); and any stroke or death 18.8%, 19.4% and 20.7% (p=0.3). No effect of heparin on the proportion of patients dead or dependent at 6 months was apparent.

Acute ischemic stroke patients with AF have a higher risk of early death, which can be explained by older age and larger infarcts, but not by a higher risk of early recurrent ischemic stroke, although slightly more patients with AF died from a fatal recurrent stroke of ischemic or unknown type (1.3% vs. 0.9%). In patients with AF the absolute risk of early recurrent stroke is low, and there is no net advantage to treatment with heparin. These data do not support the widespread use of intensive heparin regimens in the acute phase of ischemic stroke associated with AF.

The results of the studies reported in this thesis are discussed in **chapter 10**. It is concluded that:

1. The hemodynamic response caused by DCLHb increases vascular resistance, and probably eliminates its potential as a neuroprotective agent in acute ischemic stroke.

2. In the 3169 patients with AF from IST, the medium-dose (12.500 IU) heparin regimen was clearly more effective in preventing early recurrent stroke than the low-dose heparin regimen or than aspirin, but it was also the most hazardous, considering the steep increase in intracerebral hemorrhage. UFH can produce a highly variable anticoagulant response, both inter- and intraindividually. LMWH's produce a more predictable response. However, both UFH and LMWH have not been found to be useful in improving outcome in AF patients with ischemic stroke. Further trials with anticoagulation are not warranted in this group of patients. AF patients with a TIA or rapidly resolving minor ischemic stroke can be treated with anticoagulants immediately after the event on the basis of the European Atrial Fibrillation Trial results.

Samenvatting

Het herseninfarct is een belangrijke doodsoorzaak in de Westerse wereld en aanleiding tot langdurige ziekenhuisopnames invaliditeit. De enige tot nog toe bekende effectieve behandeling, trombolyse met recombinant tissue plasminogen activator, kan slechts op een klein deel van de patiënten met een infarct worden toegepast.

In dit proefschrift worden twee behandelingen voor het acute herseninfarct, beide getest in gerandomiseerde gecontroleerde studies, beschreven. Als eerste werden de veiligheid, werkzaamheid en farmacodynamiek van een hemoglobine oplossing, Diaspirin Cross-linked Hemoglobin (DCLHb, HemAssist®), onderzocht in een multicenter trial van patiënten in de acute fase van het herseninfarct. Ten tweede, hebben we het effect van heparine bestudeerd bij patiënten met een acuut herseninfarct in combinatie met boezemfibrilleren bij patiënten uit een grote studie, de International Stroke Trial (IST). We waren vooral geïnteresseerd in de preventie van vroege recidief infarcten.

Hoofdstuk 2 beschrijft de ratio achter het gebruik van DCLHb en heparine bij de behandeling van het acute herseninfarct.

DCLHb is een gezuiverde, celvrije, humane hemoglobine oplossing, en is oorspronkelijk ontwikkeld als een bloedvervangend product. Nadat duidelijk werd dat DCLHb zuurstofaanvoer bevordert, terwijl het zowel de bloeddruk als de orgaanpersfusie doet toenemen, werden studies verricht in dierexperimentele herseninfarctmodellen. De gunstige resultaten hiervan hebben uiteindelijk geleid tot de start van onze fase II (veiligheid) studie: DCLHb in Acute Ischemic Stroke (DIAS). Het bloeddrukverhogende effect dat na infusie van DCLHb is geen unieke eigenschap van DCLHb, maar een eigenschap van hemoglobine oplossingen in het algemeen. Verscheidene studies verricht om het mechanisme van het bloeddrukverhogende effect van DCLHb te ontrafelen worden beschreven. De voornaamste conclusie is dat DCLHb inwerkt op het perifere vasculaire autoregulatoire systeem, en niet op het centrale zenuwstelsel. Er is aangetoond dat er interactie is tussen DCLHb en nitric oxide (NO) en zo contractie van vasculaire gladde spierweefsel cellen veroorzaakt. Studies bij ratten hebben aangetoond dat infusie van DCLHb leidt tot verhoogde endotheline (ET-1) spiegels alsmede een toename van de bloeddruk. Dit bloeddrukverhogend effect werd duidelijk verzwakt door voorbehandeling met een ET-receptor antagonist, aldus bewijs verschaffend voor de hypothese dat het bloeddrukverhogend effect in elk geval deels wordt veroorzaakt, door de verhoogde ET-1 spiegels.

Heparine wordt geregeld toegediend aan patiënten met een infarct, ondanks het gebrek aan overtuigend bewijs voor de veiligheid of effectiviteit van deze behandeling. Heparine wordt o.a. gegeven om vroege lysis van de thrombus te bewerkstelligen en om thrombo-embolische complicaties zoals vroege recidieven te voorkomen. Aangezien de recidiefkans soms hoger wordt geacht bij patiënten met atrium fibrillatie (AF), zijn clinici bij een infarct in aanwezigheid van AF nog meer geneigd over te gaan op behandeling met heparine. In dit deel van hoofdstuk 2 wordt een overzicht gegeven van het bewijs betreffende de veiligheid en effectiviteit van heparine in de acute fase van het infarct. De conclusie is dat er slechts één groep van infarct patiënten is, waarbij het heilzame effecten van heparine onomstotelijk is vastgesteld: in de Europese Atrium Fibrillatie Trial (EAFT) bleek behandeling met orale antistolling bij patiënten met niet-reumatisch AF en een recente TIA of niet invaliderend herseninfarct de kans op een recidief infarct met twee derde te reduceren. Maar de EAFT verschaft geen data over de balans tussen de voor- en nadelen van antistolling in de acute fase na het ontstaan van de symptomen.

Voor zover mij bekend, is er slechts een studie geweest die zich heeft geconcentreerd op behandeling met antistolling in de acute fase van het herseninfarct in aanwezigheid van AF. In de HAEST studie hebben Berge et al. onderzocht of behandeling met gefractioneerde heparine (dalteparine) beter is dan aspirine om recidief infarcten gedurende de eerste 14 dagen te voorkomen bij patiënten met AF. Er werden 449 patiënten gerandomiseerd. De behandeling werd binnen 30 uur na aanvang van symptomen gestart. De auteurs concludeerden dat hoewel de data geen bewijs leverden dat LMWH beter is dan aspirine in deze groep van patiënten, de mogelijkheid van een kleiner, maar toch nog steeds klinisch waardevolle effect van een beide middelen niet kon worden uitgesloten. Ook was de trial niet groot genoeg om een betrouwbare schatting te geven van een eventueel verhoogd risico op hersenbloedingen door dalteparine.

Aangezien er 3169 patiënten met AF waren gerandomiseerd in de International Stroke Trial (IST), hebben wij besloten deze groep uitgebreider te bestuderen. Hoewel het een subgroep analyse betreft, is het waardevol omdat er nog veel vragen rond AF in associatie met een acuut infarct onbeantwoord zijn.

In **hoofdstuk 3** worden de opzet, uitvoering en de hoofdresultaten van de DIAS trial beschreven. Dit was een multi-center, gerandomiseerde, enkelblinde, placebogecontroleerde, fase II studie bestaande uit 3 delen. Gedurende een periode van 72 uur werden twaalf doseringen van 25, 50 of 100mg/kg DCLHb of eenzelfde volume fysiologische zoutoplossing werden toegediend aan patiënten met een infarct in de voorste circulatie. Behandeling werd binnen 18 uur na ontstaan van de symptomen gestart. Het primaire doel van de studie was te bepalen of toediening van DCLHb aan patiënten met een infarct veilig is. DCLHb veroorzaakte een snelle stijging van de bloeddruk. Deze verhoging van de bloeddruk ging niet gepaard met complicaties en werd goed verdragen. De plasmaconcentratie van lactaat dehydrogenase (LDH), creatine kinase (CK), bilirubine and aspartaat aminotransferase (AST) waren dosisafhankelijk verhoogd. Deze laboratoriumafwijkingen waren asymptomatisch en verdwenen binnen een week. Twee patiënten, beide uit de 100 mg/kg groep, hadden een 'adverse event' dat mogelijk gerelateerd was aan DCLHb. Het betrof een patiënt met fataal hersen- en longoedeem, en één met voorbijgaande nier- en pancreasinsufficiëntie. Geelzucht (gele verkleuring van huid en slerae, zonder aanwijzingen voor leverinsufficiëntie) en hemoglobinurie waren minor 'adverse drug reactions', die voornamelijk optraden in de 100 mg/kg groep. Hoewel de patiënten in de DCLHb groep ertoe negen ernstiger infarcten bij baseline te hebben, toonde multivariate logistische regressie analyse aan dat een ernstige infarct bij binnenkomst (odds ratio (OR) 20.9;confidence interval (CI) 4.1-102.4) en behandeling met DCLHb (OR 3.9; CI 1.4-12.0) onafhankelijke voorspellers waren van een slechte uitkomst (Rankin 3-6) op 3 maanden. Concluderende waren de uitkomst schalen slechter in de DCLHb groep en er traden meer 'serious adverse events' en doden op bij patiënten behandeld met DCLHb dan bij controle patiënten.

De bloeddruk is vaak verhoogd in de acute fase van het herseninfarct. Verlagen van de bloeddruk wordt in het algemeen afgeraden aangezien dit het herstel mogelijk negatief kan beïnvloeden. Aangezien de DIAS trial voor zover ik weet de eerste studie was, waarin de bloeddruk op een gerandomiseerde gecontroleerde manier werd verhoogd, hebben we het pressor effect van DCLHb in **hoofdstuk 4** wat uitgebreider bestudeerd. DCLHb (25, 50 and 100 mg/kg, n=10, 10 and 20, respectievelijk) of placebo (n=45) werd intraveneus toegediend, iedere 6 uur gedurende 72 uur. Behandeling werd binnen 18 uur na het ontstaan van de symptomen begonnen. De bloeddruk en hartfrequentie werden iedere 15 minuten gemeten. Voorts werd herhaaldelijk algemeen

lichamelijk en neurologisch onderzoek verricht en werden CT- en MRI-scans gemaakt.

DCLHb veroorzaakte een snelle stijging van de gemiddelde arteriële bloeddruk van 113±14 op baseline naar 129±21, 135±11 en 135±24mmHg voor de 25, 50 and 100mg/kg groepen respectievelijk, versus. 109±16mmHg in de controle groep. De duur van dit effect op de bloeddruk was dosisafhankelijk. Het pressor effect werd niet vergezeld door complicaties zoals hemorrhagische transformatie van het infarct, hersenoedeem, hypertensieve encephalopathie of toename van het gebruik van anti-hypertensiva gebruikt. Echter, de klinische uitkomstvariabelen waren slechter in de behandelde groep.

Hoewel het pressor effect op zich goed getolereerd, was de klinische uitkomst, besproken in hoofdstuk 3, slechter bij de patiënten behandeld met DCLHb. Toekomstige studies zouden andere methodes van geïnduceerde hypertensie moeten bestuderen en de klinisch effect hiervan moeten evalueren.

Al meer dan 50 jaar is bekend dat gehemolyseerd bloed de bloeddruk kan verhogen. Hoewel dierexperimentele studies suggereren dat de verhoging van de bloeddruk wordt veroorzaakt door interactie van hemoglobine met uit het endotheel afkomstige vaso-actieve substanties, is het mechanisme bij de mens onbekend. Wij onderzochten de betrokkenheid van endotheline-1 (ET-1) bij het bloeddrukverhogende effect van DCLHb. Dit wordt beschreven in **hoofdstuk 5**. Deze data zijn alleen verzameld bij de patiënten gerandomiseerd in Rotterdam.

In een gerandomiseerde fase II studie, werd DCLHb in verschillende doseringen (25, 50 and 100 mg/kg, n=8, 8 and 11, respectievelijk) of placebo (n=26) intraveneus toegediend, iedere 6 uur gedurende 72 uur aan patiënten met een acuut herseninfarct. De bloeddruk en hartfrequentie (iedere 15 minuten) en plasma concentraties van ET-1, catecholamines, renine, vasopressine and atriaal natriuretisch peptide (vóór en 24 en 66 uur ná het starten van de infusies) werden gemeten.

In de placebo groep, daalde de gemiddelde arteriële bloeddruk (MAP) van 112 (109-115) mmHg (gemiddelde en 95%BI) bij baseline spontaan met 11.4 (5.4 - 17.5) en 12.5 (5.4-19.5) mmHg na 24 en 66 uur, respectievelijk. Deze daling van de MAP was verminderd bij patiënten behandeld met DCLHb. Dit verschil was statistisch significant in de groep die de hoogste dosering DCLHb ontving. Plasma ET-1 concentraties daalden licht in de placebo groep van 4.2 (3.1-5.3) pg/ml (mediaan en range) bij baseline naar 2.4(1.9-3.7) pg/ml na 24 uur

(p=0.0044) en 2.8 (1.9-3.7) pg/ml na 66 uur (p=0.0042), maar stegen dosisafhankelijk als reactie op de DCLHb infusies. Bij de hoogste dosering van DCLHb, steeg de plasma ET-1 concentratie van 4.8 (0.1-7.8) pg/ml bij baseline naar 21.2 (13.4-53.2) pg/ml na 24 uur (p<0.001) en naar 27.6 (11.9-47.8) pg/ml na 66 uur (p<0.001). De toename van de plasma ET-1 concentratie en van de MAP waren gecorreleerd (r=0.30, p=0.02). De concentraties van de overige vasoactieve hormonen werden niet beïnvloed door DCLHb.

Infusie van DCLHb bij patiënten met een acuut herseninfarct gaat gepaard met een dosisafhankelijke stijging van de plasma ET-1 concentratie. Wij doen de suggestie dat deze toename de natuurlijke daling van de bloeddruk bij deze patiënten tegengaat.

Patiënten met een acuut herseninfarct hadden geen baat bij toediening van DCLHb, terwijl het wel duidelijk hemodynamisch actief was. In **hoofdstuk 6**, wilden we uitzoeken of het negatieve effect van DCLHb op de uitkomst werd veroorzaakt door een negatief effect op de cardiale functies. Continue Holter-ECG monitoring werd verricht gedurende 84 uur. Standaard ECG's werden gemaakt bij opname, na 24 uur en één keer tussen dag 3 en 7. Patiënten werden dagelijks gecontroleerd op 'adverse events'. Plasma concentraties van creatine kinase (CK), lactaat dehydrogenase (LDH) inclusief subfracties werden op vaste tijden bepaald.

DCLHb veroorzaakte een snelle stijging van de gemiddelde arteriële bloeddruk, die niet gepaard ging met complicaties of een overmaat aan behandeling met anti-hypertensiva. Cardiale functies, gemeten door middel van ECG's, Holter registraties, en lichamelijk onderzoek waren niet aangedaan. Cardiale 'adverse events', zoals hartfalen en arrhythmieën kwamen even vaak voor in beide groepen. De plasmaconcentraties van LDH en CPK stegen dosisafhankelijk, maar zonder een toename van de cardiale subfracties, CK-MB en LDH-1. De conclusie is dat de slechtere uitkomst van de patiënten behandeld met DCLHb niet veroorzaakt werd door een negatief effect op de functie van de hart.

Hoofdstuk 7 is de introductie tot het tweede deel van dit proefschrift en bespreekt vroege recidief infarcten, één van de gevreesde complicaties in de acute fase van het herseninfarct. De frequentie van vroege recidieven varieert in verschillende studies en er wordt vaak aangenomen dat deze hoger is bij patiënten met een potentiële cardiale emboliebron. In dit hoofdstuk worden de

definitie en epidemiologie van vroege recidief infarcten besproken, wordt gepoogd risicofactoren voor vroege recidief infarcten te identificeren en worden de kosten en baten van anti-trombotische therapie voor de preventie van deze complicatie tegen elkaar afgewogen.

De diagnose vroeg recidief infarct is niet altijd gemakkelijk bij patiënten met een acuut herseninfarct, aangezien tot 20% van de patiënten spontane fluctuaties vertonen. Een deel van deze fluctuaties wordt veroorzaakt door een recidief embolus, maar velen hebben een andere oorzaak, bijvoorbeeld een metabole stoornissen. Het risico van een recidief infarct lijkt hoger te zijn bij patiënten die geïncludeerd zijn in klinische trials dan in populatie studies of stroke data banken. Het aggregaat van de resultaten van gerandomiseerde klinische studies van heparine bij het acute herseninfarct suggereert dat deze behandeling het risico van een vroeg recidief met ongeveer een kwart doet verminderen, maar dat dit voordeel volledig teniet wordt gedaan door een even grote verhoging van de kans op een intracerebrale bloeding. De huidige data suggereren noch een duidelijke subgroep, m.n. die met een cardioembolisch infarct, met een hoger risico op een vroeg recidief infarct, noch een subgroep die wel baat zou hebben bij vroege behandeling met anticoagulantia. Het enige tot nog toe effectief gebleken middel om voege recidief infarcten te voorkomen is aspirine. De bevindingen van twee grote studies tonen aan dat behandeling met aspirine gedurende de eerste 14 dagen na het herseninfarct 10 vroege recidief infarcten en doden per 1000 behandelde patiënten voorkomt.

Atrium fibrilleren (AF), zowel continu als paroxysmaal (PAF), is een grote risicofactor voor een infarct. Het is nog onduidelijk wat de meest effectieve manier is om PAF te diagnosticeren bij patiënten met een acuut herseninfarct zonder een voorgeschiedenis van AF. In **hoofdstuk 8** wordt de additionele diagnostische opbrengt van langdurige Holter-ECG monitoring in de acute fase van herseninfarct om PAF op te sporen, vergeleken met herhaalde ECG's gedurende de eerste week en Holter-ECG gedurende de eerste 24 uur.

Wij bestudeerden prospectief 85 patiënten met een herseninfarct in de voorste circulatie binnen 18 uur na het begin van de symptomen d.m.v. continue ECG monitoring gedurende 84 uur in de setting van de DIAS-trial. De voorgeschiedenis, waaronder AF, werd afgenomen van patiënten, hun huisartsen en de beschikbare geautomatiseerde informatie van het ziekenhuis. Standaard ECG's werden gemaakt bij opname, na 24 uur en nog één keer tussen dag 3 en 7. De patiënten met AF werden onderverdeeld in 2 groepen: met of zonder AF in de voorgeschiedenis.

Eenentwintig patiënten hadden AF, waarvan 11 bekend wegens AF in de voorgeschiedenis. Acht van deze 21 patiënten hadden PAF. Bij 5 van hen was AF nooit eerder geconstateerd. Zeven van de 8 patiënten met PAF werden geïdentificeerd d.m.v. 84-uur Holter-ECG, 5 d.m.v. herhaalde ECG's, en niet één door Holter-ECG registratie gedurende de eerste 24 uur. Er wordt geconcludeerd dat PAF regelmatig voorkomt bij patiënten met een acuut herseninfarct, en vaak een nieuwe bevinding is. De meest effectieve opsporingsmethode is die d.m.v. 84-uurs Holter-ECG registratie en de herhaalde ECG's zijn slecht iets minder effectief. De opbrengst van Holter-ECG monitoring gedurende de eerste 24 uur is extreem laag.

In **hoofdstuk 9** hebben we het hoge kans op vroege dood na een herseninfarct bij patiënten met AF bestudeerd, de belangrijkste factoren geassocieerd met vroege dood geïdentificeerd, en het effect van behandeling met verschillende doseringen subcutaan toegediende heparine geëvalueerd. Wij onderzochten het optreden van 'major clinical events' binnen 14 dagen onder 18.451 patiënten uit de International Stroke Trial (IST), in eerste instantie voor alle behandelingsgroepen gecombineerd. Vervolgens bestudeerden we onder patiënten met AF, het effect van behandeling met ongefractioneerde heparine (UFH) begonnen binnen 48 uur na start van de symptomen en voortgezet tot en met 14 dagen erna.

3.169 (17%) patiënten hadden AF. In totaal kregen 784 patiënten behandeling met UFH 12.500 IE sc 2 dd toegewezen, 773 UFH 5.000 IE sc 2dd, en 1612 kregen 'geen heparine'. Binnen al deze groepen kreeg de helft van de patiënten op gerandomiseerde wijze 300mg aspirine per dag. Vergeleken met patiënten zonder AF, waren patiënten met AF vaker: vrouw (56% vs. 45%), ouder (gemiddeld 78 vs. 71 jaar), hadden een infarct op de pre-randomisatie CT-scan (57% vs. 47%), en een gedaald bewustzijn (37% vs. 21%). Het initiële herseninfarct was vaker een corticaal infarct (36% vs. 21%). Lacunaire syndroom kwam minder vaak voor (13% vs. 26%). Dood binnen 14 dagen kwam vaker voor bij patiënten met AF (17% vs. 8%) en werd vaker toegeschreven aan neurologische schade van het oorspronkelijke infarct (10% vs. 4%). De frequentie van recidief herseninfarct of niet nader gespecificeerde beroerte was niet significant anders (3.9% vs. 3.3%).

De proportie van AF patiënten met 'major clinical events' events binnen 14 dagen per behandelingsgroep was als volgt; UFH 12.500 IE (n=784), UFH 5.000 IE (n=773) en 'geen heparine' (n=1612) respectievelijk: herseninfarct 2.3%, 3.4%, 4.9% (p=0.001); hersenbloeding 2.8%, 1.3%, 0.4% (p<0.0001); en

hersenbloeding, -infarct of dood 18.8%, 19.4% and 20.7% (p=0.3). Behandeling met heparine had geen effect op de proportie patiënten die op 6 dood of afhankelijk op tijdstip 6 maanden.

Concluderend stellen we dat patiënten met een acuut herseninfarct in combinatie met AF een hoger risico hebben op vroege dood. Dit kan worden verklaard door hun oudere leeftijd en grotere infarcten, maar niet door een grotere kans op een recidief herseninfarct, hoewel iets meer patiënten met AF overleden als gevolg van een recidief beroerte met een ischemische of onbekende oorzaak (1.3% vs. 0.9%). Bij patiënten met AF is het absolute risico op een vroeg recidief infarct laag, en er is geen netto voordeel van behandeling met heparine. Deze data ondersteunen het wijdverbreide gebruik van intensieve heparine regimes in the acute fase van het herseninfarct bij aanwezigheid van AF niet.

De resultaten van de studies gepresenteerd in dit proefschrift worden besproken **hoofdstuk 10**. Er wordt geconcludeerd dat:

1. DCLHb een bloeddrukverhoging veroorzaakt die hoogstwaarschijnlijk gepaard gaat met een verhoogde vaatweerstand. Hierdoor is DCLHb niet bruikbaar als neuroprotectivum voor patiënten met een acuut herseninfarct.

2. Onder de 3169 patiënten met AF uit de IST, was de medium-dose (12.500 IU) heparine duidelijk effectiever in het voorkomen van vroege recidief infarcten dan de lage dosering heparine en dan aspirine, maar het was ook de meest riskante behandeling, gezien de forse toename van hersenbloedingen. Het is bekend dat ongefractioneerde heparine een zeer variabel effect heeft op de stolling, zowel inter- als intraindividueel. Laagmoleculaire heparines geven een meer voorspelbaar effect Maar, van zowel ongefractioneerde heparine als van laagmoleculaire heparines is aangetoond dat deze niet effectief zijn om de uitkomst van patiënten met een herseninfarct en AF te verbeteren. Verdere studies met heparine in deze groep patiënten bieden weinig perspectief. AF patiënten met een TIA of een snel herstellend minor stroke moeten op basis van de resultaten van de European Atrial Fibrillation Trial direct met anticoagulantia worden behandeld. Voor alle andere patiënten is aspirine de beste optie.

Dankwoord en Curriculum vitae

Dankwoord

Velen hebben bijgedragen aan de totstandkoming van dit proefschrift. Een aantal wil ik bij naam noemen.

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Ton van den Meiracker, lid van de grote commissie, heeft mijn eerste artikel bijna volledig herschreven. Ik heb er veel van geleerd.

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Curriculum vitae

De schrijfster van dit proefschrift werd geboren op 29 april 1968 te Nijmegen. Zij volgde haar middelbare schoolopleiding aan de Scholengemeenschap "De Krimpenerwaard" te Krimpen aan den IJssel, alwaar zij in 1986 het VWO (Gymnasium) diploma haalde.

In 1987 begon zij met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. In april 1994 haalde zij haar artsexamen. Van april 1994 tot maart 1996 was zij werkzaam als arts-onderzoeker aan de afdeling Neurologie van het Academisch Ziekenhuis Dijkzigt, te Rotterdam. Tijdens deze periode werd het grootste gedeelte van de data van het in dit proefschrift beschreven DIAS onderzoek verzameld. Na een periode van zes maanden als AGNIO werd de opleiding tot neuroloog gevolgd van juli 1996 tot 15 januari 2002 (opleiders: prof. dr F.G.A. van der Meché en prof. dr P.J. Koudstaal).

Zij zal haar werk als neuroloog vanaf februari 2002 continueren in het Medisch Centrum Rijnmond Zuid te Rotterdam.