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Controlled Safety Study of a Hemoglobin-Based Oxygen Carrier, DCLHb, in Acute Ischemic Stroke

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Background and Purpose—Diaspirin cross-linked hemoglobin (DCLHb) is a purified, cell-free human hemoglobin solution. In animal stroke models its use led to 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.

Methods—DCLHb or saline was administered to 85 patients with acute ischemic stroke in the anterior circulation, within 18 hours of onset of symptoms, in a multicenter, randomized, single-blind, dose-finding, controlled safety trial, consisting of 3 parts: 12 doses of 25, 50, and 100 mg/kg DCLHb over 72 hours.

Results—DCLHb caused a rapid rise in mean arterial blood pressure. The pressor effect was not accompanied by complications or excessive need for antihypertensive treatment. Two patients in the 100 mg/kg group had adverse events that were possibly drug related: one suffered fatal brain and pulmonary edema, the other transient renal and pancreatic insufficiency. Multivariate logistic regression analysis showed that a severe stroke at baseline and treatment with DCLHb (OR, 4.0; CI, 1.4 to 12.0) were independent predictors of a worse outcome (Rankin Scale score of 3 to 6) at 3 months.

Conclusions—Outcome scale scores were worse in the DCLHb group, and more serious adverse events and deaths occurred in DCLHb-treated patients than in control patients. We recommend that additional safety studies be performed, preferably with a second generation, genetically engineered hemoglobin. **(*Stroke*. 1999;30:993-996.)**

Key Words: blood substitutes ■ hemoglobins ■ safety ■ stroke, acute ■ stroke, ischemic

Diaspirin cross-linked hemoglobin (DCLHb) is a cell-free, hemoglobin-based oxygen-carrying solution. In animal stroke models, hemodilution with DCLHb induced a hypertensive response and resulted in significant reductions in the extent of the brain injury.[1,2] Hypertension has been used in the treatment of stroke to increase blood flow, but its use has not been widely adopted.[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 suggest that DCLHb scavenges nitric oxide (NO),[6] thereby inhibiting NO-related neurotoxicity.

DCLHb is being developed as a hemoglobin therapeutic for high-blood-loss surgery, sepsis, hemodialysis,[7] cardiac surgery, and trauma. In a phase 1 study in which healthy volunteers received a single dose of DCLHb (25, 50, or 100 mg/kg), dose-dependent increases in mean arterial pressure were reported. No significant adverse events or toxicity were observed in the phase 1 study,[8] in several phase 2 surgical and ICU studies, or in the completed cardiac surgery study or ongoing phase 3 perioperative study. However, a North American study in trauma patients was recently terminated prematurely because of higher mortality in the treatment group, and enrollment in a European trauma study has been suspended.[9]

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

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 participating centers were the neurology departments 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.

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Patients

Patients with clinical symptoms of an acute ischemic stroke in the anterior circulation were eligible if they were aged >20 years, could be treated within 18 hours after the 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 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 hematologic cause of the symptoms, congestive heart failure or acute myocardial infarction, systolic BP >230 mm Hg or diastolic BP >130 mm Hg, renal or liver disease, spontaneous improvement of symptoms by at least 2 grades on the modified Rankin Scale,[10] 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.[11] DCLHb was prepared and provided by Baxter Healthcare Corp, Deerfield, Ill (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 blinded because of the prominent color of the drug and the difficulty in manufacturing a proper placebo. Three doses were tested: 25, 50, and 100 mg/kg 10% DCLHb (n=10, n=10, and n=20, respectively) or an equal volume of saline (n=45) every 6 hours for 72 hours (12 doses) intravenously at a rate of 2 mL/min.

Assessments

Baseline assessment consisted of a medical history, general physical and neurological examinations, ECG, CT scan, urinalysis, and hematologic and biochemical tests. Neurological status was assessed by means of the modified National Institutes of Health Stroke Scale (NIHSS).[12] Functional ability was scored by means of the Barthel Activities of Daily Living Index[13] and the modified Rankin scale. Blood pressure and heart rate were measured every 15 minutes during infusions with an automatic, oscillometric blood pressure device.

The physical examination was repeated at days 3, 7, and 14 and at the 3-month follow-up. The NIHSS was assessed at days 1, 3, and 14 and at 3 months. Rankin and Barthel scores were measured at day 14 and at 3 months. Safety was further monitored by regular blood tests, urinalysis, repeat EKGs, 84-hour Holter monitoring, and repeat CT and MRI scans.

Antibody titers to DCLHb were measured at the 3-month visit.

Statistics

Values are expressed as mean±SD unless otherwise indicated. The Student unpaired t test, χ^2 test, or Fisher exact test were used as appropriate. Regression analysis was used to identify factors independently related to outcome at 3 months. A value of $P<0.05$ was considered statistically significant.

Results

Patient characteristics are presented in the Table. The groups were well matched with respect to all baseline variables, although patients who received control treatment tended to have experienced less severe strokes.

Adverse Events

Two patients had unexpected events, possibly related to DCLHb. The first was a 65-year-old patient with a severe stroke (NIHSS score of 23) and cardiomegaly; slightly elevated SGOT, SGPT, and GGT; and obstructive breathing at baseline. Blood pressure was 167/92. The patient received 5 doses of DCLHb of 80 mL each. Subsequently scleral icterus, hypertension, fever, 2 episodes of pulmonary edema, and cerebral edema developed, leading to death.

The other patient was a 53-year-old woman with a moderately severe lacunar stroke (NIHSS score of 9) and a medical history of untreated hypertension. Baseline examination showed a slightly elevated amylase (171 U/L). This patient was treated with 12 doses of DCLHb. She developed renal and pancreatic insufficiency, nausea, and anemia within 24 hours. All signs and symptoms resolved except for the amylase, which was still asymptomatically elevated (215 U/L) at 3 months.

Minor adverse drug reactions independently related to DCLHb occurred predominantly in the 100 mg/kg group. Jaundice occurred in 0 of 10, 1 of 10, and 17 of 20 patients in the 25, 50, and 100 mg/kg groups, respectively, versus 0 of 45 in the control group ($P=0.00$); it resolved around day 5. There was no associated hepatotoxicity, and it was considered to be due to the extravasation of DCLHb and the rise in bilirubin. Hemoglobinuria was found in 22 of 40 patients

Characteristics of Patients With Acute Ischemic Stroke Randomized to DCLHb or Normal Saline

Characteristic	Saline	DCLHb	*P*
Patients, n	45	40	
Age, y	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
Stroke side			
Right	19 (42)	25 (63)	0.10
Left	26 (58)	15 (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.

versus 13 of 45 controls ($P=0.03$). There was no associated renal dysfunction, and the abnormalities resolved by day 7.

Laboratory Parameters

Dose-dependent increases of LDH, CPK, bilirubin, AST were found. All laboratory abnormalities were clinically asymptomatic and disappeared within a week. There was also a dose-dependent rise in endothelin-1.[14]

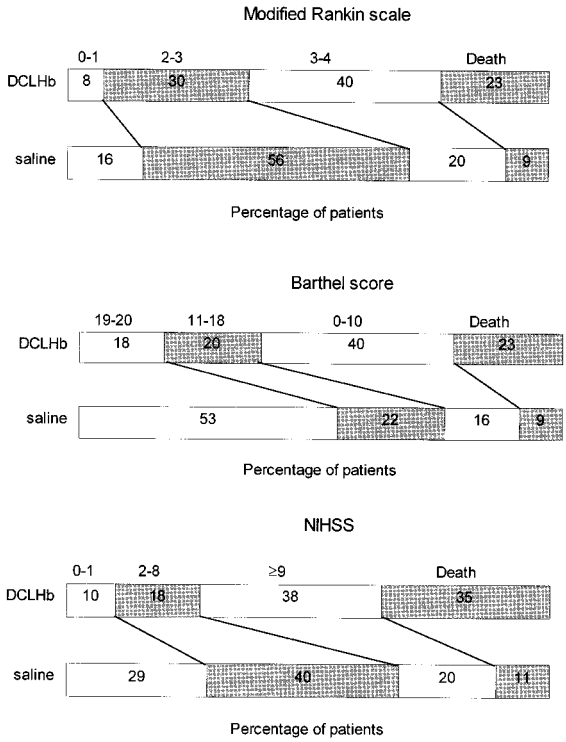
All other laboratory measurements showed no statistically significant difference between groups. At 3 months no DCLHb antibodies were found.

Blood Pressure

DCLHb produced a rapid rise in mean arterial pressure, which reached a maximum within 2 hours after the first infusion. The blood pressure increased from 113±14 at baseline to 134±20, versus 109±16 mm Hg in controls. The magnitude of the increases caused by the different doses was similar, but the duration of the pressor was dose dependent. 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 requiring pharmacological intervention occurred in 3 patients treated with DCLHb versus 3 in the saline group.

Outcome

Outcome at 3 months was significantly worse in the treatment group. Thirty-four patients (85%) had an unfavorable outcome (Rankin score, 3 to 6) versus 23 (51%) in controls ($P=0.002$). Multivariate logistic regression analysis showed that a severe stroke at baseline (Rankin score, 4 to 5; $P<0.001$; OR, 20.9; CI, 4.1 to 102.4) and treatment with DCLHb ($P=0.015$; OR, 3.9; CI, 1.4 to 12.0) were independent predictors of a worse outcome (Rankin score, 3 to 6). Outcome was not related to the dose of DCLHb. The Barthel Index and NIHSS showed similar results (Figure).



Clinical outcome at 3 months by modified Rankin Scale, Barthel Index, and NIHSS scores, according to treatment.

Discussion

We conducted a safety study on the use of DCLHb in patients with acute ischemic stroke. DCLHb produced a rapid rise in blood pressure. The duration of the effect was dose dependent. The hypertensive effect was not accompanied by complications such as hypertensive encephalopathy or hemorrhagic transformation of the infarction. Side effects that were independently related to the use of DCLHb were jaundice, hemoglobinuria, and some laboratory abnormalities. These were all transient and clinically asymptomatic.

However, treatment with DCLHb was an independent predictor of an unfavorable outcome at 3 months. The cause of the worse outcome is unclear. We have recently reported elevated ET-1 levels in response to DCLHb,[14] which may have contributed to the ischemic damage through the potent vasoconstrictor effect of ET-1.[15] However, there is also evidence that a systemic increase in endothelin causes a vasodilator effect in the brain.[16] The dose-dependent increase in ET-1 may have been promoted by the 72-hour duration of treatment or by the treatment delay of 18 hours. Alternative explanations of the worse outcome in DCLHb patients are the role of chance in this small study; the imbalance, although not statistically significant, of stroke severity at randomization; or bias due to the single-blinded nature of the study. Furthermore, in analogy with other stroke treatments,[17,18] it may be beneficial to administer DCLHb immediately after the onset of ischemia but harmful to give it 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 1 hour after initiation of ischemia. Very recently, such high doses were found to be safe in patients after they underwent coronary bypass surgery.[19]

In conclusion, infusion of low doses of DCLHb over 3 days adversely affected outcome in acute ischemic stroke patients, and more serious adverse events and deaths occurred. A difference in baseline stroke severity scores may have contributed to this imbalance. 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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