Effects of diaspirin cross-linked haemoglobin on post-traumatic cerebral perfusion pressure and blood flow in a rodent model of diffuse brain injury

I. R. Piper, M. A. Garrioch, M. J. Souter, P. J. D. Andrews and D. Thomson

Summary
Diaspirin cross-linked haemoglobin (DCLHb) is a new oxygen carrying blood substitute with vasoactive properties. Vasoactive properties may be mediated via high affinity binding of nitric oxide by the haem moiety. Using a rodent model of head injury combined with ischaemia, we studied the effects of DCLHb on cerebral blood flow (CBF) and intracranial pressure (ICP). Twenty anaesthetized rats were allocated randomly to receive treatment with DCLHb 400 mg kg\(^{-1}\) i.v. or placebo (oncologically matched plasma protein substitute 4.5\% i.v.). To produce diffusely increased ICP, after a severe weight drop injury, all animals underwent a 30-min period of bilateral carotid ligation combined with a period of induced hypotension. After reperfusion, DCLHb or placebo was infused and the animals instrumented for measurement of intraventricular ICP and CBF in the region of the sensorimotor cortex using the hydrogen clearance technique. Mean arterial pressure (MAP), ICP, cerebral perfusion pressure (CPP) (CPP=MAP–ICP) and CBF were measured 4 h after injury in all animals. DCLHb significantly reduced ICP from mean 13 (SEM 2) to 3 (1) mm Hg (P<0.001), increased CPP from 52 (8) to 95 (6) mm Hg (P<0.001) and increased CBF from 21 (2) to 29 (2) ml 100 g\(^{-1}\) min\(^{-1}\) (P=0.032). We conclude that DCLHb improved CPP without a reduction in CBF in a rodent model of post-traumatic brain swelling. (Br. J. Anaesth. 1998; 80: 639–643)

Materials and methods
The study conformed to the UK Home Office Animals Scientific Procedures Act 1986 (PPL 60/01228). We studied 20 male Sprague–Dawley rats (350–450 g), allocated randomly to a placebo (n=10) or treatment group (n=10). For induction of anaesthesia, all animals were placed in a holding cage containing 4% halothane in oxygen. When anaesthesia was induced, the animals were given diazepam 2.5 mg kg\(^{-1}\) i.p., in Hypnorm (fentanyl-based 0.3 ml kg\(^{-1}\)) to provide surgical anaesthesia for the time required to obtain i.v. access. Anaesthesia was maintained order to better perfuse the brain, the range of effective agents for treating increased ICP is limited. Diaspirin cross-linked haemoglobin (DCLHb) is a new solution manufactured from human haemoglobin, harvested from outdated donated blood. The haemoglobin molecules are cross-linked at the \(\alpha\) chains by treatment with the salicylate bis(3,5-dibromosalicyl) fumarate. This process forms a solution which is chemically stable, has low viscosity, a longer intravascular retention time than haemoglobin monomers or dimers, and an oxygen carrying capacity similar to whole blood.

During preclinical studies DCLHb increased arterial pressure and preferentially redistributed blood flow to the brain and other vital organs. This increase in arterial pressure was caused in part by generalized vasoconstriction which may include a cerebral vasoconstrictor component. Therefore, DCLHb seems to have potential as a neuroprotective agent by its ability to vasoconstrict, increase arterial pressure and yet maintain or augment oxygen delivery to tissues by virtue of its low viscosity and oxygen carrying capacity. To test the hypothesis that DCLHb may have beneficial effects on cerebral blood flow (CBF) and ICP without worsening brain perfusion, we compared its effects with standard treatment in an established rodent model of head injury-induced diffuse brain swelling.

Keywords: blood, replacement; brain, blood flow; brain, injury; brain, intracranial pressure; rat, model, rat

Head injury is one of the commonest causes of death and disability associated with trauma. The non-surgical management of the severely head injured patient involves prevention of secondary insults such as arterial hypotension and hypoxaemia. Increased intracranial pressure (ICP) also occurs frequently after head injury and is a common secondary insult which significantly contributes to patient morbidity and mortality. More than 70% of severely head injured patients exhibit one or more episodes of increased ICP (ICP > 20 mm Hg). Although there are several useful adjuncts for the management of increased ICP, including the use of osmotic and other diuretics to attempt to control tissue oedema, and agents which modify the rheology of blood in order to better perfuse the brain, the range of effective agents for treating increased ICP is limited. Diaspirin cross-linked haemoglobin (DCLHb) is a new solution manufactured from human haemoglobin, harvested from outdated donated blood. The haemoglobin molecules are cross-linked at the \(\alpha\) chains by treatment with the salicylate bis(3,5-dibromosalicyl) fumarate. This process forms a solution which is chemically stable, has low viscosity, a longer intravascular retention time than haemoglobin monomers or dimers, and an oxygen carrying capacity similar to whole blood.

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throughout the experiment by continuous infusion of propofol 2.5 µg kg⁻¹ min⁻¹. All animals received the same total dose of propofol. Intubation, paralysis with pancuronium 4 mg kg⁻¹ h⁻¹ and mechanical ventilation with an oxygen–air mix followed for the duration of the study. Bilateral arterial and venous femoral cannulae were inserted for administration of fluids and blood sampling for measurement of arterial blood-gas tensions, packed cell volume (PCV) and serum glucose and lactate concentrations. In preparation for receiving a closed head injury using the Richmond impact acceleration model, all animals had the musculature overlying the cranium surgically reflected to expose the bregma. All bleeding was stopped and the skull surface dried. In accordance with the standard method, a 1-cm diameter circular metal disk helmet was fixed, using cold curing dental acrylic, to the skull immediately overlying the bregma.

At the start of the study all animals received a severe (450 g x 2 m) weight drop head injury using the Richmond impact acceleration model. Following a 30-min stabilization period after the injury, all animals received 30 min of "secondary insults" consisting of bilateral carotid artery ligation coupled with a lowering of mean arterial pressure (MAP) to 40–50 mm Hg. This has been shown previously to produce diffusely increased ICP in this model. Arterial pressure was lowered by phlebotomy. Blood withdrawn was heparinized and kept warm at 37°C in a water bath. After the 30 min of secondary insults, the carotid clips were removed and blood reinfused. Arterial pressure was lowered by phlebotomy. Blood withdrawn was heparinized and kept warm at 37°C in a water bath. After the 30 min of secondary insults, the carotid clips were removed and blood reinfused.

### Table 1

<table>
<thead>
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<th></th>
<th>MAP (mm Hg)</th>
<th>P&lt;sub&gt;P&lt;/sub&gt;(H&lt;sub&gt;2&lt;/sub&gt;) (kPa)</th>
<th>Lactate (mmol litre⁻¹)</th>
<th>Glucose (mmol litre⁻¹)</th>
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<tbody>
<tr>
<td>Placebo group</td>
<td>46.1 (2.5)</td>
<td>26.3 (4.7)</td>
<td>5.0 (1.6)</td>
<td>4.7 (0.93)</td>
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<td>(n=9)</td>
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<td>DCLHb group</td>
<td>48.1 (0.66)</td>
<td>29.1 (5.1)</td>
<td>5.1 (1.1)</td>
<td>7.8 (1.3)</td>
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<td>(n=9)</td>
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After treatment, animals were instrumented for measurement of ICP (24-gauge Sprotte needle placed into a lateral ventricle) and CBF using the hydrogen clearance technique. Bur holes were placed bilaterally, 7 mm posterior and 4 mm lateral to the bregma for placement of hydrogen clearance electrodes in the region of the sensorimotor cortex. Electodes were positioned with the aid of an operating microscope and inserted using a microdrive, directly through the unopened dura to a depth of 1 mm into the cerebral cortex. Electrodes were sealed in place with cold curing dental acrylic. A reference electrode was wrapped in saline-soaked gauze and placed in a subcutaneous pouch. The hydrogen electrodes were polarized (+400 mV relative to the reference electrode) and allowed to stabilize for 1 h after insertion. During CBF measurement, hydrogen gas was administered at a concentration of 10 % for 10 min. The hydrogen electrode current during the desaturation phase was sampled online at 1 Hz for the entire desaturation period by a microcomputer-based CBF measurement system. After re-establishment of a stable baseline, a baseline correction algorithm was used to correct for drift in the hydrogen electrode during desaturation compared with pre-hydrogen baseline. The natural log plot was then calculated from the logarithm of the difference between each sampled point and its corresponding baseline value. The first 20 s of desaturation were discarded as potentially contaminated with arterial recirculation artefact. The initial slope index (ISI) was calculated from the slope of the subsequent 1-min period. As this head injury model is one of diffuse brain injury, to obtain a measure of a single cortical CBF value per study, ISI values reported were the average of the two electrodes recorded from both cerebral hemispheres.

A CBF measurement was obtained 4 h after the injury. This took into account a 30-min recovery period, 30 min of secondary insults, 15 min for infusion of DCLHb or HPPS (and a 10-min infusion set-up time), 60 min for surgical preparation of the animal for placement of the CBF electrodes and an intraventricular needle for measuring ICP, and 60 min for the H2 electrodes to stabilize. During this period of preparation, continuous minute by minute computerized monitoring of arterial pressure and core temperature was performed to ensure a stable basic physiology with no further secondary insults. Monitoring alarms were set to detect if MAP decreased to less than 70 mm Hg or core temperature increased to greater than 38.5°C. Arterial blood-gas tensions were obtained every 30 min. The CBF measurement 4 h after injury was recorded and this timing also coincided with previous work.

### Table 2

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<th>ICP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>CPP (mm Hg)</th>
<th>CBF (ml min⁻¹ 100 g⁻¹)</th>
<th>CVR (mm Hg)</th>
<th>ICP pulse (mm Hg)</th>
<th>P&lt;sub&gt;P&lt;/sub&gt;(H&lt;sub&gt;2&lt;/sub&gt;) (kPa)</th>
<th>P&lt;sub&gt;P&lt;/sub&gt;(H&lt;sub&gt;O&lt;/sub&gt;) (kPa)</th>
<th>pH</th>
<th>Temp (°C)</th>
<th>SaO&lt;sub&gt;2&lt;/sub&gt; (%)</th>
<th>HCO&lt;sub&gt;3&lt;/sub&gt; (mmol litre⁻¹)</th>
<th>Lactate (mmol litre⁻¹)</th>
<th>Glucose (mmol litre⁻¹)</th>
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<tr>
<td>Placebo</td>
<td>13.4 (1.6)</td>
<td>65.5 (7.0)</td>
<td>52 (7.8)</td>
<td>21.2 (1.9)</td>
<td>2.6 (0.44)</td>
<td>1.7 (0.1)</td>
<td>5.6 (0.3)</td>
<td>20.9 (3.1)</td>
<td>7.22</td>
<td>37.7</td>
<td>97.7</td>
<td>17.6</td>
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<tr>
<td>DCLHb</td>
<td>3.4** (0.8)</td>
<td>98.3** (5.8)</td>
<td>95.1** (6.0)</td>
<td>29.7* (2.3)</td>
<td>3.3 (0.3)</td>
<td>0.64** (0.1)</td>
<td>5.7 (0.3)</td>
<td>20.8 (3.9)</td>
<td>7.21</td>
<td>37.3</td>
<td>96.3</td>
<td>18.2</td>
<td>5.6</td>
<td>3.2</td>
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using this injury paradigm showing the presence of increased CBF by 4 h after injury relative to non-injured controls. Blood-gas tensions, and serum concentrations of glucose and lactate were sampled during the initial slope phase of the CBF measurement, 4 h after injury. The study ended when the CBF measurement 4 h after injury was complete. Blood PCV was measured at the start and end of the study. The animal was killed at the end of the study.

Analysis of variance was used to test for significant differences between the DCLHb and placebo groups. All values are reported as mean (SEM).

Results

Data from two animals were excluded; one animal in the DCLHb group accidentally received a large i.v. bolus of a pressor agent which caused severe arterial hypertension and subsequent metabolic acidosis. This animal died before the end of the study. One animal in the placebo group became extremely hypercapnic (P_CO2 > 9.3 kPa) which could not be controlled by adjustments in mechanical ventilation. As carbon dioxide reactivity is impaired after injury with this model, it would not be valid to normalize CBF data to a normal range of P_CO2 values. On completion of the study there were nine animals in each group.

Table 1 summarizes MAP, partial pressure of oxygen in blood (P_O2), and serum concentrations of lactate and glucose in the two groups, averaged over the 30 min of secondary insults (carotid occlusion with hypotension). There were no significant differences between the placebo and treatment groups in terms of the burden of secondary insults received.

Table 2 summarizes the physiological data (core temperature, P_Ao2, P_ACO2, pH, arterial oxygen saturation, serum concentrations of bicarbonate (HCO3), lactate and glucose, and PCV recorded at the time of the CBF measurement, 4 h after the injury. There were no significant differences between the placebo and treatment groups at 4 h for these variables. Table 2 also shows MAP, ICP, ICP pulse, CPP and CBF values recorded at 4 h. The DCLHb group had a significantly higher (P=0.002) MAP (98 (6) mm Hg) compared with the placebo group (65 (7) mm Hg). In addition, a plot of mean ICP showed that the DCLHb group had a significantly lower (P<0.001) mean ICP (3.4 (0.8) mm Hg) compared with the placebo group (13.4 (1.6) mm Hg) (fig. 1).

Mean ICP pulse amplitude 4 h after injury, also obtained at the time of CBF measurement, showed that the DCLHb group had a significantly lower (P=0.001) mean ICP pulse amplitude (0.64 (0.1) mm Hg) compared with the placebo group (1.7 (0.15) mm Hg). As would be expected from the combined results of ICP and MAP, mean CPP (CPP = MAP – ICP) was significantly higher (P<0.001) in the DCLHb group (95 (6) mm Hg) compared with the placebo group (52 (8) mm Hg) (fig. 1).

CBF, calculated as the mean of left and right cortical electrode flow values, was significantly higher (P=0.032) in the DCLHb group (29 (2.3) ml 100 g⁻¹ min⁻¹) compared with controls (21 (1.9) ml 100 g⁻¹ min⁻¹) (fig. 1). There was no significant change in cerebral vascular resistance (CVR = CPP/CBF) with treatment. CVR was 3.3 (0.3) mm Hg ml⁻¹ 100 g⁻¹ min⁻¹ in the DCLHb group compared with 2.6 (0.4) mm Hg ml⁻¹ 100 g⁻¹ min⁻¹ in the placebo group.

Discussion

Increased ICP is a frequent complication of post-traumatic brain injury and can often be difficult to control. It represents a frequently encountered secondary insult which contributes to patient morbidity and mortality. More than 70% of severely head injured patients experience one or more episodes of increased ICP (ICP > 20 mm Hg) of which 50–60% exhibit signs of diffuse brain swelling on CT scan. Of
these it has been estimated that more than 70% of increased ICP found in diffusely brain injured patients is attributable to vascular mechanisms.14

Intracranial hypertension, in the absence of surgically remediable causes, may be treated by several different approaches to reduce ICP while attempting to prevent cerebral hypoperfusion. These include mild hypocapnia, which helps to reduce the contribution of cerebral blood volume to the cause of increased ICP by means of vasoconstriction. Artificial increases in arterial pressure by pressor agents such as norepinephrine or methoxamine encourage perfusion, and osmotic and diuretic treatments may be used to achieve a reduction in the oedematous process associated with disruption of cerebral tissue.

All of the above therapeutic manoeuvres have inherent problems. Osmotic and diuretic methods of reducing brain oedema can lead to high serum osmolarity which may make renal toxicity a risk. Any agent or manoeuvre which causes cerebral vasoconstriction, such as hypocapnia, reduces cerebrovascular blood volume and leads to at least a temporary reduction in ICP. This approach, however, is not without risk. Reducing ICP by cerebral vasoconstriction in individuals who already have compromised brain perfusion can reduce CBF to a degree that brain ischaemia may occur with resultant neurological damage. An approach that induces mild cerebral vasoconstriction yet maintains adequate oxygen delivery to the tissue would be an important advance in this field.

Our work with this model has shown that DCLHb has a significant beneficial effect on reduction of ICP and enhancement of CPP. The way in which DCLHb produces these effects may be via a variety of mechanisms. The vascular tone of the cerebral tree may be altered beneficially to cause a reduction in ICP and thus enhance CPP. This reduction in ICP may have been achieved by cerebral vasoconstriction. The effect of DCLHb on pulse amplitude supports this, as an increase in vasoconstriction leads to a reduction in ICP pulse amplitude by increasing pre-capillary arterial tone.15 16 However, a significant increase in cerebrovascular resistance was not found, although a trend towards an increase was noted. Despite this, cortical CBF was not only maintained but increased. An increase in CBF and CBF with no significant increase in vascular resistance argues in favour of impaired autoregulation and a pressure passive state. However, with increased arterial pressure, such a scenario invariably results in increased ICP (not a reduction, as was the case in these studies). This suggests active autoregulatory vasoconstriction. If autoregulation were preserved in these animals, the decrease in ICP may be a reflection of a compensatory cerebral vasoconstriction caused by increased upstream MAP produced by DCLHb. However, work carried out by ourselves and others, using this weight drop model of head injury, has shown that CBF values observed by 4 h were indicative of hyperaemia and that impairment of pressure autoregulation exists.13 17 Taken together, this work supports the concept that there is vasoconstriction caused by a direct action of DCLHb on the cerebrovasculature rather than via a compensatory autoregulatory response to increased MAP. It could also be argued that propofol anaesthesia may effect the autoregulatory response, however, there is evidence that in rodents, although absolute CBF is reduced, pressure autoregulation remains intact.18

Regardless of the mechanism and whether or not a pressure passive state exists, our data showed that after administration of DCLHb, perfusion was improved in the face of reduced ICP. We speculate, however, that DCLHb was beneficial in other ways. It may carry significantly more oxygen to damaged tissues than previous therapies and, in addition, by virtue of its low viscosity7 may penetrate areas of brain that would have previously been ischaemic. Work done in a rabbit model of spinal cord ischaemia19 showed that there was a reduction in the incidence of permanent paralysis when animals were treated with DCLHb. Studies in rats also showed a significant reduction in the size of an infarct after haemodilution with DCLHb.20

The mechanisms responsible for local control of autoregulatory vasomotor tone involve several constitutive endothelial and perivascular agents, including nitric oxide and the endothelins.21 22 Haemoglobin is known to bind avidly to nitric oxide and this scavenging effect may explain the increase in vascular tone. This hypothesis has been explored in the rat24 but as yet has to be substantiated in humans. Thus it is feasible that infusion of DCLHb may lead to an increase in the amount of haemoglobin free of any encapsulating membrane, which is likely to increase binding of circulating NO.

In summary, we have demonstrated a beneficial effect of DCLHb on ICP and perfusion pressure in a rodent model of severe head injury. Further work needs to be done to identify the optimal dose level for this agent and to determine if enhanced cerebral perfusion translates into increased oxygen delivery to the tissues and to improved clinical outcome.

Acknowledgements

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Effects of DCLHb on post-traumatic cerebral perfusion


