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Experimental Subarachnoid Hemorrhage in Rats

Effect of Intravenous α-α Diaspirin Crosslinked Hemoglobin on Hypoperfusion and Neuronal Death

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Background: **Hemodilution with diaspirin crosslinked hemoglobin (DCLHb) ameliorates occlusive cerebral ischemia. However, subarachnoid hemoglobin has been implicated as a cause of cerebral hypoperfusion. The effect of intravenous DCLHb on cerebral perfusion and neuronal death after experimental subarachnoid hemorrhage was evaluated.**

Methods: **Rats (n = 48) were anesthetized with isoflurane and subarachnoid hemorrhage was induced by injecting 0.3 ml of autologous blood into the cistema magna. Each animal received one of the following regimens: Control, no hematocrit manipulation; DCLHb, hematocrit concentration decreased to 30% with DCLHb; or Alb, hematocrit concentration decreased to 30% with human serum albumin. The experiments had two parts, A and B. In part A, after 20 min, cerebral blood flow (CBF) was assessed with ^{14}C-iodoantipyrine autoradiography. In part B, after 96 h, in separate animals, the number of dead neurons was determined in predetermined coronal sections by hematoxylin and eosin staining.**

Results: **Cerebral blood flow was greater for the DCLHb group than for the control group; and CBF was greater for the Alb group than the other two groups ($P < 0.05$). In one section, CBF was 45.5 ± 10.9 ml · 100 g^{-1} · min^{-1} (mean ± SD) for the control group, 95.3 ± 16.6 ml · 100 g^{-1} · min^{-1} for the DCLHb group, and 138.1 ± 18.7 ml · 100 g^{-1} · min^{-1} for the Alb group. The number of dead neurons was less in the Alb group (611 ± 84) than in the control group (1,097 ± 211), and was less in the DCLHb group (305 ± 38) than in the other two groups ($P < 0.05$).**

Conclusions: **These data support a hypothesis that hemodilution decreases hypoperfusion and neuronal death after subarachnoid hemorrhage. The data do not support the notion that intravascular molecular hemoglobin has an adverse effect on brain injury after subarachnoid hemorrhage. (Key words: Brain: cerebral blood flow; cerebral vasospasm; subarachnoid hemorrhage. Hemodilution: hemoglobin-based oxygen carrier.**

THE prevalence of intracranial aneurysms in North America is estimated at 2,000 per 100,000 population, with an annual incidence of subarachnoid hemorrhage of 12 per 100,000 population.[1] Aneurysmal subarachnoid hemorrhage has a mortality rate of 40-50%.[1,2] If patients survive the initial hemorrhagic injury, vasospasm with delayed ischemic neurologic deficits is a major cause of death and disability.[3] Furthermore, subarachnoid hemorrhage with ischemia has a prevalence of 12-53% in patients after traumatic head injury.[4,5] After subarachnoid hemorrhage, erythrocyte breakdown products, principally hemoglobin,[6-11] are postulated to be a principal factor in the pathogenesis of cerebral vasospasm.

Hypervolemic hemodilution is reported to be effective in ameliorating neurologic deficits due to cerebral vasospasm.[12,13] The rationale for hemodilution therapy is based on an inverse correlation between blood viscosity and cerebral blood flow (CBF).[14,15] Various colloid solutions have been used to achieve hemodilution. Re-

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cently several unmodified and modified hemoglobin solutions were developed for use as therapeutic agents and are in various stages of experimental and clinical trials. Molecular hemoglobin, by decreasing viscosity while maintaining oxygen carrying capacity, might in theory be an ideal hemodiluent.[16] Recent data from our laboratory suggest that α-α diaspirin crosslinked hemoglobin (DCLHb; Baxter Healthcare Corp., Round Lake, IL) has a minimal effect on CBF in normal brain, whereas in ischemic areas of the brain DCLHb effects an increased CBF that is viscosity dependent.[15]

However, *in vitro* data suggest that hemoglobin is neurotoxic.[17,18] Furthermore, hemoglobin is known to bind the cerebral vasodilator nitric oxide, which might, in theory, aggravate cerebral vasospasm.[19,20] Given the potential for an adverse effect of molecular hemoglobin in the setting of subarachnoid hemorrhage, we evaluated the effect of intravenous DCLHb[21] on cerebral perfusion deficits and neuronal death after experimental subarachnoid hemorrhage in rats.

Materials and Methods

The DCLHb solution was supplied by Baxter Healthcare. Briefly, DCLHb was prepared by exposure of outdated human erythrocytes to hypotonic buffer. The stroma was removed by ultrafiltration and the hemoglobin was crosslinked at the α chains by bis(3,5-dibromosalicyl) fumarate. Viral inactivation and protein purification was achieved by heat pasteurization.[22,23] The solution was formulated to a concentration of 10 g/dl by diafiltration against electrolyte and buffer solution. The DCLHb was stored at −70°C. On the day of the study, it was thawed to 5°C and then passively warmed to room temperature. The viscosity of 10% DCLHb (1.1 centipoiseuilles, at 37°C and 225 s shear rate) is comparable to that of serum albumin.[24]

After we attained approval of the Institutional Research Committee of Loma Linda University, male spontaneously hypertensive rats (weighing 350–400 g; aged 16–20 weeks) were anesthetized with isoflurane by face mask. *Via* the atlantooccipital membrane, a polyethylene catheter (PE-10) was inserted into the cisterna magna and secured in place. A 72-h recovery period was allowed, and normal neurologic function was verified. The position of the catheter in the cisterna magna was verified at necropsy. The animals were then reanesthetized with isoflurane (1.44% end-tidal concentration), orotracheally intubated, and mechanically ventilated. The femoral vessels were cannulated for blood pressure monitoring (Full Scale Transducer/TA 2000 Recorder; Gould, Cerritos, CA), blood sampling, and fluid administration. Cranial temperature was servocontrolled at 37°C (Mona-Therm temperature sensor; Mallinckrodt Anesthesia Products, St. Louis, MO). Before CBF was determined, arterial blood (125 μl) was analyzed for pH, partial pressure of carbon dioxide, partial pressure of oxygen, glucose level, and hematocrit concentration (using a IL-1306 pH blood gas analyzer [Instrumentation Laboratory, Lexington, MA]; YSI model 23-A glucose analyzer [Yellow Springs Instruments, Yellow Springs, OH]; and a IEC MB centrifuge microhematocrit [DAMON/IEC Division, Needham Heights, MA], respectively).

Subarachnoid hemorrhage was induced by injecting 0.3 ml fresh autologous blood into the cistema magna.[25] The blood was injected over a 10-min period with the animal in a 20° head-down position. Pilot data in the development of this model indicated that, using this unique method of a slow injection, the intracranial pressure was typically <10 mmHg. Sometimes the intracranial pressure would transiently (<60 s) increase to 10–20 mmHg, but on no occasion was it >20 mmHg. The rats were assigned randomly to one of the following hypervolemic volume regimens, each of which was initiated immediately after subarachnoid hemorrhage and completed in a 10-min period:

- Control (n = 16): Eight milliliters fresh donor blood was given (the hematocrit concentration was not manipulated).
- DCLHb (n = 16): Blood volume and hematocrit (30%) were manipulated by a 5-ml exchange transfusion with 10% DCLHb (oncotic pressure, 43 mmHg), followed by an additional 8-ml bolus.
- Alb (n = 16): Blood volume and hematocrit (30%) were manipulated by a 5-exchange transfusion with 7.5% human serum albumin (Armour Pharmaceutical Co., Kankakee, IL; oncotic pressure, 43 mmHg), followed by an additional 8-ml bolus.

The initial dose of DCLHb was given as an exchange transfusion to maintain a stable mean arterial blood pressure. If DCLHb is initially given as a simple bolus, the mean arterial blood pressure increases by 25–30 mmHg for ≈120 min. However, if initially administered as an exchange transfusion, normotension is maintained.

Part A

Twenty minutes after subarachnoid injection of blood, at a time known to coincide with maximal vasospasm,[26-28] CBF was assessed with ^{14}C-iodoantipyrine.[29] Briefly, 100 μCi/kg ^{14}C-iodoantipyrine (New England Nuclear, Boston, MA) was given at a constantly increasing rate over 46 s. Twenty-one arterial blood samples were collected, and ^{14}C activity was determined with a quench correction (Beckman 8000 liquid scintillation spectrometer, Brea, CA). After ^{14}C infusion, the animals were killed, and the brains were rapidly removed and placed in 2-methyl-butane (at −35°C). Twenty-micrometer sections surrounding five standardized coronal planes were exposed to x-ray film (Kodak OM-1, Rochester, NY) for 21 days. The five coronal planes were at 2-mm increments spanning the distribution of the middle cerebral artery.[30] Using of a Drexel/DUMAS image analysis system (Drexel University, Philadelphia, PA) and the equation of Sakurada *et al.*,[29] CBF was assessed in each anatomic section, and the absolute CBF and the areas of hypoperfusion (CBF 0-20 or 21-40 ml · 100 $g^{-1} \cdot min^{-1}$) were determined for each section. All image analyses were performed by an independent observer who was blinded to the study protocol.

Part B

The anesthetic was discontinued, the tracheas were extubated, and the rats recovered in an incubator with automatic temperature control and humidified oxygen. Cranial temperature was servocontrolled at 37°C for the first 6 h of recovery. Although pilot studies with this model showed that after 60 min of recovery the animals maintained normal cranial temperature, cranial temperature was servocontrolled for 6 h as a reasonable margin of assurance that cranial temperature was not different among the groups.

After 96 h the animals were reanesthetized with 4% isoflurane, and the brains were perfusion fixed with 50 ml phosphate-buffered 10% formalin. The brains were removed, dehydrated in graded concentrations of ethanol and butanol, and embedded in paraffin. Six standardized coronal brain sections (8 μm thick) were prepared and stained with hematoxylin and eosin (fig. 1). The six predetermined coronal brain sections correspond to plates 18, 29, 32, 37, 52, and 60, respectively, of the atlas of the rat brain by Palkovits and Brownstein.[31]

Neuronal injury was evaluated at a magnification of ×400 by an observer who was blinded to the treatment protocols. Ischemic neurons were identified by the presence of eosinophilic cytoplasm, pyknotic nuclei, karyorrhexis, and karyolysis. The number of damaged neurons is expressed as an absolute number for all of the anatomic areas assessed in each coronal plane for each group (fig. 1) and as a total for each group of all six coronal sections.

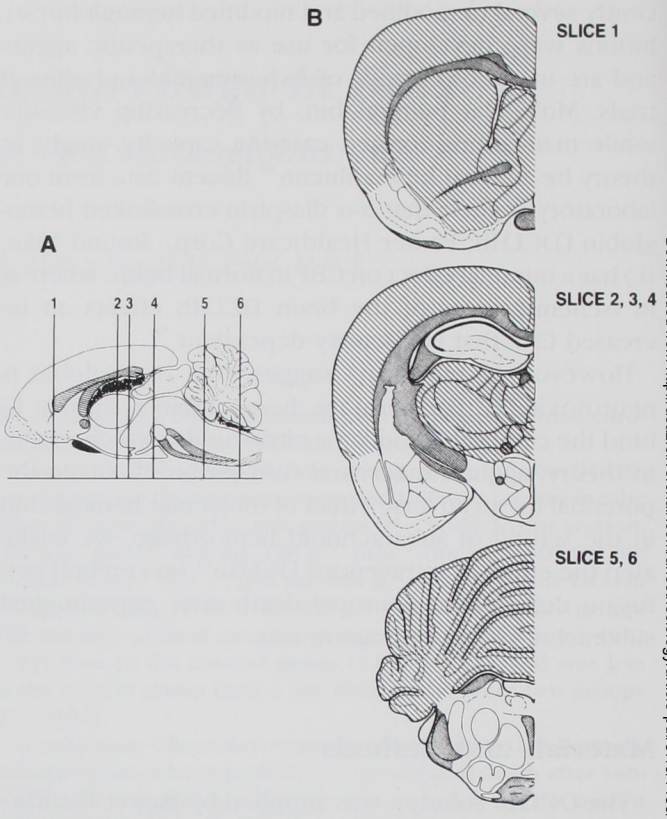

Fig. 1. (*A*) Sagittal view of the rat brain showing the six predetermined coronal brain sections used for hematoxylin and eosin staining. Sections 1–6 correspond to plates 18, 29, 32, 37, 52, and 60, respectively, of the atlas of the rat brain by Palkovits and Brownstein.[31] (*B*) Coronal views of the areas (*hatched*) of each brain section that were microscopically analyzed for neuronal necrosis.

All between-group data were evaluated using analysis of variance, and, as appropriate, mean values were compared by *t* tests with a Bonferroni correction factor for multiple comparisons. $P < 0.05$ was considered significant.

Results

All data are presented as mean ± SD. Except for hematocrit concentration, there were no between-groups differences in the physiologic data (table 1).

Table 1. Physiologic Data Immediately prior to CBF Determination (Part A), and during the Treatment Period for Part B

	Control	DCLHb	Alb
Part A (before CBF determination)			
*p*Ha	7.41 ± 0.01	7.42 ± 0.02	7.42 ± 0.01
Pa_{O_2} (mmHg)	148 ± 24	137 ± 21	144 ± 29
Pa_{CO_2} (mmHg)	38.8 ± 1.2	38.1 ± 1.1	38.0 ± 0.9
Mean arterial blood pressure (mmHg)	139 ± 8	143 ± 10	144 ± 9
Hematocrit (%)	44 ± 1	30 ± 1*	30 ± 1*
Glucose ($mg \cdot dl^{-1}$)	129 ± 19	118 ± 19	128 ± 22
Part B (during treatment period)			
*p*Ha	7.41 ± 0.01	7.41 ± 0.02	7.40 ± 0.01
Pa_{O_2} (mmHg)	139 ± 17	145 ± 26	133 ± 22
Pa_{CO_2} (mmHg)	39.1 ± 1.0	38.9 ± 1.3	39.5 ± 1.1
Mean arterial blood pressure (mmHg)	135 ± 7	139 ± 9	136 ± 5
Hematocrit (%)	45 ± 1	29 ± 2*	30 ± 1*
Glucose ($mg \cdot dl^{-1}$)	110 ± 13	112 ± 14	119 ± 20

Values are mean ± SD.

CBF = cerebral blood flow.

* $P < 0.05$ *versus* the control group.

Part A

The areas of hypoperfusion (CBF 0–20 or 21–40 $ml \cdot 100\ g^{-1} \cdot min^{-1}$) for each of the five sections are presented in table 2. Hypoperfusion was less in the DCLHb and Alb groups than in the Control group. For section 3, the areas of hypoperfusion were less in the Alb group than in the other two groups; and in section 2, the 0–20 ml/100 g area of CBF was less in the Alb group than in the DCLHb group.

The absolute CBF was greater for the DCLHb group than for the Control group, and it was greater for the Alb group than for the other two groups ($P < 0.05$). In section 3, CBF was 45.5 ± 10.9 $ml \cdot 100\ g^{-1} \cdot min^{-1}$ for the Control group, 95.3 ± 16.6 $ml \cdot 100\ g^{-1} \cdot min^{-1}$ for the DCLHb group, and 138.1 ± 18.7 $ml \cdot 100\ g^{-1} \cdot min^{-1}$ for the Alb group ($P < 0.05$; table 3).

Table 2. Area of Hypoperfusion (CBF 0–20 and 21–40 $ml \cdot 100\ g^{-1} \cdot min^{-1}$) in Coronal Brain Sections (% of Total Area [Both Hemispheres])

	Control	DCLHb	Alb
Section 1 (%)			
0–20*	19.5 ± 3.9	0.6 ± 0.2†	0.9 ± 0.3†
21–40*	45.9 ± 8.1	5.9 ± 3.8†	5.8 ± 1.7†
Section 2 (%)			
0–20*	17.4 ± 5.0	2.8 ± 1.8†	0.6 ± 0.3‡
21–40*	29.4 ± 6.1	3.8 ± 4.1†	3.4 ± 2.4†
Section 3 (%)			
0–20*	17.6 ± 6.1	2.9 ± 1.1†	1.2 ± 0.5‡
21–40*	32.0 ± 6.2	8.3 ± 3.4†	4.0 ± 2.1‡
Section 4 (%)			
0–20*	10.1 ± 4.5	1.4 ± 1.5†	1.1 ± 0.5†
21–40*	25.2 ± 8.2	4.9 ± 3.4†	4.6 ± 3.1†
Section 5 (%)			
0–20*	5.3 ± 2.2	1.2 ± 1.8†	0.6 ± 0.8†
21–40*	19.5 ± 8.1	3.3 ± 3.1†	2.4 ± 1.2†

Values are mean ± SD.

* $ml \cdot 100\ g^{-1} \cdot min^{-1}$.

† $P < 0.05$ *versus* the control group.

‡ $P < 0.05$ *versus* the other two groups.

Part B

In the control group of animals there was extensive injury in sections 1–4; however, there was minimal injury in sections 5 and 6, which correspond to cerebellar areas of the brain (fig. 1). The number of dead neu-

Table 3. Absolute CBF ($ml \cdot 100\ g^{-1} \cdot min^{-1}$) for Each of the Five Sections

Section	Control	DCLHb	Alb
1	37.6 ± 11.6	99.2 ± 14.5*	133.9 ± 16.9†
2	48.0 ± 10.8	108.2 ± 12.4*	140.1 ± 14.8†
3	45.5 ± 10.9	95.3 ± 16.6*	138.1 ± 18.7†
4	62.1 ± 8.3	99.1 ± 12.8*	138.4 ± 20.2†
5	70.5 ± 10.1	103.2 ± 13.4*	145.8 ± 15.3†

Values are mean ± SD.

* $P < 0.05$ *versus* the control group.

† $P < 0.05$ *versus* the other two groups.

Table 4. Number of Dead Neurons in Coronal Brain Sections

Section	Control	DCLHb	Alb
1	214 ± 38	89 ± 25†	172 ± 34
2	311 ± 52	68 ± 20†	121 ± 24*
3	271 ± 55	71 ± 31†	172 ± 34*
4	233 ± 43	63 ± 21†	125 ± 26*
5	33 ± 6	7 ± 6*	10 ± 5*
6	35 ± 7	7 ± 8*	11 ± 5*
Total	1097 ± 211	305 ± 38†	611 ± 84*

Values are mean ± SD; see figure 1.

* $P < 0.05$ *versus* the control group.

† $P < 0.05$ *versus* the other two groups.

rons was less for five of the six sections in the albumin group and for all of the sections in the DCLHb group compared with the control group ($P < 0.05$; table 4). In addition, for sections 1–4 the number of dead neurons was less in the DCLHb group than in the albumin group ($P < 0.05$; table 4).

When evaluated as a whole, the number of dead neurons was less in the albumin group (611 ± 84) than in the control group (1,097 ± 211), and was less in the DCLHb group (305 ± 38) than in the other two groups ($P < 0.05$; table 4).

Discussion

These data support a hypothesis that hemodilution can reduce the extent of hypoperfusion after experimental subarachnoid hemorrhage. Our results are consistent with previous data in rodent models of middle cerebral artery occlusion, which indicate that DCLHb decreases cerebral ischemia and infarct volume.[15,32–35] When non-oxygen-binding fluids are used as hemodiluents, hemodilution is postulated to augment CBF during ischemia by improving microvascular hemorheology.[14,15] Molecular hemoglobin is a potentially unique hemodiluent because, although it decreases viscosity, it can simultaneously maintain oxygen-carrying capacity. However, the possible disadvantage also exists that the ability of hemoglobin to bind nitric oxide might act to worsen the ischemia associated with cerebral vasospasm.[19,20,36]

Substantial evidence suggests that a substance(s) liberated during blood clot lysis after subarachnoid hemorrhage is responsible for cerebral vasospasm, perfusion deficits, and delayed neurologic injury.[6–9] Oxyhemoglobin is thought to be a key factor in the development of cerebral vasospasm after subarachnoid hemorrhage.[6] Although the exact mechanism whereby oxyhemoglobin induces cerebral vasospasm has not been defined precisely, hypotheses include an interaction with another spasmogen, release of iron, free radical generation, endothelin release, decreased production or function of nitric oxide, endothelial or smooth muscle dysfunction, or an imbalance between vasoconstrictive and vasodilatory substances.[6–11] Several noteworthy hypotheses may account for cerebral vasospasm after subarachnoid hemorrhage.

A Direct Cerebral Vasoconstrictor Effect of Hemoglobin

In vitro studies have documented a direct vasoconstrictor response of cerebral vessels to hemoglobin.[7,8,36] However, previous studies on the cerebral vasoactive properties of hemoglobin used hemoglobin preparations, which may contain impurities or lytic by-products that may elicit or exacerbate the vasoconstrictive response attributed to hemoglobin.[7,8,36] This may, in part, explain the negligible cerebral vasoconstriction observed in studies using highly purified preparations of hemoglobin.[37,38]

Cofactors of Hemoglobin-induced Vasoconstriction

When compared with the cerebral vasoactive response observed with isolated hemoglobin, Aoki *et al.*[38] observed a more profound vasoconstrictive response when hemoglobin was applied to cerebral vessels in combination with a low molecular weight fractionate released from the subarachnoid clot. Based on these results, they postulated that the vasoconstrictive response of cerebral vessels to isolated hemoglobin is inadequate to entirely explain vasospasm after subarachnoid hemorrhage.[38]

Hemoglobin's (DCLHb) Effect on Nitric Oxide–Endothelin

Hemoglobin may bind nitric oxide, stimulate endothelin production, and inhibit cGMP-dependent vasodilation.[19,39] In theory, binding of nitric oxide might worsen vasospasm.[20] However, the interactive effect of hemoglobin and nitric oxide with cerebral vasospasm may be more complex.

Usually basal synthesis and release of nitric oxide from the vascular endothelium is accomplished by an isoform of nitric oxide synthase (NOS) that is expressed constitutively and depends on aerobic metabolism.[40] Subarachnoid hemorrhage has been associated with histo-

pathologic evidence of damage to cerebral endothelial cells as well as functional impairment of endothelial-dependent relaxation.[20] Two other isoforms of NOS also have been described (neuronal and inducible NOS). Normally neuronal NOS is constitutively expressed. However, under pathologic conditions such as cerebral ischemia, this isoform of NOS is inducible and may contribute to early neuronal injury.[41] In the present paradigm, for DCLHb to bind nitric oxide produced by neuronal NOS, DCLHb given intravascularly would have to cross the blood-brain barrier. During experimental conditions with an intact blood-brain barrier, Keipert *et al.*[42] observed minute, but detectable, amounts of diaspirin cross-linked hemoglobin administered intravascularly in the brain tissue of rats. We are not aware of data that define the amount of cerebral tissue hemoglobin necessary to effectively scavenge nitric oxide produced by neuronal NOS. Accordingly, the possibility that DCLHb scavenged neuronal nitric oxide in the present study remains speculative.

A third isoform of NOS, inducible NOS, has also been described and may contribute to late neuronal injury.[41] During ischemia, inducible NOS is expressed in various tissues, including neurons and astrocytes.[43] However, inducible NOS activity is not detectable until at least 12 h, with a peak value at 48 h, after cerebral ischemia.[44] Based on limited data suggesting an intravascular retention half-life of 24 h for this dose of DCLHb in rats,[45] we suggest, speculatively, that DCLHb may affect nitric oxide produced by inducible NOS.

There are no ideal animal models to evaluate cerebral vasospasm. The present model is widely used and the time course of vasospasm is documented (a reduction of vessel caliber by 50% at 72 h,[26] 46% at 10 min and 20% at 48 h,[27] and 40% at 10 min and 27% at 48 h[28]). Previous studies have not observed cerebral vasoconstriction when subarachnoid injection of saline was performed,[26,28] which is consistent with data from CBF studies from our laboratory that failed to observed significant areas of hypoperfusion when either mock cerebrospinal fluid[46] or albumin[47] was injected into the subarachnoid space.

Nonetheless, the shortcoming of this model is that the human scenario of vascular disease and rupture is not replicated, thus limiting interpretation of the data. In addition, rodents have anatomic differences in the cerebral vasculature compared with humans that make them more resistant to vasospastic-induced cerebral ischemia.[48] Furthermore, vasospasm in this experimental model does not temporarily match the human condition. The clinical scenario differs, in part, from the present model by providing a time delay from the onset of vessel rupture in which blood clot lysis with release of erythrocyte breakdown products is required before cerebral vasospasm is documented to occur.[6-11] In pilot studies with this model, we observed that the mechanical stress on the erythrocytes accomplished by withdrawal and injection of the autologous blood results in erythrocyte lysis. This may in part account for the early vasospasm observed in this model of experimental subarachnoid hemorrhage. An additional critique of this study is that we evaluated only one preparation of hemoglobin. It is possible that other hemoglobin preparations might have properties that would potentiate an inherent cerebral vasoconstrictive response to hemoglobin.

Finally, readers may question the apparent differences in the magnitude of the effect on hypoperfusion *versus* absolute CBF for DCLHb or albumin (tables 2 and 3). Although some statistical differences were evident, in general the areas of hypoperfusion were similar for the DCLHb and Alb groups, whereas the differences in absolute CBF between the DCLHb and Alb groups were not only statistically but prominently different. This might be explained in part by the specificity of the method for assessing hypoperfusion or absolute CBF. It would be expected that the method for determining the area of hypoperfusion would favor the discrimination of ischemic areas of the brain, whereas the absolute CBF method would factor in not only the area of hypoperfusion but also the effect of the intervention (*e.g.*, DCLHb or albumin) on relatively normal areas of the brain for the reported CBF value. Data suggest that in normal brain DCLHb has a modest or negligible effect on CBF, whereas a similar dose of albumin effects a greater increase in CBF.[15,49] Thus, when assessing the absolute CBF data, the potential for an adverse effect at the low-flow end of the CBF range to be masked by a meaningless effect at the normal or the high-flow end of the CBF range is possible.

In conclusion, in an animal model of subarachnoid hemorrhage, the effect of hemodilution with DCLHb or albumin on cerebral hypoperfusion and neuronal death was assessed. The data indicate that hemodilution decreased hypoperfusion after experimental subarachnoid hemorrhage, and, in terms of CBF, albumin is a more effective hemodiluent than DCLHb. Conversely, neuronal death was less in DCLHb-hemodiluted animals than in those that were hemodiluted with albumin. Hemoglobin is known to bind nitric oxide, which may

account for the difference in hypoperfusion between the DCLHb and Alb groups. Although hemoglobin has been implicated as a cerebral spasmogen, it is possible that impurities of hemoglobin preparations used in previous studies have quantitatively magnified any inherent vasoconstrictive property. In addition, there may be physiologically endogenous cofactors of hemoglobin that are necessary to produce cerebral vasospasm. Finally, it is plausible that hemoglobin may exert a dual effect on nitric oxide's influence on brain injury after subarachnoid hemorrhage. By binding nitric oxide, hemoglobin may initially neutralize favorable vasodilatory properties but result in an overall beneficial effect by reducing the local concentration of nitric oxide and its potential neurotoxic effect. Nonetheless, the present data do not support the notion that intravascular molecular hemoglobin has an adverse effect on brain injury after subarachnoid hemorrhage.

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