

EFFECTS OF HYPERTONIC ARGININE ON CEREBRAL BLOOD FLOW AND INTRACRANIAL PRESSURE AFTER TRAUMATIC BRAIN INJURY COMBINED WITH HEMORRHAGIC HYPOTENSION

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ABSTRACT—Hypertonic saline solutions improve cerebral blood flow (CBF) when used for acute resuscitation from hemorrhagic hypotension accompanying some models of traumatic brain injury (TBI); however, the duration of increased CBF is brief. Because the nitric oxide synthase substrate L-arginine provides prolonged improvement in CBF after TBI, we investigated whether a hypertonic resuscitation fluid containing L-arginine would improve CBF in comparison to hypertonic saline without L-arginine in a model of moderate, paramedian, fluid-percussion TBI followed immediately by hemorrhagic hypotension (mean arterial pressure [MAP] = 60 mm Hg for 45 min). Sprague-Dawley rats were anesthetized with 4.0% isoflurane, intubated and ventilated with 1.5%–2.0% isoflurane in oxygen/air (50:50). After preparation for TBI and measurement of CBF using laser Doppler flowmetry and measurement of intracranial pressure (ICP) using an implanted transducer, rats were subjected to moderate (2.0 atm) TBI, hemorrhaged for 45 min, and randomly assigned to receive an infusion of hypertonic saline (7.5%, 2,400 mOsm total; 6 mL/kg; n = 6) or hypertonic saline with 50, 100, or 300 mg/kg L-arginine (2,400 mOsm; 6 mL/kg; n = 6 in each of the three dose groups) and then monitored for 120 min after the end of infusion. CBF was measured continuously and calculated as a percent of the pre-TBI baseline during the hemorrhage period, after reinfusion of one of the hypertonic arginine solutions, and 30, 60, and 120 min after reinfusion. All four hypertonic solutions initially improved MAP, which, by 120 min after infusion, had decreased nearly to the levels observed during hemorrhage. ICP remained below baseline levels during resuscitation in all groups, although ICP was slightly greater ($P = NS$) than baseline in the hypertonic saline group. CBF increased similarly in all groups during infusion and then decreased similarly in all groups. At 120 min after infusion, CBF was highest in the group infused with hypertonic saline, but the difference was not significant. We conclude that the improvement of MAP, ICP, and CBF produced by hypertonic saline alone after TBI and hemorrhagic hypotension is not significantly enhanced by the addition of L-arginine at these doses.

KEYWORDS—Cerebral blood flow, hemorrhagic hypotension, hypertonic arginine, intracranial pressure, rats, traumatic brain injury

INTRODUCTION

After clinical traumatic brain injury (TBI), secondary ischemia increases mortality. Clinical factors associated with secondary ischemic injury include systemic hypotension and hypoxemia and intracranial hypertension (1–3). Hypotension after clinical TBI, usually attributable to hemorrhage associated with systemic injuries, has been strongly associated with poor outcome. In patients after TBI, systolic blood pressures <90 mm Hg between the occurrence of TBI and arrival in the emergency department more than doubled mortality and nearly halved the percentage of favorable clinical outcomes (2).

The adverse influence of hypotension on outcome has been associated with reduced cerebral blood flow (CBF) and impaired cerebral vascular reactivity. Cerebral ischemia is caused by complex interactions between reduced blood pressure, impaired cerebral vasodilatory responsiveness, cerebral swelling, and increased intracranial pressure (ICP). Optimal treatment would

correct all of these physiologic deficits. Small volumes of hypertonic solutions, which we (4–6) and others (7, 8) have used to improve blood pressure and reduce ICP in experimental TBI, represent an effective method of rapidly resuscitating hypotensive, head-injured patients. Secondary analyses of clinical trials of hypertonic resuscitation of hypotensive trauma patients suggest that outcome is improved in the subset of patients with TBI (9, 10). Such characteristics are especially desirable in situations such as battlefield casualties in which only small volumes of fluids may be available. A multidisciplinary review panel, convened by the National Academy of Sciences to address the issue of resuscitation of hemorrhage in a battlefield environment, stated that hypertonic solutions should be used for initial resuscitation of the hemorrhaging battlefield casualty and emphasized the need for continuing research into novel small-volume solutions (11).

Unfortunately, hypertonic saline solutions, when used as initial treatment of hemorrhage and TBI, only transiently improve blood pressure and CBF. Moreover, small-volume resuscitation using hypertonic saline does not correct the loss of cerebral vasodilatory responsiveness after TBI (12, 13). Therefore, addition of pharmacologically active components to small-volume resuscitation solutions could improve CBF and restore cerebral vascular reactivity. Evidence suggests the involvement of the endothelium-dependent relaxing factor

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nitric oxide (NO) (14, 15) and other oxygen-free radicals in posttraumatic cerebral hypoperfusion (16, 17). Posttraumatic hypoperfusion may result from impairment or destruction of a cerebral vasodilatory mechanism such as NO (18, 19). Continuous production of NO may provide a resting cerebral vasodilatory "tone," as is suggested by evidence that inhibition of NO synthesis decreases CBF below baseline levels (20–23).

However, addition of L-arginine to resuscitation fluid also could exert undesirable side effects, including increased ICP related to increased CBF and potentially increased production of peroxynitrite. In this study, we investigated the hypothesis that addition of L-arginine to hypertonic saline resuscitation after TBI and hemorrhage would increase the duration of increased CBF after resuscitation without increasing ICP.

MATERIALS AND METHODS

Animal preparation

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch. The experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. Male Sprague-Dawley rats weighing 350 to 450 g were anesthetized with isoflurane in an anesthetic chamber, intubated, and mechanically ventilated with 1.5% to 2.0% isoflurane in O₂/room air (50:50) using a volume ventilator (EDCO Scientific, Chapel Hill, NC). Polyethylene cannulae (PE 50) were placed in both femoral arteries and one femoral vein for arterial pressure monitoring and hemorrhage and for drug infusion, respectively. Rectal and temporalis muscle temperatures were monitored using a telethermometer (Yellow Springs Instruments, Yellow Springs, OH), and rectal temperature was maintained using a thermostatically controlled water blanket (Gaymar, Orchard Park, NY).

Rats were prepared for paramedian fluid-percussion TBI as previously described (14, 24). Briefly, the rats were placed in a stereotaxic frame and the scalp was sagittally incised. A 4.0-mm hole was trephined into the skull 2.0 mm to the right of the sagittal suture and midway between lambda and bregma, and a modified LuerLok syringe hub was placed over the exposed dura, bonded in place with cyanoacrylic adhesive, and covered with dental acrylic. An additional 2.0-mm craniotomy was drilled for placement of a probe for measuring ICP (Camino 4F Model 110 transducer probe) connected to a Camino 420V ICP Monitor (Camino, Inc., San Diego, CA). Rats were then prepared for placement of a laser Doppler flow probe as described below. Isoflurane was lowered to 1.5%; the rats were connected to the trauma device and subjected to moderate, paramedian (2.0 atm) TBI. Five minutes after TBI, mean arterial blood pressure (MAP) was reduced to 60 mm Hg for 45 min by removing blood from one femoral artery. After 45 min, the shed blood was replaced by one of the solutions listed below (see "Experimental Design"), and MAP, ICP, CBF, and temperature were monitored continuously for 2 h. Arterial pH, pO₂, pCO₂, hematocrit, and glucose levels were measured every 30 min.

Laser doppler flowmetry

CBF was measured using laser Doppler flowmetry (LDF) as described elsewhere (14, 25). Briefly, the left calvaria lateral and slightly posterior to the injury adapter was thinned using an air-cooled drill and topical cooling with room temperature water (Dremel, Racine, WI). Using an electrode holder on a stereotaxic head holder (Stoelting Co., Wood Dale, IL), a fiberoptic needle probe (Perimed, Stockholm, Sweden) was placed over the shaved parietal calvaria and carefully positioned away from large vessels visible through the remaining calvaria. The probe emits monochromatic red light (632.8 nm) that is reflected by moving erythrocytes. The power and frequency of the reflected signal, detected by optodes in the needle probe head, are proportional to the blood volume and blood velocity, respectively. Blood velocity is calculated based upon the Doppler shift created by red blood cells moving in the area illuminated by the laser and reflected back to the receiver in the same probe. Perfusion is calculated as the product of blood volume and velocity in a 1.0-mm³ tissue volume under the probe (25). Measurements were recorded on a PeriFlux PF3 Laser Doppler Perfusion Monitor (Perimed). After probe placement, the responsiveness of the experimental

TABLE 1. Arterial Blood Gases, Hematocrit and Plasma Glucose in Rats After Moderate (2.0 atm) Fluid-Percussion TBI, Hemorrhagic Hypotension (60 mm Hg for 45 min), and Resuscitation With Hypertonic Saline (n = 6) or Hypertonic Saline With 50 (n = 6), 100 (n = 6), or 300 (n = 6) mg/kg L-Arginine

	Group	BL	HEM	30	60	120
pH	HS	7.44 ± 0.02	7.38 ± 0.03	7.35 ± 0.02	7.37 ± 0.02	7.31 ± 0.05
	HA50	7.43 ± 0.01	7.38 ± 0.02	7.37 ± 0.02	7.38 ± 0.03	7.37 ± 0.04
	HA100	7.43 ± 0.03	7.43 ± 0.03	7.37 ± 0.02	7.37 ± 0.03	7.39 ± 0.02
	HA300	7.46 ± 0.01	7.41 ± 0.03	7.31 ± 0.02	7.31 ± 0.03	7.34 ± 0.03
pCO ₂	HS	36.2 ± 0.7	33.2 ± 1.7	35.4 ± 0.5	33.4 ± 1.1	30.0 ± 2.9
	HA50	38.8 ± 1.2	34.3 ± 1.0	34.0 ± 0.9	32.5 ± 1.0	29.1 ± 2.3
	HA100	36.3 ± 0.9	31.2 ± 1.2	33.0 ± 3.4	36.1 ± 1.6	32.3 ± 1.4
	HA300	36.7 ± 1.2	30.0 ± 1.3	34.3 ± 1.8	33.2 ± 1.0	28.6 ± 2.0
pO ₂	HS	184 ± 4	190 ± 4	183 ± 5	178 ± 6	183 ± 5
	HA50	170 ± 6	166 ± 13	165 ± 10	173 ± 5	179 ± 2
	HA100	162 ± 9	178 ± 4	162 ± 3	164 ± 5	162 ± 7
	HA300	169 ± 5	158 ± 14	153 ± 17	149 ± 21	155 ± 22
Hct	HS	44.3 ± 0.9	28.5 ± 1.7	28.5 ± 1.2	27.5 ± 1.3	25.8 ± 0.7
	HA50	44.2 ± 1.1	30.0 ± 0.9	27.2 ± 1.0	26.5 ± 0.9	25.0 ± 1.2
	HA100	41.5 ± 0.6	30.7 ± 1.2	28.3 ± 1.6	28.8 ± 1.8	28.8 ± 1.5
	HA300	42.8 ± 0.8	29.7 ± 1.2	27.2 ± 1.3	27.8 ± 1.5	28.2 ± 1.1
glu	HS	176 ± 24	265 ± 42	247 ± 32	231 ± 38	139 ± 42
	HA50	173 ± 23	272 ± 38	241 ± 41	241 ± 34	171 ± 32
	HA100	192 ± 33	236 ± 45	198 ± 25	183 ± 23	164 ± 21
	HA300	176 ± 16	255 ± 35	193 ± 39	214 ± 20	137 ± 9

BL indicates pre-TBI baseline; HEM, end of hemorrhage; (30, 60, 120), 30, 60, and 120 min after the end of infusion of resuscitation fluid; pH, arterial pH (in pH units); HS, hypertonic saline; HA50, hypertonic saline with 50 mg/kg L-arginine; HA100, hypertonic saline with 100 mg/kg L-arginine; HA300, hypertonic saline with 300 mg/kg L-arginine; pCO₂, arterial pCO₂ (in mm Hg); pO₂, arterial pO₂ (in mm Hg); Hct, hematocrit; glu, plasma glucose.

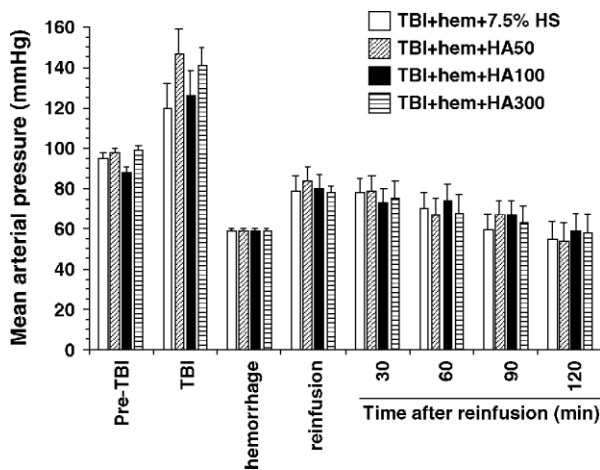


FIG. 1. MAP in rats after moderate, paramedian, fluid-percussion TBI, hemorrhage (hem) for 45 min, and infusion with either 7.5% hypertonic saline (TBI + hem + HS) ($n = 6$) or hypertonic saline with 50 (TBI + hem + HA50) ($n = 6$), 100 (TBI + hem + HA100) ($n = 6$), or 300 mg/kg (TBI + hem + HA300) ($n = 6$) and 30, 60, and 120 min after infusion. All values are mean \pm SEM.

preparation to changes in cerebral perfusion was determined by decreasing respiratory rate. If perfusion did not increase in response to increased PaCO₂, the probe was moved slightly until a perfusion increase was achieved. If a hyperemic response could not be obtained, the experiment was terminated. Rats also were excluded from the analysis if CBF did not decrease by at least 20% during hemorrhage after TBI. Because TBI caused cerebral hypoperfusion (14, 26–28) and impaired autoregulation in rats (29, 30) and other species (31–33), rats not exhibiting CBF decreases during hypotension after TBI were considered to be insufficiently injured to warrant further study.

Experimental design

After moderate TBI (2.0 atm) and hemorrhagic hypotension (60 mm Hg for 45 min), resuscitation solutions were administered in a blinded, randomized fashion to the treatment groups listed as follows: (1) HS—7.5% hypertonic saline (2,400 mOsm, 6 mL/kg) was infused into a femoral vein at a rate of 1.0 mL·min⁻¹·kg⁻¹; (2) HA50—50 mg/kg of L-arginine in hypertonic saline solution (sufficient sodium chloride added to produce total osmolality = 2,400 mOsm, 6 mL/kg) was infused into a femoral vein at a rate of 1.0 mL·min⁻¹·kg⁻¹; (3) HA100—100 mg/kg of L-arginine in hypertonic saline solution (sufficient sodium chloride added to produce total osmolality = 2,400 mOsm, 6 mL/kg) was infused into a femoral vein at a rate of 1.0 mL·min⁻¹·kg⁻¹; and (4) HA300—300 mg/kg of L-arginine in hypertonic saline solution (sufficient sodium chloride added to produce total osmolality = 2,400 mOsm, 6 mL/kg) was infused into a femoral vein at a rate of 1.0 mL·min⁻¹·kg⁻¹.

Data from the measurements of ICP and MAP are expressed as mean \pm standard error of the mean (SEM) absolute values recorded before (Baseline) TBI; within 1 min of TBI (TBI); immediately after hemorrhage (Hem); immediately after infusion of the hypertonic saline or arginine solutions (Inf); or 30, 60, or 120 min after infusion. Data from the measurements of laser Doppler CBF are expressed as mean \pm SEM of the percent of the pre-TBI (Baseline) measurements at the same measurement intervals as those used for ICP and MAP.

Statistical methods

MPA, CBF, and ICP were analyzed using analysis of variance for a two-factor experiment with repeated measures on time. The two factors were treatment group (HS, HA50, HA100, and HA300) and time (8 time points). First-order autoregressive covariance was used for a covariance structure. Main effects were assessed at the 0.05 level of significance, and the interaction between treatment group and time was assessed at the 0.15 level of significance. Fisher's least significant difference procedure was used for multiple comparisons with Bonferroni adjustment for the number of comparisons. Computations were carried out using PROC MIXED with LSMEANS option and Satterthwaite approximation for the denominator degrees of freedom in SAS (SAS Institute Inc., SAS/STAT 9.1 User's Guide, Cary, NC: SAS Institute Inc., 2004).

RESULTS

Thirty-eight rats were subjected to moderate TBI, hemorrhage, and resuscitation. Fourteen rats were excluded from the

analysis because LDF did not decrease by at least 20% during hemorrhage after TBI.

There were no significant differences in MAP, ICP, arterial pH, pO₂, pCO₂, hematocrit, or glucose levels at Baseline among the experimental groups as shown in Table 1. Arterial pO₂ did not change significantly over time, nor did the experimental groups differ significantly from one another. Arterial pCO₂, arterial pH, arterial glucose, and hematocrit decreased significantly ($P < 0.0001$ for all variables) in all four groups over time, but there were no significant differences in pairwise comparisons between groups. MPA changed significantly ($P < 0.0001$), increasing immediately after TBI, decreasing during hemorrhage, and then increasing toward baseline, but there were no significant differences in MAP among groups as shown in Figure 1.

There were significant changes in ICP ($P < 0.0001$) over time in all groups, with ICP increasing after TBI, then decreasing during hemorrhage and resuscitation as shown in Figure 2. In addition, there was a statistically significant group effect for ICP ($P = 0.049$). In individual comparisons, mean ICP over time in the HA300 group was statistically significantly lower than that in the HS group. In individual comparisons between the HA50, HA100, and HS groups, mean ICP across all time points was not statistically different. However, if the number of animals for each group were 29, there would be sufficient power (0.80) to statistically confirm a reduction of ICP of the observed magnitude in the HA50 in comparison to the HS group with a one-sided Fisher's least significant difference procedure at the 0.005 level of comparisonwise error rate (Elashoff, JD, nQuery Advisor Version 4.0 User's Guide, Los Angeles, CA, 2000). If the number of animals for each group were 14, there would be sufficient power (0.80) to statistically confirm a reduction of ICP of the observed magnitude in the HA100 in comparison to the HS group with a one-sided Fisher's least significant difference procedure at the 0.005 level of comparisonwise error rate.

CBF changed significantly ($P = 0.0011$) after TBI and hemorrhage, decreasing during hemorrhage and then increasing

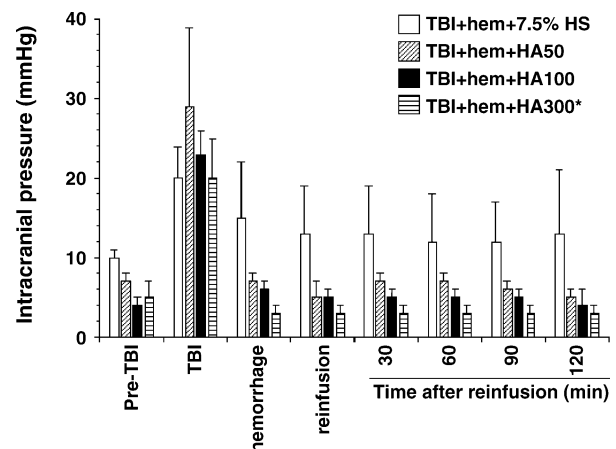


FIG. 2. ICP in rats after moderate, paramedian, fluid-percussion TBI, hem for 45 min, and infusion with either 7.5% hypertonic saline (TBI + hem + HS) ($n = 6$) or hypertonic saline with 50 (TBI + hem + HA50) ($n = 6$), 100 (TBI + hem + HA100) ($n = 6$), or 300 mg/kg (TBI + hem + HA300) ($n = 6$) and 30, 60, and 120 min after infusion. All values are mean \pm SEM. $P < 0.05$, HA300 versus HS.

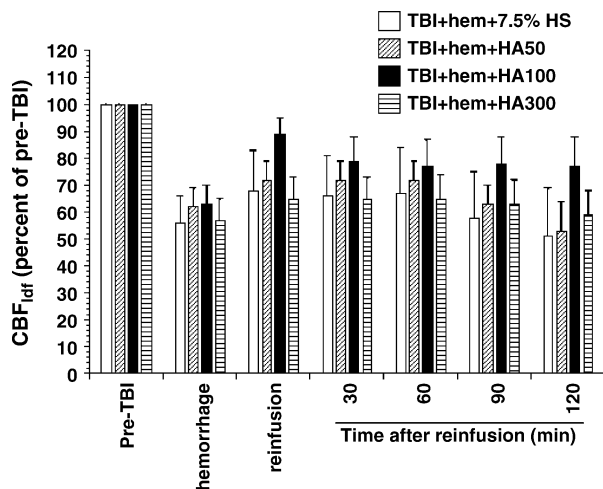


FIG. 3. Laser Doppler CBF (CBF_{ldf}) in rats after moderate, paramedian, fluid-percussion TBI, hem for 45 min, and infusion with either 7.5% hypertonic saline (TBI + hem + 7.5% HS) ($n = 6$), or hypertonic saline with 50 (TBI + hem + HA50) ($n = 6$), 100 (TBI + hem + HA100) ($n = 6$), or 300 mg/kg (TBI + hem + HA300) ($n = 6$) and 30, 60, and 120 min after infusion. All values are mean \pm SEM percent of baseline.

toward baseline after resuscitation as shown in Figure 3. Although CBF was statistically insignificantly higher in the group resuscitated with hypertonic saline with 100 mg/kg L-arginine immediately and 120 min after infusion, there were no significant differences among the groups.

DISCUSSION

These data are the first to examine the hypothesis that addition of L-arginine to hypertonic saline resuscitation after TBI and hemorrhage would increase the duration of increased CBF after resuscitation without increasing ICP. Surprisingly, addition of L-arginine did not enhance either the magnitude or the duration of improvement in CBF when compared with 7.5% saline alone. However, L-arginine-containing hypertonic solutions were not associated with higher ICP than hypertonic saline alone; in fact, ICP tended to be lower after resuscitation in the groups receiving L-arginine, with the difference attaining statistical significance in the HA300 group. Moreover, adding L-arginine to hypertonic resuscitation did not reduce the effectiveness of hypertonic saline at restoring MAP after hemorrhage. This observation is important because previous studies in which L-arginine was given to rats after cortical contusion injury showed similarly low MAP to that usually following cortical contusion injury (34).

Although L-arginine did not improve CBF when added to hypertonic saline, there still could be a role for adding L-arginine to conventional resuscitation solutions. The control resuscitation regimen (hypertonic saline without added L-arginine) used in this study has been associated with higher CBF and lower ICP than conventional crystalloid or colloid resuscitation solutions. Because hypertonic saline solutions are not approved for use in resuscitating trauma patients in the United States, it is important to determine whether adding L-arginine to conventional crystalloids or colloids could improve CBF and ICP without reducing MAP. In addition, endpoints other than CBF and ICP (e.g., inflammatory

cytokine expression, protein nitration, and gene and protein expressions) also should be examined to determine whether adding L-arginine to conventional hypertonic resuscitation offers any benefit to hypotensive, head-injured patients.

Resuscitation of hypotensive patients with TBI is an important clinical topic. When TBI is accompanied by hemorrhagic hypotension, the mortality rate is doubled (3, 35). The physiologic deficit most likely responsible for the strikingly adverse influence of hypotension on outcome after TBI is cerebral ischemia caused by complex interactions between hypotension, cerebral vasoconstriction, and impaired cerebral vasodilatory responsiveness. The impairment of cerebral vascular reactivity associated with fluid-percussion TBI increases with increasing severity of injury. Moderate fluid-percussion TBI reduces, and severe TBI abolishes: increases in CBF that normally occur in response to hypoxia or hypercapnia (36). Similarly, CBF autoregulation in response to decreased arterial blood pressure was reduced by moderate TBI and abolished by severe TBI (32). Optimal resuscitation would correct all of these physiologic deficits. However, after hemorrhagic shock, fluid resuscitation with conventional fluids is associated with a rapid increase in ICP, which does not occur with hypertonic resuscitation (4, 37, 38).

To avoid intracranial hypertension occurring as a consequence of resuscitation with conventional fluids we (4–6), and others (7, 8) have used low-volume hypertonic saline for initial fluid resuscitation of hemorrhage after experimental TBI and have demonstrated that hypertonic saline expanded blood volume, increased blood pressure, and lowered ICP but only transiently improved CBF and did not correct TBI-induced loss of cerebral vasodilatory responsiveness. Failure to produce sustained improvement in CBF and cerebrovascular function may partially explain the failure of the most recent clinical trial of hypertonic saline in hypotensive, head-injured patients (39). An agent that effectively restores CBF, cerebral vasodilatory responsiveness, and provides systemic hemodynamic stability in the period immediately after injury (i.e., the period preceding definitive hospital care) remains to be developed.

Posttraumatic hypoperfusion may result from impairment or destruction of a cerebral vasodilator such as the endothelium-dependent relaxing factor NO (18, 19). In uninjured experimental animals, inhibition of NO synthesis decreased CBF, (20–23) which suggests that continuous production of NO generates a tonic cerebral vasodilatory influence. However, NO is an oxygen-free radical that is inactivated by contact with other free radicals (40) such as superoxide anion ($\bullet O_2^-$) radicals that may be generated after TBI as a byproduct of increases in prostaglandin synthesis (17, 41). We simultaneously directly measured reduced CBF and increased $\bullet O_2^-$ levels after TBI in rats (14, 17), thereby suggesting an association between increased $\bullet O_2^-$ levels and posttraumatic cerebral hypoperfusion. Even in the absence of TBI, hemorrhagic hypotension reduced cortical NO levels and NO synthase (NOS) activity in cats (42). *In vitro* treatment with SOD (43) or L-arginine (42) restored vasodilatory responses to acetylcholine that had been impaired by hemorrhagic hypotension.

In addition to resuscitation fluids of L-arginine, the physiologic substrate that is converted to NO by NOS could

exert favorable effects on the cerebral circulation after TBI. After cortical impact TBI, L-arginine (300 mg/kg) improved CBF without increasing cerebral perfusion pressure (44). L-Arginine also increased CBF during cerebral ischemia (45, 46) and reduced infarct volume after cerebral ischemia (46, 47) in rats. After experimental fluid-percussion TBI, L-arginine completely prevented cerebral hypoperfusion (14). Previous data from our laboratories demonstrated that CBF was preserved after TBI in rats treated with L-arginine but not D-arginine, thereby supporting the hypothesis that TBI reduced CBF by reducing an NO-mediated cerebral vasodilatory tone (14).

However, provision of exogenous L-arginine could potentially increase production of the highly reactive radical peroxynitrite (ONOO^-), the highly toxic product of the reaction between NO and $\bullet\text{O}_2^-$. The adverse effects of $\bullet\text{O}_2^-$ on NO levels and on cerebral vasoreactivity are at least partially mediated by combining $\bullet\text{O}_2^-$ with NO to form ONOO^- (48, 49), which may have adverse effects in addition to a simple reduction of NO levels. A powerful oxidizing agent (50), ONOO^- , when protonated, decays homolytically to form a highly reactive hydroxyl-type radical. Recent evidence also indicates that ONOO^- constricts the cerebral circulation (51). We have observed that ONOO^- produces dose-dependent antagonism of cerebral vasodilatory responses to progressive hypotension *in vitro* (52).

The interactions between NO, $\bullet\text{O}_2^-$, ONOO^- , and the cerebral circulation are important, but each of these constituents also exerts important effects on neuronal tissue. Most importantly, neuronal NOS (nNOS), which is upregulated after TBI (53, 54), is associated with neuronal injury (55–57). Thus, improvements in cerebral perfusion associated with increased substrate for NO production by endothelial NOS (eNOS) could be offset by adverse effects of additional NO production by nNOS in neurons and by increased production of ONOO^- . Inhibition of NOS has been reported to be beneficial after TBI. The eNOS inhibitor L-NAME (*N*^ω-nitro L-arginine methyl ester) and the nNOS inhibitor 7-NI (7-nitroindazole) improved neurologic outcome in mice (58), and 7-NI improved behavioral outcome (forelimb placing) in rats after TBI (54). The effects of L-arginine or NOS inhibitors on behavioral outcome after TBI combined with hemorrhage have not yet been reported. Alternatively, rapid, effective restoration of CBF after TBI and hypotension by reducing secondary cerebral hypoperfusion could reduce the formation of deleterious agents such as $\bullet\text{O}_2^-$ and ONOO^- as well as cytokines and other inflammatory mediators.

We chose doses of L-arginine for this study in an attempt to achieve satisfactory cerebral vasodilation while avoiding potentially adverse consequences of L-arginine. Hypertonic saline transiently improved CBF when used for resuscitation from hemorrhagic shock (4, 5, 59, 60). After focal cerebral ischemia in rats, 300 mg/kg L-arginine increased CBF and reduced lesion volume but caused cerebral vasodilation and increased CBF in uninjured rats as well (45, 46). In a dose of 100 mg/kg, L-arginine completely prevented reductions in CBF after moderate fluid-percussion TBI in rats without increasing CBF in sham-injured rats (14). Because even mild cerebral vasodilation could increase ICP after TBI, we

included a lower dose of 50 mg/kg in our study. Our data suggest that 100 mg/kg of L-arginine is the most promising dose in this model of TBI, but proving this would require larger groups of rats. CBF tended to be higher in the 100-mg/kg group than in either the hypertonic saline or the 50- or 300-mg/kg groups, and ICP was similarly low in all three L-arginine groups. There also were no differences in their effects on MAP between the three doses.

These data require comparisons with previously reported results in rats receiving a cortical contusion injury followed by administration of L-arginine (300 mg/kg). In that study, L-arginine was associated with a sustained increase in CBF. Possible explanations for the differences between the present study and the study by Cherian et al. (44) are that cortical contusion more profoundly reduces CBF than fluid-percussion injury and that Cherian et al. (44) infused L-arginine in animals that had not been hemorrhaged (although cortical contusion injury is associated with moderately severe, non-hemorrhagic systemic hypotension). In addition, in that study, Cherian et al. (44) compared L-arginine with a continuous infusion of phenylephrine sufficient to reverse the usual hypotension associated with cortical contusion injury. A comparison of these studies suggests the need for additional studies to determine whether L-arginine would be effective in increasing CBF after cortical contusion plus hemorrhage and whether hypertonic saline would be comparably effective to hypertonic L-arginine after contusion injury.

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