Neuroprotective Effects of L-Arginine Administration after Cortical Impact Injury in Rats: Dose Response and Time Window

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ABSTRACT

Administration of L-arginine has been shown to increase cerebral blood flow and reduce neurological damage after experimental traumatic brain injury. The purpose of this study was to examine the optimal dose and time window for these neuroprotective effects. In a dose response experiment, doses of L-arginine ranging from 37.5 to 600 mg/kg were administered 5 min after a 5-m/s, 3-mm, controlled cortical impact in rats. The amount of brain injury found at 2 weeks after injury, both at the contusion site and in the ipsilateral hippocampus, were inversely related to the dose of L-arginine administered. Both 300- and 600-mg/kg doses of L-arginine significantly reduced contusion volume. The 300-mg/kg dose significantly increased the neuron density in the CA1 region of the hippocampus. Physiological effects of L-arginine were also dose-related. The greatest reduction in intracranial pressure occurred with the 300-mg/kg dose of L-arginine. Doses up to 300 mg/kg were well tolerated, but the 600-mg/kg dose resulted in transient hypotension. In another experiment, 300 mg/kg L-arginine was administered at times varying from 5 min to 48 h after injury. Contusion volume was significantly reduced when the L-arginine was given at 5 min and 1 h after injury. The protective effect was less when the same dose was given at the later times, but there was no evidence of an adverse effect even when the L-arginine was administered 48 h after injury.

Traumatic brain injury (TBI) causes a reduction in cerebral blood flow (CBF), potentially leading to oxygen and nutrient deprivation and initiation of a cascade of secondary ischemia (Bouma et al., 1991; Marion et al., 1991). In the controlled cortical impact injury model of TBI, the reduction in blood flow is related to the severity of injury. Following a mild 3-m/s, 2.5-mm deformation cortical impact injury, blood flow is reduced by approximately 50% at the impact site but remains well preserved elsewhere in the brain (Giri et al., 2000). This reduction in CBF is not sufficient to cause ischemic damage; however, it does make the injured brain more susceptible to secondary ischemic insults. Following a more severe 5-m/s, 3-mm deformation cortical impact injury, blood flow at the impact site decreased more than 75% below preinjury levels (Cherian et al., 1994). This reduction in CBF is more likely to result in ischemic damage.

L-Arginine administered intravenously in a dose of 300 mg/kg 5 min after the impact injury restores CBF to near preinjury levels and also significantly reduces the volume of contused brain (Cherian et al., 1999). Similar neuroprotective effects in other experimental TBI models and in some cerebral ischemia models have been observed with early administration of L-arginine (Morikawa et al., 1994; DeWitt et al., 1997; Sadoshima et al., 1997; Wada et al., 1998). Unlike induced hypertension, which is the usual clinical treatment of cerebral hypoperfusion after trauma, L-arginine administration does not have the potential to increase brain edema and, in fact, has been observed in these experimental models to significantly lower intracranial pressure (ICP) (Cherian et al., 1999). As a result of these observations, L-arginine has become an interesting potential therapeutic agent for improving cerebral perfusion after traumatic brain injury. Before L-arginine could be considered for trials in patients with traumatic brain injury, two issues are important to clarify: first is the optimal dose of the drug and second is the time window.

Although L-arginine is a nonessential amino acid that is a normal constituent of the body and is found in both enteral and parental nutrition formulas, little experience is available about the pharmacology of L-arginine administration in the doses given in these experimental studies, especially in critically ill patients. Hypotension is meticulously avoided in patients with traumatic brain injury because a reduction in
blood pressure can impair cerebral perfusion. Hypotension is a potential adverse effect of L-arginine that could limit its usefulness in patients with traumatic brain injury.

Many drugs have been shown to be neuroprotective when given before injury or within the first few minutes after injury. This ultra-early administration is not practical in the clinical setting. There is an additional concern with later administration of L-arginine. L-Arginine given in models of cerebral ischemia at times more than 6 h after the onset of ischemia have sometimes actually had adverse effects on neurological outcome (Zhang et al., 1996). This adverse effect is presumably from increased nitric oxide production by inducible nitric-oxide synthase (NOS). In all of the previous work in brain trauma, L-arginine was given within the first few minutes after injury. Studies examining the neuroprotective effect of L-arginine at later times of administration in TBI models are needed. The purpose of this study was to determine the optimal dose and time window for administration of a single dose of L-arginine after controlled cortical impact injury.

Materials and Methods

Two experiments were performed. The first experiment was a dose response study with the following endpoints: plasma concentration of arginine 15 min after infusion, blood pressure and ICP effects of the infusion of L-arginine, and neurological outcome assessed by measuring contusion volume and neuron density in the CA1 and CA3 regions of the hippocampus at 2 weeks after injury. The second experiment was a study of the time window for the neuroprotective effects of L-arginine. In both experiments, the neurological outcome was assessed by measuring contusion volume and hippocampal neuron survival. Other than these differences and the treatment groups, the methods for the two experiments were similar. The study protocol was approved by the institutional animal protocol review committee, using guidelines for humane care and use of animals developed by the National Institutes of Health.

Treatment Groups

Dose Response Experiment. To study the effects of different doses of L-arginine, a total of 37 male Evans rats were randomly assigned to one of the following doses: none (control group), 37.5, 75, 150, 300, and 600 mg/kg. The numbers of animals in each group are listed in Table 1. Each of the L-arginine doses were dissolved in 1 ml of sterile 0.9% saline so that the infusion volume was the same for each group and only the dosage of L-arginine varied. The L-arginine infusion was started 5 min after the impact injury and given over 10 min. A plasma sample was collected at 15 min after infusion for measurement of L-arginine. Mean arterial pressure (MAP) and ICP were monitored during the impact injury and for 30 min after the injury.

Time Window Experiment. To study the time window for the neuroprotective effect of L-arginine administration, a total of 82 rats were randomly assigned to the following postinjury times for the administration of a single dose: 5 min and 1, 6, 24, and 48 h. At each administration time, the animals were randomly assigned to receive either L-arginine (300 mg/kg dissolved in 1 ml of 0.9% normal saline) or an equal volume of saline. The numbers of animals in each group are summarized in Table 2.

Anesthesia and Surgical Preparation

For both experiments, Long Evans rats, weighing 300 to 400g and fasted overnight, were anesthetized with 3.5% isoflurane in 100% oxygen in a vented anesthesia chamber. Following endotracheal intubation with a 16-gauge Teflon catheter, the rats were mechanically ventilated with 2% isoflurane in 100% oxygen for the surgical preparation, for the impact injury, and for the postinjury physiological monitoring (30 min in the dose response experiment). ICP was monitored by a 3F microsensor transducer (Codman & Schurtleff, Randolph, MA) inserted in the left frontal lobe, well away from the impact site. ICP was monitored during the impact injury as a measure of the severity of the injury. Rectal temperature was maintained at 36.5–37.5°C by a heating pad, which was controlled by rectal thermistor. Brain temperature was kept constant at 37°C with the help of a heating lamp directed at the head.

In the dose response experiment, additional physiological monitoring was done. A 22-gauge catheter was placed in the tail artery to monitor arterial blood pressure and to draw arterial blood samples. Arterial blood gases were obtained preinjury to assure that ventilation and oxygenation were adequate. Both ICP and MAP were monitored for 30 min after the impact injury to assess the effects of the various L-arginine doses on these parameters.

In the time window experiment, an additional procedure was performed before the impact injury. To administer to the assigned treatment the various times postinjury, an intravenous silicone catheter (Access Technologies, Niles, IL) was implanted in the femoral vein and threaded subcutaneously to the back where it was attached to a small injection port.

Production of Brain Injury

The details of the methods to produce the impact injury have been previously described (Cherian et al., 1996). Briefly, the head of the rat was fixed in a stereotaxic frame by ear bars and incisor bar. A 10-mm diameter craniotomy was performed on the right side of the skull over the parietal cortex. The impactor tip, which had a diameter of 8 mm, was centered in the craniotomy site perpendicular to the exposed surface of the brain at an angle of approximately 45 degrees to the vertical. The tip was lowered until it just touched the dural surface. The impactor rod was then retracted, and the tip advanced an additional 3 mm to produce a brain deformation of 3 mm during the impact. The gas pressure applied to the impactor was adjusted to 150 psi, giving an impact velocity of approximately 5 m/s and duration of approximately 150 to 160 ms.

Postoperative Care

At the end of the monitoring period, after removing all catheters and suturing the surgical wounds, the rats were allowed to awaken from anesthesia. For the first 3 days postinjury, the rats were treated with butorphanol tartrate, 0.05 mg of i.m. every 12 h (twice a day), for analgesia and enrofloxacin 2.27%, 0.1 ml of IM qd, to reduce the risk of postoperative infections.

| TABLE 1 Dose response experiment: mean values for measures of impact severity |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| L-Arginine Dose Given 5 min Postinjury | None | 75 mg/kg | 150 mg/kg | 300 mg/kg | 600 mg/kg |
| Number of animals in group | 7 | 7 | 5 | 5 | 7 |
| Impact velocity (m/s) | 4.8 ± 0.1 | 4.8 ± 0.1 | 4.9 ± 0.1 | 4.9 ± 0.1 | 4.9 ± 0.2 |
| Impact duration (ms) | 160 ± 1 | 155 ± 3 | 151 ± 4 | 155 ± 3 | 156 ± 3 |
| Peak ICP (mm Hg) | 187 ± 11 | 208 ± 9 | 205 ± 12 | 201 ± 28 | 192 ± 12 |

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TABLE 2
Time window experiment: mean values for measures of impact severity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Weight (g)</th>
<th>Impact velocity (m/s)</th>
<th>Impact duration (ms)</th>
<th>Peak ICP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>8</td>
<td>348 ± 14</td>
<td>4.9 ± 0.04</td>
<td>134 ± 2.1</td>
<td>203 ± 7.5</td>
</tr>
<tr>
<td>SA</td>
<td>7</td>
<td>377 ± 14</td>
<td>4.9 ± 0.04</td>
<td>134 ± 3.2</td>
<td>201 ± 12.2</td>
</tr>
<tr>
<td>LA</td>
<td>8</td>
<td>373 ± 12</td>
<td>4.8 ± 0.06</td>
<td>130 ± 3.5</td>
<td>190 ± 11.1</td>
</tr>
<tr>
<td>SA</td>
<td>10</td>
<td>399 ± 21</td>
<td>4.8 ± 0.07</td>
<td>133 ± 2.4</td>
<td>193 ± 9.6</td>
</tr>
<tr>
<td>LA</td>
<td>7</td>
<td>372 ± 12</td>
<td>4.9 ± 0.04</td>
<td>126 ± 2</td>
<td>187 ± 11.1</td>
</tr>
<tr>
<td>SA</td>
<td>11</td>
<td>378 ± 11</td>
<td>4.8 ± 0.04</td>
<td>128 ± 2</td>
<td>187 ± 9.7</td>
</tr>
<tr>
<td>LA</td>
<td>7</td>
<td>369 ± 10</td>
<td>4.8 ± 0.05</td>
<td>131 ± 4.6</td>
<td>205 ± 9.5</td>
</tr>
<tr>
<td>SA</td>
<td>8</td>
<td>368 ± 15</td>
<td>4.8 ± 0.07</td>
<td>135 ± 3.5</td>
<td>181 ± 11.1</td>
</tr>
<tr>
<td>LA</td>
<td>8</td>
<td>369 ± 6</td>
<td>4.9 ± 0.03</td>
<td>134 ± 3.5</td>
<td>199 ± 11.1</td>
</tr>
<tr>
<td>SA</td>
<td>8</td>
<td>386 ± 8</td>
<td>4.9 ± 0.08</td>
<td>131 ± 3.1</td>
<td>194 ± 8.4</td>
</tr>
</tbody>
</table>

Treatments: LA, L-arginine; SA, saline.

Outcome Measures
The outcome measures were performed by investigators who were blinded to the treatment group. At 2 weeks after the impact, the animals were deeply anesthetized with a combination of ketamine/xylazine/acepromazine and perfused transectially with 0.9% saline, followed by 10% phosphate buffered formaldehyde. The entire brain was removed and fixed in 4% formalin. The fixed brains were examined grossly for the presence of contusion, hematoma, and herniation. The brains were photographed, sectioned at 2-mm intervals, and then embedded in paraffin. Haematoxylin and eosin stained 9-μm thick sections were prepared for histologic examination. Particular care was made to include the largest cross-sectional area of cortical injury on the cut surface of the embedded sections. The H&E-stained coronal sections were digitized using a Polaroid Sprint Scanner (Polaroid Corporation, Waltham, MA) equipped with a PathScan Enabler (Meyer Instruments, Houston, TX).

The injury volume was measured by determining the cross-sectional area of injury in each H&E-stained coronal image and multiplying by the thickness of the tissue between the slices. This slab volume technique was implemented on the image processing program Optimas 5.2 (Optimas Corporation, Seattle, WA). Neurons in the middle 1-mm segments of the CA1 and CA3 regions of the hippocampus were counted at a magnification of 200×. Neurons were identified by nuclear and cytoplasmic morphology, and individual cells were counted whether normal or damaged. Neurons with cytoplasmic shrinkage, basophilia, or eosinophilia or with loss of nuclear detail were regarded as damaged. The regions measured were 1 mm long and 1 mm wide (0.5 mm on either side of the long axis of the segment). The total number of neurons and the number of neurons that appeared normal were expressed as neurons per square millimeter.

Statistical Analysis
Summary data are expressed as mean ± S.E.M. Histological outcome measures were compared using one-way analysis of variance (dose response experiment) or two-way analysis of variance (time window experiment) followed by Tukey’s test for multiple comparisons. Physiological parameters, such as MAP and ICP, were analyzed by repeated measures analysis of variance.

Results
Dose Response Experiment
Plasma Arginine Concentration. The plasma arginine concentration at 15 min postinfusion of the assigned dose increased following L-arginine infusion in a dose-dependent manner (Fig. 1). The saline control group had a plasma arginine concentration of 393 ± 105 μM compared with 2129 ± 1963 μM after the 600-mg/kg dose of L-arginine (p = 0.04).

ICP was measured preinjury and for the first 30 min after the injury while the animal remained anesthetized and ventilated. The baseline intracranial pressure was similar in all groups and averaged 9 ± 1 mm Hg. ICP increased after the impact injury in all groups, and at 5 min postinjury just before the infusion of L-arginine, ICP averaged 32 ± 2 mm Hg, with no significant differences among the treatment groups. At the end of the 30-min monitoring period, ICP had decreased to 17 ± 1 mm Hg in the 300-mg/kg group compared...
TABLE 3
Dose response experiment physiologic variables at baseline and for 30 min after injury

<table>
<thead>
<tr>
<th>L-Arginine Dose Given 5 min Postinjury</th>
<th>None</th>
<th>37.5 mg/kg</th>
<th>75 mg/kg</th>
<th>150 mg/kg</th>
<th>300 mg/kg</th>
<th>600 mg/kg</th>
</tr>
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<tr>
<td>Number of animals in group</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>7</td>
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</tr>
<tr>
<td>Baseline period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>351 ± 12</td>
<td>356 ± 22</td>
<td>347 ± 19</td>
<td>358 ± 16</td>
<td>365 ± 20</td>
<td>356 ± 12</td>
</tr>
<tr>
<td>Arterial pO2 (mm Hg)</td>
<td>167 ± 24</td>
<td>154 ± 33</td>
<td>180 ± 37</td>
<td>238 ± 17</td>
<td>248 ± 48</td>
<td>179 ± 27</td>
</tr>
<tr>
<td>Arterial pCO2 (mm Hg)</td>
<td>36 ± 3</td>
<td>34 ± 3</td>
<td>35 ± 2</td>
<td>37 ± 3</td>
<td>37 ± 4</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.33 ± 0.01</td>
<td>7.34 ± 0.02</td>
<td>7.33 ± 0.01</td>
<td>7.31 ± 0.01</td>
<td>7.30 ± 0.03</td>
<td>7.32 ± 0.1</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>86 ± 6.5</td>
<td>93 ± 10</td>
<td>90 ± 6</td>
<td>80 ± 8</td>
<td>93 ± 6.2</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>Intracranial pressure (mm Hg)</td>
<td>9 ± 1</td>
<td>9 ± 2</td>
<td>7 ± 1.1</td>
<td>8 ± 2</td>
<td>9 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Preinfusion period (5 min after impact injury)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>80 ± 8</td>
<td>97 ± 9</td>
<td>87 ± 13</td>
<td>91 ± 13</td>
<td>92 ± 15</td>
<td>80 ± 6</td>
</tr>
<tr>
<td>Intracranial pressure (mm Hg)</td>
<td>28 ± 2</td>
<td>31 ± 7</td>
<td>34 ± 6</td>
<td>26 ± 6</td>
<td>39 ± 5</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>Postinfusion period (30 min after impact injury)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>95 ± 7</td>
<td>98 ± 8</td>
<td>101 ± 10</td>
<td>89 ± 10</td>
<td>95 ± 12</td>
<td>74 ± 5*</td>
</tr>
<tr>
<td>Intracranial pressure (mm Hg)</td>
<td>24 ± 5</td>
<td>25 ± 5</td>
<td>20 ± 3</td>
<td>24 ± 10</td>
<td>17 ± 1</td>
<td>22 ± 8</td>
</tr>
</tbody>
</table>

* Different from control group (p < 0.05).

Fig. 2. In the dose response experiment, the decrease in ICP between 5 and 30 min postinjury was greatest with the 300-mg/kg dose of L-arginine.

with 24 ± 5 mm Hg in the saline control group, but this small absolute value was not significantly different. The decrease in ICP between the preinfusion value and the 30-min postinjury value, however, was significantly greater in the 300-mg/kg group than in the saline control group (Fig. 2).

Blood pressure was also measured preinjury and for the first 30 min after the injury. The baseline mean arterial pressure for all the groups was 87 ± 4 mm Hg. Following the infusion of the assigned dose of L-arginine, there was a significant difference in the mean values among the different levels of treatment (treatment effect, p = 0.040; time effect, p = 0.573; time × treatment interaction, p = 0.746). L-Arginine in lower doses had no significant effect on mean arterial pressure, whereas a 600-mg/kg dose tended to cause hypotension (Table 1).

Histological Outcome Measures. As shown in Fig. 3, left panel, the contusion volume at 2 weeks after injury varied inversely with the dose of L-arginine administered (overall drug effect, p = 0.045) and was significantly less in the animals receiving the 300- and the 600-mg/kg doses of L-arginine than in the control group given only saline. The average contusion volume was 7.5 ± 4.0 mm³ with the 300-mg/kg dose and 11.3 ± 5.5 mm³ with the 600-mg/kg dose compared with 30.5 ± 4.7 mm³ in the control group (p < 0.05 for both comparisons).
As illustrated in Fig. 4-Left, the neuron density in the CA1 region of the hippocampus at two weeks after injury varied directly with the dose of L-arginine administered (overall dose effect, \( p = 0.01 \)). The mean neuron density after administration of the 300-mg/kg dose of L-arginine was significantly greater than in the control group. Neuron density in the CA3 region followed a similar trend (Fig. 5-Left), but the treatment effect was not statistically significant (overall dose effect, \( p = 0.233 \)).

**Time Window Experiment**

**Histological Outcome Measures.** As shown in Fig. 3, right panel, L-arginine (300 mg/kg) was the most effective in reducing contusion volume at 2 weeks postinjury when it was given early following the impact injury. The contusion volume was significantly smaller than the control group only when the dose was given at 5 min or 1 h after injury. Unlike the situation that has been observed in models of cerebral ischemia, however, there was no evidence that giving L-arginine as late as 48 h after injury had an adverse effect.

**Discussion**

**Mechanism of the Neuroprotective Effects of L-Arginine Administration.** The neuroprotective effects of L-arginine administration are presumed to occur via production of nitric oxide (NO), as L-arginine is the immediate precursor of NO in the reaction mediated by the enzyme NOS. NO is produced by many different tissues and has numerous physiological functions as well as some pathological effects. The neuroprotective effects of L-arginine administration could also occur from other effects on the vasculature, including that L-arginine is essential for the function of certain K\(_{\text{ATP}}\) channels (Kontos and Wei, 1998).

The clinical effects of nitric oxide can be beneficial or they can be detrimental, particularly when the brain is injured (Iadecola, 1997). Nitric oxide is a potent vasodilator and may increase collateral blood flow, which in some circumstances, such as early stroke, can increase survival of ischemic tissue. Nitric oxide inhibits platelet aggregation and leukocyte adhesion and may also improve blood flow by preventing mi-
Nitric oxide may inhibit Ca\(^{2+}\) influx through the N-methyl-D-aspartate receptor and may limit glutamate neurotoxicity in cerebral ischemia (Lipton et al., 1993; Fagni et al., 1995).

Nitric oxide can also have effects that are cytotoxic, however. Nitric oxide promotes oxidative damage by reacting with superoxide anion to form peroxynitrite and by perturbing iron metabolism (Beckman et al., 1990; Reif and Simmons, 1990). NOS may generate peroxynitrite directly by producing both nitric oxide and superoxide. During ischemia, nitric oxide, either directly or through its derived species, can cause energy failure, produce DNA damage, inhibit DNA synthesis, and trigger programmed cell death (Bonfoco et al., 1995). Nitric oxide may exacerbate ischemic damage by enhancing the postischemic release of excitatory amino acids (Montague et al., 1994). Finally, the redox state of the tissue may have an important impact on the effects of nitric oxide. In neuronal cultures, NO\(^{-}\), one of the redox forms of nitric oxide, is toxic by producing peroxynitrite, whereas nitrosonium (NO\(^{+}\)) is protective (Lipton et al., 1993). Additional potential concerns, particularly in the setting of traumatic brain injury, are possible effects of nitric oxide on systemic blood pressure and on ICP. In general, vasodilating agents can be anticipated to have the potential to lower blood pressure and to raise ICP.

Anesthetic agents and postoperative analgesics such as butorphanol can have neuroprotective effects in experimental models and can confound the results of a study. In the experiments reported here, however, the anesthesia and postoperative treatment were administered consistently in all animals. Therefore, these factors do not account for the changes observed.

The control animals in this study received saline. Use of D-arginine as a control for this study might have provided additional insight into the mechanism of the neuroprotective effects of L-arginine since the conversion of arginine to nitric oxide is stereospecific for the L-isomer. In previous studies with this model of trauma and in the fluid perfusion model, however, the beneficial effect of arginine administration on cerebral blood flow was only seen with the L-isomer. D-Arginine had no effect on posttraumatic cerebral blood flow or on nitric oxide concentrations in the brain (DeWitt et al., 1997; Cherian and Robertson, 2002).

**L-Arginine Dose for Neuroprotection after TBI.** In adults, arginine is a nonessential amino acid and is available in health food stores as a nutritional supplement (Reyes et al., 1994). Arginine homeostasis is normally achieved by a balance between dietary intake and degradation. When arginine degradation and/or utilization is increased, such as during growth and development and with wound healing, trauma, injury, or sepsis, arginine may become an essential amino acid (Seifert et al., 1978). Parenteral arginine supplementation in patients with trauma has been shown to improve wound healing and lymphocyte immune responses (Barbul et al., 1990). As a nutritional supplement, there are no significant adverse effects.

Nevertheless, the doses of L-arginine found to be neuroprotective in this study are considerably larger than those used in nutritional supplements. The only current clinical use of L-arginine in doses equivalent to those used in these experimental studies is a diagnostic test for growth hormone production. A dose of 30 g is typically given intravenously to an adult subject for this purpose. L-Arginine has been given to normal subjects and found to raise basal CBF (Reutens et al., 1997). L-Arginine has also been demonstrated to raise middle cerebral artery flow velocity in normal subjects (Micieli et al., 1997). There have been no significant adverse effects of L-arginine infusion in these studies of normal human subjects, although a slight reduction in blood pressure is commonly observed. Hypotension in a patient with traumatic brain injury, however, may be a serious adverse effect of a drug treatment because it can reduce cerebral perfusion. The present study suggests that in the rat, the 300-mg/kg dose of L-arginine provides the best balance between neuroprotection and minimal hypotensive action.

Another potential adverse effect of L-arginine administration in a patient with traumatic brain injury is that the improvement in cerebral blood flow could be associated with an increase in intracranial pressure. All cerebral vasodilators have this potential adverse effect if they increase cerebral blood volume. Nevertheless, this has not been reported with L-arginine administration in the cortical impact injury model. In fact, as was observed in this study, there is often a reduction in ICP. A reduction in ICP has also been observed with d-arginine administration, suggesting that this is a nonspecific effect and probably unrelated to nitric oxide production (Cherian and Robertson, 2002). L-Arginine in the 300- and 600-mg/kg dose is hypertonic. It is possible that the L-arginine may reduce the edema formation in the contused tissue, although when brain water content has been measured after L-arginine administration, it has not been found to be significantly reduced (Cherian et al., 1999).

**Time Window for L-Arginine Administration after TBI.** Factors to be considered in the timing of L-arginine are two fold. First, when CBF is low enough to result in additional ischemic damage to the brain after trauma. Most of the clinical literature suggests that the lowest values of CBF after TBI occur within the first 6 to 12 h after injury (Bouma et al., 1991, 1992; Kelly et al., 1997). Following that time, transient ischemia due to secondary insults such as hypotension or intracranial hypertension may occur, but the proper treatment of these secondary events is correction of the underlying abnormality (Gopinath et al., 1994). This pattern of the evolution of CBF after trauma would suggest that early administration of L-arginine would be likely to have the best chance for reducing neurological injury. The present study confirms this intuition, suggesting that the best protective effects occur when the drug is given within 5 min of injury.

The second issue of interest to the time window question is whether other later pathological processes induced by trauma might be augmented by L-arginine administration. As recently reviewed by Iadecola (1997), the early effects of nitric oxide produced by the constitutive forms of NOS are typically physiological and protective by increasing CBF, whereas the late effects of nitric oxide produced by the inducible form of NOS (iNOS) are often pathological and cause additional cytotoxic damage to the already injured tissue.

In stroke models, the effect of NO is clearly time-dependent given that during the 1st h after the onset of ischemia, the administration of NO donors increases CBF and reduces the size of infarction (Zhang and Iadecola, 1993, 1994; Zhang et al., 1994). Nevertheless, administration of L-arginine more than 6 h after the onset of a stroke has detrimental effects,
and drugs that reduce NO metabolism have a protective effect (Zhang et al., 1996). Mice that are deficient in iNOS have a smaller infract volume than wild-type mice (Iadecola et al., 1997).

In trauma models, the role of iNOS in the evolution of injury has been more controversial. In some studies, administration of an iNOS inhibitor has had protective effects (Wada et al., 1998). In other studies, it has had an adverse effect (Sinz et al., 1999). Mice that are deficient in iNOS have a worse outcome after TBI than wild-type mice (Sinz et al., 1999). The present study would be more consistent with these latter findings. No adverse effect of L-arginine was observed even when it was administered as late as 48 h after injury.

Summary. L-Arginine administration has beneficial effects on CBF at the impact site. The dose of L-arginine that has the best neuroprotective effect is 300 mg/kg. The neuroprotective effect is also time-dependent, with the best results occurring with administration of the L-arginine as soon after the injury as possible. No adverse effects were observed with the administration of L-arginine.

References


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