L-Arginine Improves Diminished Cerebral CO₂ Reactivity in Patients

C. Zimmermann, MD; R.L. Haberl, MD

Background and Purpose—There is experimental evidence that L-arginine restores diminished CO₂ reactivity after mild traumatic brain injury in rats. This effect is believed to be mediated by L-arginine–derived nitric oxide, which is a permissive substrate for CO₂ reactivity. To clarify whether these findings can be transferred to the clinical situation and have beneficial effects in patients, we studied the effects of L-arginine on CO₂ reactivity of the cerebral vessels in patients with impaired vasomotor reactivity (VMR) and compared them with patients with normal VMR.

Methods—Twenty-two patients with cardiovascular risk factors and VMR <50% with no extracranial or intracranial stenoses were examined by bilateral transcranial Doppler sonography of the right and left middle cerebral arteries and compared with 20 age- and risk-matched patients with normal VMR (>50%). VMR was tested by 1-minute hyperventilation, followed by a 3-minute inhalation of 5% CO₂. Examinations were performed before and after infusion of 30 g L-arginine over 30 minutes. The 22 patients with reduced VMR (<50%) were compared with 20 patients with normal VMR (>50%).

Results—Initial mean VMR of the 42 patients was 50±12%. There was no difference between the right- and the left-side VMR. In the 22 patients with reduced VMR in the first examination (42±8%), VMR increased significantly after infusion of L-arginine (52±14%, P=0.005). In contrast, values did not change after infusion of L-arginine in the 20 patients with normal VMR (59±8% before versus 59±13% after L-arginine). There was a negative correlation of initial CO₂ vasoreactivity and the percentage of VMR increase after infusion of L-arginine.

Conclusions—Our data support the hypothesis that in humans L-arginine is able to improve impaired CO₂ reactivity of the cerebral vessels. This effect can be found in patients at cardiovascular risk with impaired VMR and might have therapeutic implications in the future. (Stroke. 2003;34:643-647.)

Key Words: arginine ■ risk ■ vasomotor reactivity

Cerebral blood flow is thought to be regulated by 2 major structural elements: endothelium and vascular smooth muscle cells. Resting cerebrovascular blood flow is dependent mainly on endothelial function and is believed to be influenced by nitric oxide (NO) availability. NO is known to be the most effective constituent vasodilating agent and is responsible for normal vascular tone. In addition to NO, changes in PCO₂ and pH are fundamental mechanisms in inducing vasodilation or vasoconstriction of the cerebral vessels and are involved in maintaining adequate cerebral blood flow.

Experimental data suggest that CO₂ reactivity is depressed shortly after mild brain injury but can return to normal levels over time. In humans, CO₂ reactivity is known to be depressed in patients with lacunar syndromes and cerebral small-vessel disease. Golding et al recently published the experimental finding that L-arginine restores diminished CO₂ reactivity in adult rats after mild traumatic brain injury. Beneficial effects of L-arginine are attributed to the fact that it is the substrate for endothelial NO synthase, which increases local NO production and might be involved in scavenging free radicals.

In patients, improvement in cerebral vasomotor reactivity (VMR) was found after intake of pravastatin, an HMG CoA inhibitor leading to upregulation of endothelial NO synthase.

Although it is known that L-arginine itself can temporarily increase cerebral blood flow, it is not clear whether it affects CO₂ reactivity in humans and can improve impaired CO₂ reactivity in patients. The purpose of the present study was to examine whether L-arginine can restore diminished CO₂ reactivity in patients. Therefore, we studied the effects of L-arginine on VMR in patients with impaired VMR and compared them with patients with normal VMR.

Methods

In total, 42 patients with cardiovascular risk factors such as arterial hypertension, dyslipidemia, diabetes mellitus, smoking, coronary heart disease, and/or history of previous cerebrovascular event were included in the study. Patients were asked for drug intake, history of hypertension, smoking habits, history of coronary heart disease, and previous cerebrovascular events. They did not have any neurological...
deficit and were enrolled only when a transient ischemic attack or minor stroke had occurred >6 months ago. An ECG was recorded to assess left ventricular hypertrophy or other cardiac pathologies. Blood tests were done for cholesterol, low-density and high-density cholesterol, and HbA1c. All patients underwent extracranial Duplex sonography to exclude stenoses or occlusions of extracerebral vessels and were screened for intracranial stenosis by transcranial Doppler (TCD) sonography. The study was approved by the local ethics committee, and all patients gave written, informed consent.

In a subgroup of patients (n=13), cerebral computed tomography (CCT) or MRI scans were available to assess the degree of small-vessel disease. Scans were analyzed by a blinded neurologist to classify the amount of deep white matter hyperintensities according to a white matter lesion rating scale suggested by van Swieten et al.12 Left- and right-hemisphere lesions were graded as 0 for no lesions, 1 for partly hypodense lesions in CCT/multiple focal lesions in T2-weighted MRI, or 2 for lesions reaching the subcortical regions in CT/Multiple confluent lesions in T2-weighted MRI according to the white matter lesion rating scale of van Swieten et al.12 The sum of the rating points was used to compare different patient groups.

VMR was assessed by TCD sonography according to standardized methods described elsewhere.7,13 Examinations were performed in the late morning with patients sitting in a half-supine position under bilateral TCD sonography (Multidop X2, DWL). Probes were fixed with a bandage device. During TCD examination, blood pressure, pulse rate (N-Cat Monitoring Unit, Nellcor), and end-expiratory CO2 (CO2 Analyzer, DWL) were continuously monitored and registered to control patient cooperation and CO2 inhalation. Bilateral TCD curves of the left and right middle cerebral arteries were recorded simultaneously by TCD7 software (DWL) and analyzed offline. Venous blood samples were taken before and after infusion of L-arginine to determine pH.

VMR was assessed by measurement of maximum increase of mean flow velocity (MFV) while the patient breathed a 5% CO2/95% O2 mixture through a handheld mask pressed tightly over the mouth and nose for at least 3 minutes, as well as by measurement of the maximal decrease during hyperventilation for at least 1 minute or until a steady state was reached. Values were calculated as percentage of the maximal rise and fall compared with baseline as described elsewhere.7,11,14

Of the 42 patients, 22 patients had slightly reduced VMR values (<50%), and they were compared with the 20 patients showing normal VMR values (>50%). Reference values for normal VMR were based on reference values suggested by Widder et al indicating a normal VMR of 50±10% and internal reference values for VMR in our laboratory from healthy volunteers showing normal VMR performance >50% (mean±SD, 62±13%). In both patient groups, VMR maneuver was performed twice, once before and once after an infusion of 30 g L-arginine. The second examination was performed about 1 hour after the first examination, 15 minutes after the termination of the intravenous infusion of L-arginine hydrochloride (Fresenius Kabi) dissolved in 500 mL saline (L-arginine infusion velocity, 1 g/min). During the whole examination period, neither patient nor probe position was changed, and VMR was calculated exactly the same way both times.

Results

VMRs of the right and left middle cerebral arteries were similar (51.1±13.6% and 48.4±13.4%, respectively). Twenty-two patients with VMR <50% were compared with 20 age- and risk-matched patients with VMR >50%. Patient data and cardiovascular risk factors for the 2 groups are shown in Table 1.

### Table 1. Patient Characteristics and Vascular Risk Profile of 42 Patients With Vasomotor Reactivity Maneuver Before and After L-Arginine

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Mean±SD</th>
<th>Range</th>
<th>VMR &gt;50%</th>
<th>VMR &lt;50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (male/female)</td>
<td>n=42</td>
<td>n=20 (21/1)</td>
<td>n=22 (14/6)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>63.6±7.4</td>
<td>52–81</td>
<td>61.5±6.9</td>
<td>65.5±7.5</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>138.5±17.4</td>
<td>101–176</td>
<td>137.1±19.4</td>
<td>139.6±15.6</td>
</tr>
<tr>
<td>Arterial hypertension, y</td>
<td>6.9±8.9</td>
<td>0–30</td>
<td>8.0±9.8</td>
<td>6.0±8.1</td>
</tr>
<tr>
<td>HbA1c, % Hb</td>
<td>5.8±0.8</td>
<td>3.8–9.5</td>
<td>6.0±1.0</td>
<td>5.8±0.7</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>203.3±36.0</td>
<td>135–295</td>
<td>205.3±39.1</td>
<td>201.3±33.7</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>153.0±87.8</td>
<td>59–532</td>
<td>142.2±56.3</td>
<td>162.9±109.4</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.0±3.6</td>
<td>16.9–32.6</td>
<td>22.3±3.5</td>
<td>23.7±3.6</td>
</tr>
<tr>
<td>CCHS</td>
<td>13.8±7.3</td>
<td>0–30</td>
<td>12.6±7.4</td>
<td>15.0±7.1</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>0.10±0.02</td>
<td>0.06–0.17</td>
<td>0.09±0.02</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>Previous cardiovascular events (TIA/PRIND/Stroke)</td>
<td>8/8/3</td>
<td>6/3/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease/left ventricular hypertrophy</td>
<td>2/3</td>
<td>4/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking habit</td>
<td>4 yes/18 no</td>
<td>20 no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>11 yes/11 no</td>
<td>11 yes/9 no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiaggregation/antiplatelet therapy</td>
<td>8/5/6</td>
<td>12/3/4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CCHS indicates Copenhagen City Heart Study prospective stroke risk score; a risk evaluation by age, blood pressure, atrial fibrillation, smoking, left ventricular hypertrophy, treatment of hypertension, cardiovascular disease and diabetes mellitus; IMT, intima-media thickness of the dorsal wall of the common carotid artery (CCA) 2 cm below the bifurcation.24
The absolute MFV values at baseline and after L-arginine did not differ between groups (43.2 ± 8.8 and 42.3 ± 10.5 cm/s; Table 2). Forty minutes after the start of L-arginine infusion, the reduced VMR group showed absolute levels of MFV of 52.4 ± 9.9 cm/s (P = 0.02), whereas the MFV in the normal VMR group had increased only to 48.2 ± 12.8 cm/s (P = 0.09). However, at the beginning of the second VMR maneuver, absolute MFV returned to baseline levels in both groups (Table 2).

After the infusion of L-arginine, the reduced VMR group showed significantly improved VMR. In contrast, the normal VMR group did not show any change in VMR after the infusion of L-arginine (the Figure). Improvement in vasoreactivity was due mainly to the improvement in vasodilation in response to CO₂, whereas hyperventilation-mediated vasoconstriction did not show statistically significant differences (Table 3). There was no statistical difference between the 2 groups concerning heart rate, mean arterial blood pressure, venous pH, or absolute MFV of the middle cerebral artery, although arterial blood pressure and venous pH were significantly decreased after the infusion of L-arginine in both groups (Table 2).

There was a weak but statistically significant negative correlation for VMR and the increase in VMR after L-arginine (right side: β = −0.452, P = 0.003; left side: β = −0.406, P = 0.008).

Assessment of a possible association between cerebral microangiopathy and VMR performance was done in a subgroup of patients, including 8 scans (6 MRI and 2 CCT) from patients showing VMR <50% and 5 scans (2 MRI and 3 CCT) from patients showing VMR >50%. White matter lesions were slightly more pronounced in the group of patients with initially reduced VMR [1.60 ± 1.52 (n = 8; median, 1.5) versus 0.88 ± 1.13 (n = 5; median, 0.5)], although the differences were not statistically significant.

### Discussion

The major finding of our study was that diminished CO₂ reactivity of the cerebral vessels in patients with cardiovascular risk factors was restored by infusion of 30 g L-arginine. Similar findings have been described for rats with reduced cerebral CO₂ reactivity after mild cortical injury.⁴,¹⁵ Cerebral microangiopathy is one of the known pathological states leading to disturbed CO₂ reactivity in humans, and CO₂ reactivity may already be impaired in subclinical small-vessel disease.⁵–⁷ Our patients had cardiovascular risk factors associated with the risk of cerebral microangiopathic changes, and most patients had a history of transient ischemic attack or minor stroke several months before the study and did not show any neurological deficit at the time of entering the study. We can only speculate about the reasons for the impaired VMR in some of the patients because we were able to analyze cerebral neuroimaging findings in only a subgroup.

### Table 2. Physiological Variables Before and After Infusion of L-Arginine

| Treatment Effects of L-Arginine Were Seen for Mean Arterial Blood Pressure and Venous pH in Both Groups (Wilcoxon Tests). |
|---|---|---|
| **Patients With VMR>50% Normal VMR Group** | **Patients With VMR<50% Reduced VMR Group** | **Statistical Significance** |
| Mean blood pressure, mm Hg | Baseline | After L-Arginine | Baseline | After L-Arginine | Baseline | After L-Arginine |
| 101.4 ± 13.2 | 94.1 ± 12.1 | 104.3 ± 14.5 | 94.7 ± 15.6 | 0.002 | 0.004 |
| Heart rate, per min | 60.9 ± 10.3 | 61.4 ± 9.4 | 59.4 ± 9.4 | 59.1 ± 9.5 | NS |
| Venous pH | 7.389 ± 0.037 | 7.367 ± 0.037 | 7.381 ± 0.050 | 7.360 ± 0.031 | 0.04 | 0.05 |
| Absolute mean flow velocity of the MCA, cm/s | 43.2 ± 8.8 | 45.5 ± 10.2 | 42.3 ± 10.5 | 43.6 ± 11.2 | NS |

MCA indicates middle cerebral artery. *There was no statistical group effect (Mann Whitney U tests) between the 2 groups.

Treatment effects of L-arginine were seen for mean arterial blood pressure and venous pH in both groups (Wilcoxon tests).

### Table 3. Percent of Decrease of Baseline Mean Flow Velocity (MFV) During Hyperventilation as Well as Increase in Percent Hypercapnia Before and After Infusion of L-Arginine in Patients With Reduced vs Normal VMR

<table>
<thead>
<tr>
<th>HV-Mediated Vasodilation</th>
<th>CO₂-Mediated Vasodilation</th>
<th>VMR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients (n=22) with Reduced VMR&lt;50%</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before L-arginine</td>
<td>14.3 ± 8.0</td>
<td>26.9 ± 8.1</td>
</tr>
<tr>
<td>After L-arginine</td>
<td>17.4 ± 8.7</td>
<td>34.2 ± 12.3</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Patients (n=20) with Normal VMR&gt;50%</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before L-arginine</td>
<td>19.2 ± 7.5</td>
<td>40.1 ± 10.6</td>
</tr>
<tr>
<td>After L-arginine</td>
<td>19.1 ± 6.2</td>
<td>40.2 ± 16.2</td>
</tr>
</tbody>
</table>

HV indicates hyperventilation for at least 1 minute or until a steady state was reached; CO₂ by inhalation of a 5% CO₂/95% O₂ mixture for at least 3 minutes.
of patients. Our major interest in this study was to assess cerebral vasoreactivity. Grading of CCT or MRI deep white matter lesions showed a nonstatistical difference between the 2 groups. Nevertheless, our exploratory analysis is in line with recently published reports,5–7 and extracranial and intracranial stenoses or other pathological changes associated with diminished CO2 reactivity had been excluded by duplex and TCD sonography before study inclusion.

In accordance with previous studies, L-arginine temporarily increased absolute MFV of the middle cerebral artery.10,11 This effect of L-arginine was statistically significant in the group of patients with impaired VMR. Absolute MFV levels had returned to values similar to baseline before the second VMR maneuver.

L-Arginine–induced increases in MFV and cerebral blood flow have been found by PET and TCD studies, and it has been suggested that the beneficial effects of L-arginine are mediated by a combination of providing substrate for endothelial NO synthase and scavenging free radicals.4,10,11 NO is generated by endothelial NO synthase, which cleaves L-arginine into NO and L-citrulline. Proper NO synthase activity and NO bioavailability are known to be essential for the regulation of the resting tone of all vessels.16–18 Arteriosclerosis and cardiovascular disease are associated with a lack of NO; e.g., reduced coronary blood flow velocity can be improved by infusion of L-arginine in hypercholesteremic patients.19 Iadecola20 and Maccario et al21 have found NO to be a permissive factor in cerebral CO2 reactivity, showing that resting NO levels facilitate the action of vasodilators.

The mechanisms of CO2 reactivity have not been elucidated completely. However, it is known that an increase in inspiratory CO2 is followed by an extracellular increase in H+ ions and a decrease in pH and muscular vasodilatation, which are thought to be the major mechanisms of CO2-mediated vasodilatation. Other mechanisms such as NO, prostaglandins, or neurogenic components might also be involved in CO2 reactivity.16,20 The L-arginine–induced improvement in MFV changes during hypercapnia was more pronounced than during hyperventilation. Hypercapnia dilates smaller cerebral arterioles more than larger ones, whereas the hypocapnic vasoconstrictor effect is independent of size.16 L-Arginine might especially improve the “small vessel function,” affecting their ability for vasodilatation.

Endothelial NO synthase activity is dependent on pH in isolated rat hearts.17 We have measured venous pH in the patients before and after administration of L-arginine, finding no difference between the 2 groups. The effects of L-arginine seen in the patients with reduced VMR could not be explained by changes in arterial blood pressure or by variations in heart rate. All parameters were within a normal range, and there were no significant differences between the 2 groups after administration of L-arginine. However, both groups of patients had a significant decrease in mean arterial blood pressure and venous pH after infusion of L-arginine. Both changes have been described and discussed before as typical side effects of L-arginine.22

There was a weak but significant correlation between initial VMR and improvement after L-arginine, showing that the beneficial effects of L-arginine were most pronounced in patients with initially low levels of vasoreactivity. Similar findings have been published from Sterzer et al9 for improvement of VMR by pravastatin.

In summary, this is the first study to show that previous findings from animal studies can be transferred to the clinical situation: L-arginine is able to normalize cerebral VMR and CO2 reactivity in patients with reduced VMR but not in patients with normal VMR. The exact mechanisms involved in the improvement in vasoreactivity have not been fully elucidated, but there is evidence that L-arginine–mediated NO might have a beneficial role in cerebral CO2 reactivity in patients and might have therapeutic implications in the future.23,24

Acknowledgments

This study was supported by a fund from the German Bundesministerium für Bildung und Forschung Kompetenznetz Schlaganfall, project A6. Special thanks are due Dr Wanner, Laboratory of Clinical Chemistry, Krankenhaus München-Haselau, for cooperation and technical analysis of laboratory tests and Dr Andreas Ströhle and Martin Wimmer for critical review of the manuscript.

References

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Stroke. 2003;34:643-647; originally published online February 20, 2003;
doi: 10.1161/01.STR.0000056526.35630.47
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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