Concentration Changes of Malondialdehyde Across the Cerebral Vascular Bed and Shedding of L-Selectin During Carotid Endarterectomy

Markus A. Weigand, MD; Andreas Laipple; Konstanze Plaschke, PhD; Hans-Henning Eckstein, MD; Elke Martin, MD, FANZCA; Hubert J. Bardenheuer, MD

Background and Purpose—Oxidative stress has been postulated to account for delayed neuronal death due to ischemia/reperfusion. We investigated cerebral formation of malondialdehyde as an index of lipid peroxidation in relation to different sources of reactive oxygen species in patients undergoing carotid endarterectomy.

Methods—In 25 patients undergoing carotid endarterectomy, jugular venous–arterial concentration differences of brain metabolites, malondialdehyde, plasma total antioxidant status, and soluble P-selectin and L-selectin were measured. A carotid artery shunt (n=5) was placed only after complete loss of somatosensory evoked potentials, indicating a focal cerebral blood flow <15 mL/min per 100 g.

Results—As an indication of cerebral lipid peroxidation, jugular venous–arterial malondialdehyde concentration differences were significantly enhanced before reperfusion, and an additional rise was observed 15 minutes after reperfusion. Plasma total antioxidant status significantly decreased during carotid artery occlusion only in patients with carotid artery shunt. This decrease was matched by cerebral formation of adenosine, hypoxanthine, and nitrite/nitrate. While jugular venous–arterial concentration differences of soluble P-selectin showed changes similar to those of malondialdehyde, the concentration difference for soluble L-selectin was enhanced exclusively at 15 minutes after reperfusion.

Conclusions—Short-term incomplete cerebral ischemia/reperfusion significantly enhanced cerebral lipid peroxidation, as indicated by malondialdehyde formation. The generation of reactive oxygen species by xanthine oxidase or nitric oxide metabolism might be involved in the induction of lipid peroxidation. The additional rise in cerebral release of malondialdehyde was found to coincide with a significant activation of polymorphonuclear leukocytes across the cerebral circulation. (Stroke. 1999;30:306-311.)

Key Words: adenosine ■ adhesion molecules ■ carotid endarterectomy ■ lipid peroxidation ■ nitric oxide ■ oxygen radicals

Carotid endarterectomy has been proven to reduce the incidence of ipsilateral strokes in patients with symptomatic and asymptomatic carotid stenoses ≥70%.1-3 On average, however, 2% to 6% of all patients undergoing carotid endarterectomy sustain a stroke in the perioperative period.4 Intraoperative embolism and hyperperfusion are possible causes of a perioperative neurological deficit due to clamping of the carotid artery.

Two major hypotheses have been developed to account for the phenomenon of ischemia/reperfusion–induced neuronal death. The neurotransmitter hypothesis is related to the role of excitotoxic amino acids and is preferentially aimed at events during the acute period of ischemia. The free radical hypothesis is directed at events during reperfusion.5 The generation of reactive oxygen species (ROS) initiates a vicious cascade of tissue injury. In particular, ROS lead to peroxidation of phospholipids with consecutive alteration of membrane structure. These events provide a conceptual basis to explain delayed neuronal death after periods of ischemia/reperfusion.6 In animal studies it has been shown that endothelial adhesion of polymorphonuclear leukocytes (PMN), which generate ROS and reactive nitrogen species, significantly contributes to the pathogenesis of reperfusion injury after focal ischemia.7,8

This clinical study investigates the interrelation between cerebral energy metabolism, nitric oxide (NO) metabolism, cellular activation, and cerebral lipid peroxidation as indicated by the formation of malondialdehyde (MDA) in patients undergoing carotid endarterectomy.

Focal cerebral ischemia was induced by acute vascular occlusion of the common carotid artery, and the extent of ischemia was verified by monitoring of somatosensory evoked potentials (SSEP). During carotid surgery a vascular shunt was placed only under conditions when total loss of
SSEP amplitude occurred, indicating a regional cerebral blood flow <15 mL/min per 100 g. Carotid endarterectomy is a relevant clinical model to study focal cerebral ischemia/reperfusion injury in patients.

**Subjects and Methods**

After institutional approval, informed consent was obtained from 25 patients (mean age, 65±2 years; age range, 40 to 82 years) undergoing elective carotid endarterectomy. The group included 6 women and 19 men. In all but 5 patients without prior symptoms, the indication for carotid endarterectomy was symptomatic carotid artery stenosis ≥70%. Thirteen patients took aspirin as antiplatelet medication, whereas none of the patients took antioxidants. After premedication with midazolam (3.75 to 7.5 mg), anesthesia was induced with 1 to 3 mg midazolam, 2 to 5 μg·kg⁻¹ fentanyl, 0.15 to 0.3 mg·kg⁻¹ etomidate, and 0.5 mg·kg⁻¹ atracurium. After induction, anesthesia was maintained with nitrous oxide in oxygen (N₂O:O₂ = 50:50) and 0.2% to 0.6% isoflurane. Atracurium and fentanyl were administered intraoperatively as necessary. All patients were mechanically ventilated to maintain normocapnia with PaCO₂ of 38 to 41 mm Hg. In each patient, ECG, end-tidal capnometry, and arterial blood pressure changes were continuously recorded. A thorough neurological examination was performed immediately after the patient awakened, 1 hour later, and then daily until the patients were discharged.

Intraoperatively, SSEP were continuously recorded after contralateral median nerve stimulation (Nicolet Spirit) to detect critical isoelectricity, and arterial blood pressure changes were continuously recorded. A thorough neurological examination was performed immediately after the patient awakened, 1 hour later, and then daily until the patients were discharged.

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**TABLE 1. Parameters of Hemodynamic Data and Blood Gas Analysis**

<table>
<thead>
<tr>
<th>No-Shunt Group (n=20)</th>
<th>Shunt Group (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time of Occlusion, min</strong></td>
<td><strong>Time of Occlusion, min</strong></td>
</tr>
<tr>
<td>C₁</td>
<td>10</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>95±3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>69±3</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>205±8</td>
</tr>
<tr>
<td>PacO₂, mm Hg</td>
<td>39.4±0.7</td>
</tr>
<tr>
<td>Arterial hemoglobin, mg/dL</td>
<td>10.8±0.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. C₁ indicates before carotid cross-clamping; C₂, 15 minutes after reperfusion; and MAP, mean arterial pressure. There were no significant differences between groups.

**Statistical Analysis**

Results are expressed as mean±SEM. Differences within or between the patient groups were examined by ANOVA followed by Scheffé multiple comparisons. Statistical significance is at the P=0.05 level.

**Results**

In the present study a total loss of SSEP amplitude occurred in 5 patients at 6±1 minutes after carotid artery occlusion. Therefore, intraoperative shunt placement was performed in 5 of 25 patients (shunt group [n=5] versus no-shunt group [n=20], respectively). As can be seen in Table 1, no significant differences between the groups were obtained in hemodynamic parameters, PaO₂, PacO₂, or hemoglobin concentrations throughout the study period. In patients in the shunt group, SSEP amplitude remained depressed at 15 minutes after reperfusion despite reperfusion (Table 2). One patient in the shunt group suffered postoperatively from a transient
TABLE 2. Changes of Jugular Venous–Arterial Concentrations of Lactate, Nucleosides, Nitrite/Nitrate Ratio, and Plasma Total Antioxidant Status

<table>
<thead>
<tr>
<th></th>
<th>No-Shunt Group (n=20)</th>
<th>Shunt Group (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of Occlusion, min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>10</td>
</tr>
<tr>
<td>SSEP, %</td>
<td>93±6</td>
<td>74±6</td>
</tr>
<tr>
<td>ΔLAC, mmol/L</td>
<td>0.04±0.02</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>ΔADO, mmol/L</td>
<td>6±6</td>
<td>36±13</td>
</tr>
<tr>
<td>ΔHYPO, nmol/L</td>
<td>26±88</td>
<td>89±135</td>
</tr>
<tr>
<td>ΔNO2⁵⁻/NO3²⁻, μmol/L</td>
<td>0.34±0.3</td>
<td>1.3±0.4</td>
</tr>
<tr>
<td>ΔTAS, mmol/L</td>
<td>0.00±0.02</td>
<td>−0.01±0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM. C1 indicates before carotid cross-clamping; C2, 15 minutes after reperfusion; and ΔHYPO, jugular venous–arterial concentration difference of hypoxanthine.

*P<0.05 vs C1; †P<0.05, shunt vs no-shunt group.

15 minutes of reperfusion, there was an additional rise of ΔMDA to 291.0±70.9 nmol/L (P<0.05).

Both ΔsP-selectin and jugular venous–arterial concentration difference in Sl-selectin (ΔsL-selectin) exhibited only minor changes throughout the study period in patients with sufficient collateral perfusion (no-shunt group). Despite a significantly shorter period of vessel occlusion in the shunt group, ΔsP-selectin was enhanced in parallel with the changes in MDA during carotid occlusion and reperfusion, respectively. In contrast, ΔsL-selectin exhibited significant changes only at the end of the study period (15 minutes after reperfusion).

Discussion

In patients undergoing carotid endarterectomy, the jugular venous–arterial MDA concentration differences (ΔMDA) were significantly higher in patients in whom a total loss of SSEP amplitude occurred (shunt group). In contrast to patients with adequate collateral blood flow (no-shunt group), increased ΔMDA was obtained during an occlusion period as short as 6±1 minutes and exhibited a 6-fold increase at 15 minutes after start of reperfusion.

ROS such as superoxide anions (O₂⁻), hydrogen peroxides (H₂O₂), and the extremely toxic hydroxyl radical (·OH) are difficult to detect in patients because of their short half-life. Therefore, byproducts of lipid peroxidation or depletion of endogenous antioxidants have often been used as indirect markers for free radical generation.12–14 MDA is a 3-carbon compound, which reflects both auto-oxidation and oxygen radical–mediated peroxidation of polyunsaturated fatty acids, in particular, arachidonic acid.14,15 However, release of MDA is not specific for lipid peroxidation, because other sources of MDA formation have been described. In certain tissues, MDA can also be formed by nonenzymatic or enzymatic processes, for example, by human platelet synthetase.15–17 Nevertheless, the significant increase in ΔMDA in the shunt group together with the decrease in ΔTAS across the cerebral circulation is a strong indication that lipid peroxidation takes place in the cerebral vascular bed even after short periods of incomplete cerebral ischemia. This is even more evident.

neurological deficit. It is important to note that the mean occlusion time in the no-shunt group was 30±2 minutes, which was significantly longer than that in the shunt group (6±1 minutes). In patients in the shunt group, shunt opening was taken as the start of reperfusion.

Under baseline conditions, there was no significant difference in jugular venous–arterial lactate concentration difference (ΔLAC) between the shunt group and the no-shunt group (Table 2). In patients with inadequate collateral blood flow (shunt group), however, ΔLAC was significantly increased during carotid artery occlusion and remained elevated until 15 minutes after reperfusion. Before carotid artery occlusion, no significant difference in jugular venous–arterial adenosine difference (ΔADO) was obtained between the groups. Major concentration changes of adenosine across the cerebral vascular bed were observed during the clamping period, when ΔADO exhibited a peak value of 181±37 nmol/L at 6 minutes after carotid cross-clamping. In contrast to lactate, ΔADO returned to control levels within 15 minutes after reperfusion. In general, similar results were also obtained in the case of hypoxanthine and nitrite/nitrate in the shunt group.

Under baseline conditions, the jugular venous–arterial difference in plasma total antioxidant status (ΔTAS) was higher in patients with inadequate collateral blood flow (shunt group). While ΔTAS remained nearly unchanged in the no-shunt group, carotid artery clamping induced a significant decrease in ΔTAS in the shunt group.

In patients with adequate collateral blood flow (no-shunt group), jugular venous–arterial MDA concentration differences (ΔMDA) remained almost stable throughout the study period (Figure). In patients with inadequate collateral blood flow (shunt group), ΔMDA and jugular venous–arterial concentration difference in sP-selectin (ΔsP-selectin) were significantly different from those in patients with adequate collateral blood flow under control conditions. Furthermore, in the shunt group significant changes in ΔMDA also occurred after cross-clamping of the carotid artery. ΔMDA increased from baseline values (34±26 nmol/L) to 130±49 nmol/L at the end of the occlusion period (6±1 minutes). At
because the obtained changes in ΔTAS are temporally related to the occurrence of cerebral ischemia.14,18,19

Clinically, the occurrence of short-term incomplete cerebral ischemia in the shunt group was verified by total loss of SSEP amplitude after carotid cross-clamping. The electrophysiological changes indicate a local cerebral blood flow, 15 mL/min per 100 g and are associated with a significant impairment of cellular ion homeostasis.20,21 In addition, the significant increases in ΔLAC and ΔADO are further evidence that some degree of cerebral ischemia is present in patients with a shunt during carotid artery occlusion. In particular, adenosine has been characterized as a sensitive indicator of disturbances in tissue oxygenation in several organs, including the brain.22,23 The data indicate that metabolic parameters are altered in close parallelism with the impairment of cerebral function (SSEP) when inadequate collateral blood flow is present in patients undergoing carotid endarterectomy. In patients with shunt, the shunt was placed to restore cerebral perfusion and to avoid neuronal death due to ischemia. Since shunt placement was completed at 6 ± 1 minutes after carotid cross-clamping, it is of particular interest that the changes in cerebral lipid peroxidation were also induced after a relatively short period of focal cerebral ischemia followed by reperfusion.

Enhanced generation of ROS in the posts ischemic reperfusion period induces oxidative damage of proteins and lipids24 and impairs mitochondrial function.25 In animal experiments with 2 hours of middle cerebral artery occlusion, both mitochondrial function and the bioenergetic cellular state were shown to only partially recover in the first hour after reperfusion and to deteriorate again within 2 to 4 hours after reperfusion. Folbergrova´ et al6 have demonstrated that ROS

Changes of jugular venous–arterial concentrations of various parameters in patients with adequate collateral blood flow ([- shunt]) and patients with inadequate collateral blood flow ([+ shunt]). C1 indicates before carotid cross-clamping; C2, 15 minutes after reperfusion. Values are mean±SEM. *P≤0.05 vs C1; #P≤0.05 [+ shunt] vs [- shunt].

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are causally involved in the impairment of cellular energy metabolism because the spin-trapping agent N-tert-butyl-α-phenylnitrone (PBN) improved mitochondrial function and reduced infarct volume. The changes in lactate give additional indirect evidence that impaired cellular energy metabolism occurred under conditions of short-term carotid occlusion. In contrast to adenosine, ∆LAC remained enhanced in patients with shunt at 15 minutes after reperfusion. This ongoing lactate production by the brain could be due to ROS-dependent postischemic inhibition of the pyruvate dehydrogenase complex, which reflects impaired mitochondrial function.26 This hypothesis is further supported by clinical data demonstrating that the electrophysiological function of these patients was still depressed at 15 minutes after reperfusion, as indicated by changes in SSEP.

Few data have been presented concerning oxidant production during cerebral ischemia and reperfusion in patients. While Soong et al19 found an increase in jugular venous MDA only 60 seconds after carotid clamp removal, Bacon et al27 observed a decrease in the antioxidant capacity across the cerebral circulation after declamping of the external and internal carotid arteries. In contrast to our study, focal cerebral ischemia was documented in neither of the mentioned investigations, because a shunt was either generally inserted in all patients19 or none of the subjects required a shunt.13

In the present study the origin of MDA formation in the shunt group is difficult to determine. For instance, brain tissue itself is at particular risk of being injured by oxidant-mediated triggers because tissue contains large iron stores and high levels of polyunsaturated lipids but exhibits only poor antioxidant defenses. In addition, the cerebral vascular endothelium can also be one source for the rise in MDA. Interestingly, ∆MDA in the shunt group was already elevated at the end of the ischemic period. This finding demonstrates that molecular events leading to oxygen radical production not only occur during reperfusion but also during short-term and incomplete tissue ischemia.

Biochemically, a potential source of ROS formation is purine catabolism. During ischemia, accumulation of adenosine and its metabolite hypoxanthine (see Table 2) takes place. While in the normoxic brain hypoxanthine is metabolized by xanthine dehydrogenase to xanthise and ultimately to uric acid, the enzyme xanthine dehydrogenase is converted to xanthine oxidase during ischemia. In contrast to xanthine dehydrogenase, xanthine oxidase instead uses molecular oxygen of the nucleotide radical of NAD⁺ as its electron acceptor, thereby catalyzing the formation of O₂⁻ during reperfusion.5,27 Xia and Zweier28 have demonstrated that the free radical formation via xanthine oxidase is substrate driven. Because adenosine and hypoxanthine accumulate significantly in patients with shunt before reperfusion, the substrate-dependent conversion of hypoxanthine/xanthine to uric acid by xanthine oxidase seems to be an important source for the initial burst of free radical generation. Interestingly, this is coincident with a simultaneous decrease in plasma total antioxidant status.

Another important source of ROS is the metabolism of NO. NO reacts with superoxide to yield the peroxynitrite anion (ONOO⁻), which decomposes to OH⁻.29 Furthermore, the peroxynitrite anion itself is also a highly reactive oxidizing agent that can cause tissue damage.8 Experimental studies have demonstrated that NO mediates glutamate neurotoxicity in primary cortical cultures30 and that inhibition of NO generation can reduce infarct volume induced by transient occlusion of the middle cerebral artery.31

In this clinical study the ratio of nitrite/nitrate was taken as an indirect marker of NO production.9 Interestingly, in this clinical study the ratio of nitrite/nitrate was actually increased in the shunt group. Moreover, these results were well matched with the decrease in plasma total antioxidant status before reperfusion. Therefore, the observed changes can be taken as indirect evidence that NO metabolism might contribute to ROS generation in patients undergoing carotid endarterectomy.

del Zoppo et al32 suggested a pivotal role for PMN in cerebral ischemia. This hypothesis is supported by the finding that antibodies to PMN or adhesion molecules ameliorate infarct volume after transient ischemia in animals.33,34 In addition, Okada et al5 have shown in baboon experiments that P-selectins can be detected on the cerebral endothelium in the early phase of reperfusion after cerebral artery occlusion. Until now, however, no data have been available concerning the kinetics of adhesion molecule expression during short-term ischemia/reperfusion in patients. In this study we measured ∆sP-selectin and ∆sL-selectin to characterize the changes in cerebral expression and shedding of both adhesion molecules.

Similar to the changes in ∆MDA, we found a significant increase in ∆sP-selectin in the shunt group before reperfusion, indicating enhanced expression and shedding of P-selectin. In contrast, at this point ∆sL-selectin was nearly unchanged. P-selectin expression, which is observed within minutes after endothelial activation, increases the number of PMN rolling along the endothelium.36 Rolling brings PMN into close proximity to chemoattractants such as platelet-activating factor, which is also expressed on endothelial cells in response to ROS.37 As a result, strong attachment of PMN to the endothelium occurs. The marked elevation of ∆sL-selectin in the shunt group at 15 minutes after reperfusion provides indirect evidence that activation of PMN is likely to take place within the cerebral vascular bed.

In conclusion, we demonstrate that lipid peroxidation can occur during short-term and incomplete cerebral ischemia/reperfusion in patients undergoing carotid endarterectomy. Although the quantitative role of each compartment cannot be determined as yet, the data demonstrate that the ATP-degradation pathway, NO metabolism, as well as cellular factors such as PMN are likely to contribute to the production of ROS under conditions of cerebral ischemia/reperfusion.

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