Preventive Antibacterial Treatment Improves the General Medical and Neurological Outcome in a Mouse Model of Stroke

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Background and Purpose—Epidemiological studies have demonstrated a high incidence of infections after severe stroke and their prominent role in morbidity and mortality in stroke patients. In a mouse model, it has been shown recently that stroke is coupled with severe and long-lasting immunosuppression, which is responsible for the development of spontaneous systemic infections. Here, we investigated in the same model the effects of preventive antibiotic treatment on survival and functional outcome of experimental stroke.

Methods—Mice were subjected to experimental stroke by occlusion of the middle cerebral artery (MCAO) for 60 minutes. A group of mice received moxifloxacin (6 × 100 mg/kg body weight every 2 hours over 12 hours) either immediately or 12 hours after MCAO. Control animals received the vector only. Behavior, neurological deficit, fever, survival, and body weight were monitored over 14 days. In a subgroup, infarct volume was measured 4 days after MCAO. Microbiological assessment was based on cultures of lung tissue, blood, and feces of animals 3 days after stroke. For a dose-response study, moxifloxacin was given immediately after MCAO in different doses and at different time points.

Results—Microbiological analyses of blood and lung tissue demonstrated high bacterial burden, mainly Escherichia coli, 3 days after stroke. Accordingly, we observed clinical and histological signs of septicemia and pneumonia. Moxifloxacin prevented the development of infections and fever, significantly reduced mortality, and improved neurological outcome.

Conclusions—Preventive antibiotic treatment may be an important new therapeutical approach to improve outcome in patients with severe stroke. (Stroke. 2004;35:2-6.)

Key Words: antibiotics ■ pneumonia ■ stroke ■ mice

Among the complications of acute stroke, the risk of relapsing cerebral ischemia or complicating mass effects resulting from edema is well known.1 However, clinical experience and clinical studies also demonstrate the high incidence of infectious complications after stroke. Among patients with different types of stroke, 16% to 61% develop fever, a clinically significant infection is diagnosed in 21% to 65%, and 10% to 22% of stroke patients acquire pneumonia.2–6 Pneumonia is the most common cause of death in stroke patients.7,8 The incidence of infections among stroke patients is thus significantly higher than the general prevalence of hospital-acquired infections (4% to 9% of all hospitalized patients) and that reported among surgical patients (3%).9 The risk of developing infections is highest within the first 2 days after stroke.6 However, it persists beyond the acute phase, and complicating infections are common also during rehabilitation.10

An immunosuppressive state was postulated as a reason for the increased infectious vulnerability in stroke patients as early as 1974.11 Recently, this hypothesis was reinforced by describing a stroke-induced immunodepression syndrome (SIDS) after experimental stroke in mice.12 SIDS is characterized by a rapid and long-lasting suppression of cellular immune responses. Furthermore, SIDS is invariably accompanied by spontaneous development of pneumonia and septicemia in this model. Importantly, immunological alterations in middle cerebral artery occlusion (MCAO) mice occur before blood or lung bacteria become detectable, demonstrating that a compromised antibacterial defense gives rise to bacterial infections.

On the basis of these findings, we explored the effects of preventive antibacterial therapy on the incidence of infections and outcome in a well-established mouse model of experimental stroke.

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A patent application on preventive antinfective therapy in stroke has been filed at the European Patent Office (PCT/EP03/02246). Patent owner is Charité, Humboldt University Berlin. Patent inventors are Andreas Meisel, Christian Meisel, Konstantin Prass, Elke Halle, Hans-Dieter Volk, and Ulrich Dirnagl.

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Materials and Methods

Animal Model

For all experiments, we used 7-week-old male Sv129/J mice weighing 18 to 22 g. All experiments were performed and quantified in a randomized fashion by investigators (I.V., K.P.) blinded to treatment groups. All surgical procedures were approved by the local authorities. The surgical procedure of MCAO did not exceed 10 minutes and was performed as described elsewhere. Briefly, a monofilament was introduced into the common carotid artery under halothane narcosis, advanced to the origin of the MCA, and left there for 60 minutes until reperfusion. Occlusion and reperfusion were verified by laser Doppler flowmetry (Peri Flux 4001 Master, Perimed). During surgery and MCAO, rectal temperature was maintained between 37°C and 37.5°C with a heating pad. Mice were kept in heated cages for the next 2 hours, and rectal temperature was frequently measured. Animals were then returned to their home cages and allowed free access to food and water. Brains were removed 4 days after reperfusion, snap-frozen, and stored at −70°C. Infarct areas were quantified in 20-μm hematoxylin and eosin-stained cryostat sections with an image analysis system (Sigma Scan Pro, Jandel Scientific). Infarction volume was obtained by numeric analysis of digitized sections. Differences were considered significant at P<0.05.

Statistical Analysis

Data were analyzed with the statistical software SPSS. Differences in group survival and neurological deficit were determined by Fisher’s exact test. Differences in group weight were analyzed with repeated-measures ANOVA with Bonferroni’s correction, with time as a within-subject factor and treatment as a between-subject factor. Differences were considered significant at P<0.05.

Results

The early mortality in MXFX- and diluent-treated animals equally amounted to ~14% to 20% until day 4 after stroke. However, only another 5% of the MXFX-treated animals died, whereas 57% of mice treated with diluent were dead by day 6 (P=0.004, diluent versus MXFX 0 to 10 hours; P=0.008, diluent versus MXFX 12 to 22 hours). No significant difference in survival between the 2 MXFX treatment groups was observed (Figure 1A). All sham-operated animals remained sterile and survived for at least 42 days (n=15, data not shown). Physiological parameters (body weight, mean arterial pressure) were not significantly different between all studied groups (data not shown).

Two hours after ischemia, the rectal temperature of all animals dropped to the temperature of the heated cage (37°C) and normalized (37.5°C) by 4 hours. Administration of MXFX 0 to 10 hours completely prevented the development of fever (Figure 1B). In animals that received MXFX 12 to 22 hours, temperatures rose to 38.5°C at 12 hours but normalized soon after treatment was begun. In contrast, temperatures further increased to 39°C in diluent-treated animals and remained high until 24 hours. Two days after ischemia, however, these animals died, whereas 57% of mice treated with diluent were dead by day 6 (P=0.004, diluent versus MXFX 0 to 10 hours; P=0.008, diluent versus MXFX 12 to 22 hours). No significant difference in survival between the 2 MXFX treatment groups was observed (Figure 1A). All sham-operated animals remained sterile and survived for at least 42 days (n=15, data not shown). Physiological parameters (body weight, mean arterial pressure) were not significantly different between all studied groups (data not shown).

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TABLE 1. Spectrum of Bacteria Found 3 Days After MCAO in Blood, Lung, and Intestine of Mice Treated With Either Moxifloxacin or Diluent

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Lung</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moxifloxacin (MXFX) 0–10 hours</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Moxifloxacin (MXFX) 12–22 hours</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Administration of moxifloxacin (MXFX): 0 to 10 hours MXFX 6 mg/kg body weight; 12 to 22 hours MXFX 6 mg/kg body weight. The numbers in parentheses indicate the proportion of positive results to total number of samples.
developed hypothermia (down to 32°C) as a sign of sepsis. Normalization of rectal temperature occurred only in the surviving 43% of animals between days 5 and 8 (Figure 1B).

The course of body weight was determined as a measure of general stress induced by cerebral ischemia and recovery from it. With no significant difference between the 2 groups, MXFX-treated animals displayed a weight loss of 11±8% (mean±SD) until day 5 and had almost regained their weight (99±7%) by day 14 after ischemia (Figure 2). Diluent-treated animals showed a significantly greater weight loss (25±8% on day 5), and the surviving animals reached only 91±7% of their original weight by day 14 (P<0.001, diluent versus MXFX 0 to 10 hours; P<0.001, diluent versus MXFX 12 to 22 hours).

To assess the effect of preventive antibiotic therapy on neurological outcome, we compared infarct volumes on day 4 and, in a separate group of animals, the neurological deficit measured daily until day 7 after stroke with a modified Bederson score.14 Infarct volumes tended to be smaller in both MXFX treatment groups (Figure 3). However, a statistically significant difference was observed only in the late treatment group (107±11.9 versus 89.6±8.2 mm³, diluent versus MXFX 12 to 22 hours; P<0.05). To test whether MXFX reduces infarct size by preventing bacterial infections or by direct neuroprotective mechanisms, we performed adoptive lymphocyte transfer experiments. Transfer of splenocytes from healthy littermates restored immune function and thereby prevented infections after MCAO, as demonstrated previously.12 After lymphocyte transfer, late treatment with MXFX (12 to 22 hours) did not further reduce infarct volume significantly, although there was a trend toward smaller infarcts in the MXFX-treated group (n=14 each group; 88.5±28.8 versus 74.8±21.8 mm³, medium plus MXFX versus splenocytes plus MXFX; P=0.25, 2-tailed Student’s t test).

With respect to the functional deficit, there was no significant difference between MXFX-treated and diluent-treated animals until day 2 after stroke. However, MXFX-treated animals showed a significantly faster and better functional recovery after day 2 (Figure 4). On day 7 after MCAO, 43% of diluent-treated mice had either fully recovered (score=0) or retained a measurable neurological deficit (score=1) (57% of animals had died). In contrast, ~80% of MXFX-treated animals displayed either no or only a small deficit (score ≤1) on day 7. No significant differences in severity of neurological deficit or functional recovery were observed between the 2 MXFX-treatment groups.

Most bacterial organisms found in blood and lung cultures of diluent-treated mice 3 days after cerebral ischemia were also present in the intestinal flora (Table 1). The most commonly found bacteria were Escherichia coli and Enterococcus faecalis, which are facultative pathogens of the intestinal compartment. No bacterial growth was observed in blood and lung cultures of MXFX-treated animals. Antibacterial treatment did not measurably change the intestinal flora (Table 1).
bacteremia. However, application of at least $3 \times 100$ mg MXFX was necessary to prevent pneumonia.

**Discussion**

Recently, it has been demonstrated in this mouse model (MCAO) that focal cerebral ischemia induces a long-lasting depression of the cell-mediated immunity (monocyte deactivation, lymphopenia, Th1/Th2 shift) associated with spontaneous pneumonia and septicemia. It was shown that an impaired early NK- and T-cell response, particularly reduced IFN-$\gamma$ production, is the critical stroke-induced defect of the antibacterial defense. This immunodeficiency is mainly caused by excessive activation of the sympathetic nervous system. Because sham-operated animals remained sterile, susceptibility to infection resulted from stroke, not from surgical stress. In the present study, we demonstrated that stroke-related infections can be effectively prevented by antibacterial therapy with MXFX both immediately or 12 hours after experimental stroke. Consequently, preventive antibacterial therapy is shown to improve outcome after experimental stroke with a reduction in mortality by 40%. Moreover, in the treated groups, the proportion of animals with only mild or no deficit (80%) was almost twice that of untreated controls (43%).

In addition to their relevance for the improvement of stroke therapies (see below), the results of this study have implications for the currently used experimental stroke models as follows.

First, the commonly observed hyperthermia after experimental stroke is not necessarily a "central fever" caused by stroke-induced tissue damage (eg, in the hypothalamus) as proposed by Li et al. These authors argued that, in a rat model of focal cerebral ischemia, the occurrence of fever after stroke depended on an infarction of the hypothalamus. MCAO in our model also damaged the hypothalamus (data not shown). However, because MXFX prevented infections and fever (Figure 1B and Table 2), we conclude that hyperthermia is caused by infections that develop as a result of the stroke-induced immunodepression rather than by central nervous dysfunction of temperature regulation. We suggest that hypothalamic damage may cause excessive activation of the sympathetic nervous system, subsequently leading to SIDS.

However, our data do not rule out a central fever in other models of stroke and in (noninfected) human stroke patients.

Second, the previously reported high mortality in the MCAO model after day 4 is less likely to be a direct effect of stroke (eg, by brain edema) than a consequence of SIDS and the related severe infections. Interestingly, the time course of the survival rate shows 2 phases. In the first phase (until day 4), 14% to 20% of mice in each group died (Figure 1A). This finding suggests that mortality in this phase is not due to infections but is probably caused by the brain lesion itself. After day 4, however, only another 5% of MXFX-treated mice died, whereas mortality in the untreated group increased dramatically and reached 57% by day 6 (Figure 1A). We conclude that this second phase is due to severe infections. Hitherto, long-term studies (eg, on neuronal plasticity and regeneration) in this stroke model were hampered by a generally high mortality. Thus, preventive antibacterial approaches may facilitate such studies. Furthermore, infections have to be con-

**TABLE 2. Effect of Several Dose Regimens of Moxifloxacin on Bacterial Counts in Lung and Blood**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Survivors After</th>
<th>Lung, CFU/mL</th>
<th>Blood, CFU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Hours</td>
<td>48 Hours</td>
<td>72 Hours</td>
</tr>
<tr>
<td>10 mg 3×†</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>30 mg 1×</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>30 mg 3×†</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>100 mg 1×</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>100 mg 3×†</td>
<td>5</td>
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</table>

*Administration of moxifloxacin (mg/kg body weight) was started immediately after MCAO. Data are given as mean of colony-forming units (CFU) per milliliter blood or lung homogenate. n=5 in each group.
†Every 2 hours.
considered in ischemic stroke because varying degrees of infection may lead to significant variability in outcomes.

To date, all therapeutic efforts in the treatment of ischemic stroke have aimed at preventing further tissue loss beyond the initial core of cerebral ischemia, i.e., damage due to secondary pathological mechanisms in the penumbra. Despite promising experimental results, several pharmacological approaches to secondary neuroprotection failed to be convincing in clinical trials. Thus, for most patients, besides thrombolysis and immediate secondary stroke prophylaxis, the acute therapy of ischemic stroke remains limited to general measures such as the maintenance of a supranormal blood pressure to ensure sufficient cerebrovascular perfusion and adjustment of blood glucose levels and rectal temperature.\(^{18}\) Beyond that and apart from rehabilitation, the bulk of stroke therapy consists of the management or prevention of complications,\(^{19}\) among which infections are most prominent.\(^{20}\) Regarding the established risk factors for deterioration of outcome during the acute phase of ischemic stroke,\(^{21,22}\) infections influence at least 2, by raising the rectal temperature and lowering the blood pressure. Additional harmful mechanisms like the systemic release of proinflammatory cytokines (eg, tumor necrosis factor, interleukin-1), activation of procoagulant and inhibition of anticoagulant pathways, or disturbances in the cerebral microvasculature have to be suspected.\(^{23}\) Although our data suggest that prevention of bacterial infections leads to neuroprotection, MXFX may have other, more direct mechanisms of action (eg, antiexcitotoxic, antiinflammatory) apart from its antibacterial effects, as described for other antibiotics like minocycline.\(^{24}\)

Infectious complications, particularly pneumonia, are associated with worse functional outcome and increased mortality in stroke patients.\(^{8,25}\) Aspiration caused by dysphagia, frequently observed after severe strokes, is a known risk factor for pneumonia. However, because aspiration alone is not sufficient for the development of pneumonia, other predisposing factors such as an impaired immune response have been postulated.\(^{26,27}\) Indeed, a stroke-induced defect of the antibacterial immune defense has been demonstrated in experimental stroke.\(^{12}\) In stroke patients, the rate of infectious complications is proportional to the severity of the initial ischemic deficit.\(^{4,5,8,25}\) With respect to stroke size, our model equals large infarctions in the MCA territory, a subtype with the highest incidence of pneumonia in patients.\(^{25}\) Our results are therefore likely to be relevant especially for this subtype of ischemic stroke.

The prevention of complicating infections in our stroke model appears to salvage tissue at risk and to improve survival and functional outcome after stroke. We believe that our findings warrant clinical studies of preventive antibacterial therapy regimens in stroke patients.

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