Effects of minocycline alone and in combination with mild hypothermia in embolic stroke

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Abstract

Inflammatory reactions occurring in the brain after ischemia may contribute to secondary damage. In the present study effects of minocycline, an anti-inflammatory agent, alone or in combination with mild hypothermia on focal embolic cerebral ischemia have been examined. Administration of minocycline alone or minocycline plus mild hypothermia reduced the infarction volume significantly. However, mild hypothermia in combination with minocycline did not show any additive effect. These results suggest that minocycline is beneficial in ischemic brain injury, and the lack of the enhanced neuroprotection may reflect the brief exposure to hypothermia.

Key words: cerebral, infarction, inflammatory, ischemia, occlusion, rat

Theme: Disorders of the nervous system

Topic: ischemia
Background

Inflammatory reactions occur within the brain after ischemic injury and these may contribute to secondary cerebral damage. Because inflammation reactions occur rapidly and persists for at least a few days after ischemic brain injury [1, 3], these secondary responses are potential targets for human therapy with a sufficiently wide therapeutic window. Minocycline is a semisynthetic second-generation drug of tetracycline and it exerts anti-inflammatory effects that are completely separate and distinct from its antimicrobial action [5]. Minocycline is used clinically for treatment of severe inflammatory diseases and rheumatoid arthritis [5]. Recent studies have shown that minocycline reduces the loss of hippocampal pyramidal neurons when used in a gerbil model of global ischemia [11]. In a rat model of ischemia induced by occlusion of the middle cerebral artery (MCA) with a suture, minocycline is also an effective neuroprotective agent [12]. Mild hypothermia induced before and after ischemic brain injury reduces the structural, metabolic and behavioral changes due to ischemia [2, 6, 7]. In a repetitive forebrain ischemia, we have demonstrated that hypothermia protects neurons from damage [6]. We have also shown the effect of hypothermia can be enhanced by combination with an N-methyl-D-aspartate receptor blocker, CGS-19755 [7]. However, effects of minocycline and hypothermia on the embolic model of stroke have not been examined. In the present study, we studied whether: 1) minocycline is neuroprotective in a focal embolic model of cerebral ischemia; 2) mild hypothermia after ischemic injury is neuroprotective in a focal embolic model of cerebral ischemia; 3) combination of minocycline and mild hypothermia is superior to either minocycline or hypothermia alone.

Results

Embolizing a pre-formed thrombus resulted in an infarction in the territory irrigated by the MCA, mainly located in the cerebral cortex and striatum. In the control group that received the saline injection, the infarction volume was 32±3.2% (mean±SEM) at 48 h after embolization (Fig. 1). In the second group, administration of minocycline alone reduced the infarction volume by 42%, which was significantly smaller than in the control group (P<0.05). In the third group, administration of minocycline plus
hypothermia resulted in the infarction volume being reduced by 44% which was also significantly smaller than in the control group (P<0.05). However, the infarction volume was not significantly different between the groups that received minocycline alone and minocycline plus hypothermia. Compared to the control group, infarction volume was reduced by 14% in the ischemic animals that received hypothermia alone. This was not a significant difference.

The mortality rates (Table 1) were three out of ten rats (30%, one died within 24 h and two within 36 h after embolization) in the control group; one out of nine rats (11%, within 36 h) in the second group (minocycline alone). There was no significant difference in mortality rates between the two groups. In the third group (minocycline and hypothermia) and the fourth group (hypothermia alone), all rats reached the end of the experiments.

Discussion

In the present study, results show that minocycline, injected after embolization, was neuroprotective since the data showed that infarction volume was decreased significantly following minocycline treatment. The infarction volume was also reduced significantly in animals that received treatment of minocycline in combination with hypothermia. The reduction in infarction volume was however the same in these two groups, i.e. minocycline alone and minocycline plus hypothermia. Mild hypothermia instituted 1 h after ischemia with a period of two hours did not reduce infarction volume significantly. However, hypothermia alone or in combination with minocycline decreased mortality. There was also a trend that minocycline treatment alone decreased mortality.

The present study demonstrates that minocycline treatment benefits focal ischemia in an embolic stroke model in rats. There are several possible mechanisms for its beneficial actions. First, minocycline may reduce the infarction by inhibiting inflammatory reactions. Minocycline has been used as a anti-inflammatory agent in many diseases [5] and, recently, it has been shown that minocycline prevents microglia activation in the injured brain [11]. Minocycline also inhibits production of other inflammation
mediators, for example, prostaglandin (PG) E$_2$ [12]. Second, minocycline affects cortical spreading depression, which reflects transient depolarization of astrocytes and neurons and contributes to the evolution of infarction due to ischemia [12]. Additionally, minocycline may inhibit matrix metalloproteinases (MMP) in neutrophils. MMP may worsen ischemic damage by increasing permeability of the brain blood barrier [12].

Previously, we have shown that mild hypothermia is neuroprotective in a global model of ischemic brain injury when introduced during the induction of ischemia [6]. In the present study, mild hypothermia was instituted 1 h after ischemic injury, since this will be more clinically relevant. Data showed that mild hypothermia started 1 h after ischemia, with duration of two hours, did not significantly reduce infarction size. Hypothermia, in combination with minocycline, reduced infarction volume but it did not further improve stroke outcome when using minocycline alone. This may indicate that the neuroprotective action of mild hypothermia diminishes when started with a delay. These data may also imply that prolonged hypothermia may be required to achieve a neuroprotective effect in this model. Despite the fact that there was no reduction of infarction volume, hypothermia, either alone or in combination with minocycline, reduced mortality in the ischemic animals. One possible reason for this is that hypothermia reduce edema and intracranial pressure, which are major players in mortality in ischemic brain injury [2, 4].

**Conclusions**

The present study has demonstrated that treatment with minocycline alone or in combination with hypothermia reduced infarction volume in focal embolic brain injury. Minocycline has been used clinically for treatment of patients with inflammatory diseases and rheumatoid arthritis [5]. It has minimal side effects and is well tolerated in the patients. Minocycline, therefore, could potentially be a useful drug for treatment of stroke patients.
Materials and Methods

Male Wistar rats, weighing 300-350 g, were used. Embolic focal cerebral ischemia was induced by embolizing pre-formed clot into the MCA [8, 9]. Hypothermia was instituted by spraying 100% alcohol on the body of the rat while a fan circulated room air around the animal [6, 7]. The rectal temperature was kept at 34-35°C during the hypothermia period. Hypothermia was started at 1 h after embolization and hypothermia lasted for a period of two hours. At 48 h after embolization infarction volume was measured in the 2,3,5-triphenyltetrazolium chloride (TTC) stained brain sections [18]. The infarction volume was expressed as a percentage of the total volume from the ipsilateral hemisphere. The study consisted of four groups. In the control group (n=10), saline was injected. In the second group (n=9), minocycline (Sigma) was administered 1 and 4 h after embolization on the first day, 45 mg/kg body weight i.p., and 24 and 32 h on the second day, 22.5 mg/kg. In the third group (n=9), minocycline was administered the same as in the second group, but hypothermia was also instituted. In the fourth group (n=9), animals received hypothermia treatment alone. Minocycline was dissolved in distilled water and injection volume was 2 ml/per rat. The dose of minocycline used in the study was based on previous works that showed it effectively reduces inflammation and protected against focal cerebral ischemia in the ischemic brain [11, 12].
References


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**Figure legend**

**Figure 1. Effects of minocycline alone or in combination with hypothermia on infarction volume in the ipsilateral hemisphere following embolizing a pre-formed clot into the MCA.**

Infarction volume was measured at 48 h after embolization in TTC stained brain sections. Number of rats in each group is indicated in the parenthesis above each bar. Bar represents mean±SEM and * denotes significant difference from the saline group (p<0.05). Mino: minocycline; hypo: hypothermia.

**Table legend**

**Table 1. Mortality in different groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Saline</th>
<th>Mino</th>
<th>Mino+Hypo</th>
<th>Hypo</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>24h</td>
<td>1</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>36h</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3 (20%)</td>
<td>1(11%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*Mortality was recorded at 24 and 36 h after embolization. No death occurred later than 36 h after embolization. Mino.: minocycline; Hypo: hypothermia*
Fig. 1

% of infarction volume

saline  mino  mino + hypo  hypo

(10)  (9)  (9)  (9)