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Research article

Delayed minocycline but not delayed mild hypothermia protects against embolic stroke

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Abstract

Background: 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.

Methods: Focal ischemic injury was induced by embolizing a preformed clot into the middle cerebral artery (MCA). Infarction volume was measured at 48 h after the injury. Mortality was also recorded.

Results: Delayed administration of minocycline alone or delayed minocycline plus delayed mild hypothermia reduced the infarction volume significantly. However, delayed mild hypothermia alone was not protective and delayed mild hypothermia in combination with minocycline did not show any additive effect.

Conclusions: These results suggest that minocycline is beneficial in focal ischemic brain injury, and the lack of the enhanced neuroprotection may be due to the brief exposure to hypothermia.

Background

Inflammatory reactions occur within the brain after ischemic injury and these may contribute to secondary cerebral damage. Because inflammation reactions occur rapidly and persist for at least a few days after ischemic brain injury [2,7,11], these secondary responses are potential targets for human therapy with a sufficiently wide therapeutic window. Minocycline is a semisynthetic second-generation drug of tetracycline group and it exerts anti-inflammatory effects that are completely separate and distinct from its antimicrobial action [12]. Minocycline is used clinically for treatment of severe inflammatory diseases and rheumatoid arthritis [12]. Recent studies have shown that minocycline reduces the loss of hippocampal pyramidal neurons when used in a gerbil model of global ischemia [20]. 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 [21]. Mild hypothermia induced before and after ischemic brain injury reduces the structural, metabolic and behavioral changes due to ischemia [4,14,15]. In global ischemic injury model of repetitive forebrain ischemia, we have demonstrated that mild hypothermia protects neurons from damage [14]. We and other group have also shown that the protective effect of mild hypothermia can be enhanced by combination with other neuroprotective agents in global ischemic injury model [3,5,15]. Mild hypothermia in combination with anti-in-

flammatory treatment provides long-lasting neuroprotection of CA1 hippocampus following transient global ischemia [3,5]. Combination of mild hypothermia and drug therapy is also effective treatment for transient focal cerebral ischemia [13]. 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 minocyline or hypothermia alone.

Methods

Male Wistar rats, weighing 300–350 g, were used. Embolic focal cerebral ischemia was induced by embolizing preformed clot into the MCA [17,18]. The rectal temperature of each rat was kept at 37°C throughout the surgery procedure with a feedback controlled heating system. In hypothermia groups, temperature was instituted by spraying 100% alcohol on the body of the rat while a fan circulated room air around the animal, detailed previously [14,15]. The rectal temperature was kept at 34–35°C during the hypothermia period. A mild degree of hypothermia was chosen since it protects neurons from damage in both focal and global ischemic injury [3,5,9,10,14]. We also found that the mild hypothermia possessed an additive effect to the protective actions of anti-glutamate agent in a global ischemic injury model [15]. Furthermore, study has shown a high correlation between brain and rectal temperatures in focal ischemic brain injury model, with brain temperature being higher than rectal temperature by 0.2 to 0.7°C [9].

The procedures for assessment of infarct volume have been reported previously [19]. In brief, at the end of each experiment (48 h after embolization) the brain was removed and sectioned coronally with 2.0 mm of thickness per section. The sections were stained with 2,3,5-triphenyltetrazolium chloride (TTC) solution and scanned with a flatbed color scanner (Scanjet 4p, Hewlett-Packard). The images were analyzed using Adobe Photoshop. The total volume of infarction was determined by integration of the areas from these sections. 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 in the same fashion as in the second group, but hypothermia was also instituted. In the fourth group (n = 9), animals received hypothermia treatment alone. In the present study, hypothermia was started at 1 h after embolization and hypothermia lasted for a period of two hours. 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 protects against focal cerebral ischemia in the ischemic brain [20,21].

The differences of infarction volume were analyzed with one way ANOVA followed by Tukey test. The rates of mortality following different treatments were compared with Chi-square test. Differences were considered significant when $p < 0.05$.

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 was 32 ± 3.2% of the volume from the ipsilateral hemisphere (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 from the control group.

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 (minocylcine alone). There was no significant difference in mortality rates between those two groups (Chi-square; $P > 0.05$). In the third group (minocycline and hypothermia) and the fourth group (hypothermia alone), all rats reached the end of the experiments. However, when the data were analyzed with Chi-square test, the difference in mortality between the control group and the third or fourth group was not significant ($P > 0.05$).

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 vol-

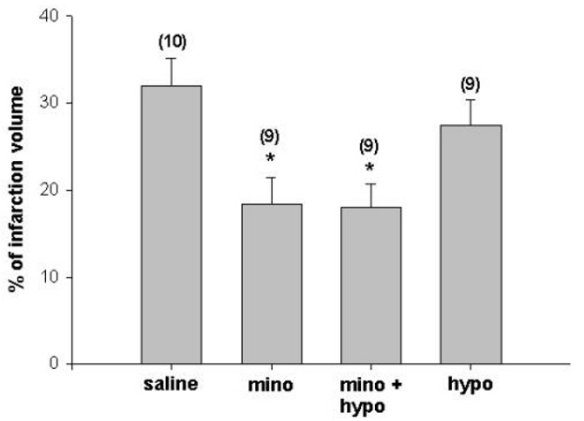

Figure 1
Effects of minocycline alone or in combination with hypothermia on percentage of the total volume from the ipsilateral hemisphere following embolizing a pre-formed clot into the MCA. Infarction volume was measured at 48 h after embolization in TTC stained brain sections. Number of rats in each group is indicated in the parenthesis above each bar. Bar represents mean ± SEM and * denotes significant difference from the saline group ($p < 0.05$). The differences of infarction volume were analyzed with one way ANOVA followed by Tukey test. Mino: minocycline; hypo: hypothermia.

Table 1: Mortality in different groups[a]

Group	Saline (n = 10)	Mino (n = 9)	Mino+hypo (n = 9)	Hypo (n = 9)
24 h	1	0	0	0
36 h	2	1	0	0
Total	3 (20%)	1 (11%)	0 (0%)	0 (0%)

[a]Mortality was recorded at 24 and 36 h after embolization. No death occurred later than 36 h after embolization. Mino.: minocycline; Hypo: hypothermia

ume 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. Interestingly, no rat died prematurely in the groups treated with hypothermia alone or in combination with minocycline.

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 and, recently, it has been shown that minocycline prevents microglia activation in the injured brain [12,16,20]. Minocycline also inhibits production of other inflammation mediators, for example, prostaglandin (PG) E_2[21]. Second, minocycline may inhibit the activity of matrix metalloproteinases (MMP) and, MMP have been found to worsen ischemic damage by increasing permeability of the brain blood barrier [21]. Additionally, the protective actions of minocycline may also arise through other mechanisms such as, inhibition of caspase, inducible nitric oxide synthase (iNOS) and p38 mitogen-activated protein kinase (MAPK) [6,8,16].

Studies have shown that mild or moderate hypothermia is protective in models of transient or permanent MCA occlusion [1,9,10]. Further, mild hypothermia in combination with drug therapy is more effective treatment than hypothermia alone in transient cerebral ischemia [5,13]. Previously, our studies showed that mild hypothermia is neuroprotective in global model of ischemic brain injury when hypothermia was introduced during the induction of ischemia [14]. 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 a 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 than using minocyline alone. Since this is the first study in which the neuroprotecive effects of hypothermia were studied in a focal embolic stroke model, the reason why delayed mild hypothermia is not neuroprotective is not clear. One reason can be that hypothermia may protect ischemic brain injury more effectively in one model than another, since the pathophysiology in different models is not identical. For example, in the model used in the present study fragments of the clots injected moved downstream and occluded the downstream microcirculation after the clots in the larger arteries, the MCA and ICA, are dissolved [18]. This is in contrast to the focal ischemic injury model induced by insertion of a suture into the ICA in which there will be no downstream movement of clots. Secondly, the lack of neuroprotection of mild hypothermia in the present study may also be due to the delay in start of the treatment. These data may also imply that prolonged hypothermia may be required to achieve neuroprotective effects in this model. Despite the fact that there was no reduction in infarction volume, hypothermia, either alone or in combination with minocy-

cline, tended to reduce mortality in the ischemic animals. No death occurred prematurely in the rats that received treatment of either hypothermia alone or in combination with minocycline, although statistical analysis did not show significant difference when compared to the control group. The lack of significant difference is probably due to small sample size in this study.

Conclusions

The present study has demonstrated that treatment with minocycline alone or in combination with hypothermia reduces infarction volume in focal embolic brain injury. Minocycline has been used clinically for treatment of patients with inflammatory diseases and rheumatoid arthritis [12]. 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.

Competing Interests

None declared.

Author's Contributions

Wang, CX participated in the design of the study, carried out statistical analysis and drafted the manuscript.

Yang, T carried out the embolization procedures, treatment administration, and participated in infarct volume assessment.

Noor, R participated in the infarct volume calculations and assisted in preparation of the manuscript.

Shuaib, A conceived of the study and participated in its design and coordination.

All authors have read and approved the final manuscript.

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