Pre-Ischemic Treatment of Pentoxifylline Reduces Infarct Volumes in Transient Focal Cerebral Ischemia in the Rat

A.A. Nekooeian¹, A. Vakili², G.A. Dehghani²,³

Departments of Pharmacology¹, Physiology² and Medicinal & Natural Products Chemistry Research Center³, Shiraz University of Medical Sciences, Shiraz, Iran.

Correspondence: Gholam A Dehghani PhD, Department of physiology, Shiraz University of Medical Sciences, Shiraz, Iran. Tel/Fax: +98 711 2302026 E-mail: dehghang@sums.ac.ir

Abstract

Background: Pentoxifylline (PTX) is used in human for intermittent claudication and cerebral vascular disorders including cerebrovascular dementia. It also inhibits the synthesis of tumor necrosis factor-α (TNF-α), which is believed to be neurotoxic in animal models of cerebral ischemia. The objective of this study was to examine the role of PTX on ischemia/reperfusion injures in rat model of transient focal cerebral ischemia induced by middle cerebral artery occlusion (MCAO).

Methods: Male Sprague Dawley rats (n=31) were assigned to sham, saline or PTX (30 or 60 mg/kg)-treated groups. Ischemia was induced by MCAO, followed by 24-hrs reperfusion. Intraperitoneal saline or PTX was administered at 30 min before ischemia. Neurological deficit score test (NDS) was performed after 24-hrs, and the animals was sacrificed for evaluation of cortical and striatal infarct volumes using triphenyltetrazolium chloride staining.

Results: The sham group did not have neural dysfunction or cerebral infarction. Cortical infarct volumes in 30 or 60 mg/kg PTX-treated groups, 149±12 and 129±19 mm³ respectively, were significantly lower than that of saline-treated group (208±12 mm³). Similar results were also obtained about the striatal infarct volumes (39±5 and 40±6 vs. 58±5 mm³). However, there was no significant difference among the neurological dysfunctions from saline and PTX-treated rats.

Conclusion: the results of this study indicate that pentoxifylline reduced cerebral infarctions, possibly by diminishing the TNF-α-induced neurotoxicity in transient focal cerebral ischemia. This finding also suggests that pentoxifylline might be suitable for clinical trials.

Keywords ● Pentoxifylline ● cerebral ischemia ● TNF-α ● infarction

Introduction

Tumor necrosis factor-alpha (TNF-α) is one of the cytokines released early in cerebral ischemia.¹ ² Although not universally agreed upon,³⁻⁵ previous studies adopting various approaches including using anti-TNF-α antibody,⁶⁻⁷ TNF-α binding proteins,⁶⁻⁸ or its exogenous application,⁵ indicated a neurotoxic role for TNF-α in cerebral ischemic injuries.
The detrimental effects of TNF-α in cerebral ischemia have been attributed to a number of mechanisms, namely increasing the blood-brain barrier permeability,10 inducing the pial artery constriction,11 stimulating the production of matrix-degrading metalloproteinase,11 increasing leukocyte adhesion to brain vessels,12 and direct toxic effects on brain capillaries.13

Pentoxifylline (PTX), a methylxanthine, is widely used for the treatment of intermittent claudication,17 as well as peripheral vascular and cerebrovascular disorders.15-16 PTX inhibits gene transcription and synthesis of TNF-α in mononuclear phagocytes and microglia.17,18 Moreover, PTX was shown to act as neuroprotective in brain trauma,18 global cerebral ischemia and hypoxic-ischemic brain injuries in the rat.19-20 In this study, we have investigated the effects of pretreated pentoxifylline on cerebral infarction during ischemia-reperfusion injury in the rat model of transient focal cerebral ischemia. The findings of this study have also specifies if TNF-α is involved in focal ischemia-related neurotoxicity.

Materials and Methods

Experiments were performed in conformity with the university research council guidelines for conducting animal studies. Male Sprague Dawley rats (300-370 g) were obtained from Razi institute (Shiraz, Iran) and housed in standard cages in room temperature (22-24°C), humidity (40-60%), and light (07.00-19.00 hr) -controlled environment. They had free access to water and food pellets until the night before the experiment which they were refrained from food.

Instrumentation and surgical procedures

Animals were anesthetized with chloral hydrate (400 mg/kg, ip). The right femoral artery was cannulated for continuous recording of blood pressure and taking blood samples (0.5 ml) for gas analysis. The surgical procedures and induction of cerebral focal ischemia were done using previously a published method.21 Under an operation microscope right common carotid artery (CCA) and external carotid artery (ECA) were exposed. The internal carotid artery (ICA) was also dissected to the level petrigpalatine artery (PA). A silk thread was placed loosely around the ECA stump, and CCA and ICA were occluded temporarily, using a microvascular clip and through a small incision, a Poly-L-Lysine-coated nylon thread (3-0) was inserted through ECA.21 Body temperature was maintained at 37±0.5°C throughout the experiment using rectal thermometer and heating lamp.

Experimental design

Rats (n=31) were randomly assigned to four groups. The first group was sham-operated rats (n=7) subjected to all surgical procedures except the induction of middle cerebral artery occlusion (MCAO). The second group (n=9) was subjected to 90 min MCAO, starting 30 min after ip injection of normal saline (1 ml/kg body weight) as a vehicle for PTX. The third (n=8) and fourth (n=7) groups were subjected to 90 min MCAO at 30 min after ip injections of 30 or 60 mg/kg PTX (Sigma, Germany).

Experimental protocol

After surgery and instrumentation, animals were allowed to recuperate for 30 min. After which, they received ip injections of saline or PTX. Then arterial blood sample was withdrawn for the measurement of blood gases and pH at 10 min before MCAO. Afterwards, ischemia was induced by inserting a nylon thread and occluding MCA. Ninety min later, the thread was gently removed, and 24-hrs reperfusion started.21 A second blood sample was withdrawn at 10 minutes after the onset of reperfusion. Finally, the incisions were sutured and the animal was kept in warm cages to recover from anesthesia.

After 24-hrs reperfusion, neurological deficit score (NDS) test was performed using five-points scoring system as described previously.21,22 Then animals was sacrificed, using an overdose of the anaesthetic, and the brain was removed and processed for the determination of infarct volumes using an established method.21

Measurement of cerebral infarct volumes

Rats’ brains were cleaned and immersed in cold saline solutions (4°C) for 5 to 20 minutes. They were then sectioned coronally into six 2-mm thick slices using a brain matrix. Afterwards, brain slices were immersed in 2% Triphenyltetrazolium chloride (TTC, Sigma; Germany) solutions, and kept in a water bath at 37°C for 15 min. The slices were then immersed in 10% buffer formalin (Merck; Germany) solution for 24 hrs. They were then photographed using a digital camera (Cannon, Japan) connected to a computer.

The infarct area of each slice was measured using an Image Analyzer Software (NIH Image Analyzer) and its infarct volume calculated by multiplying its infarct area by its 2-mm thickness. The total infarct volume of each brain then was calculated as the sum of the infarct volumes of all slices. Since brain edema might significantly affect the accuracy of the estimation of infarct volume,25,26 the calculated infarct volumes were corrected for brain edema using the formula proposed by Swanson and colleagues.26
Pentoxifylline reduces brain injuries in transient focal ischemia

Statistical analysis

Data are presented as Mean±SEM. The inter-group comparisons of the infarct volumes and the physiological variables including mean blood pressure, blood gases and blood pH values were performed using one-way analysis of variance (ANOVA). Where a significant difference was found with ANOVA, the source of the difference was located using Duncan’s multiple range tests. The intra-group comparisons of physiological variables were done using paired t-test. Neurological deficit scores were analyzed using Kruskal-Wallis followed by Dunn’s test for pair-wise comparison. The probability of committing type one error (α value) of p<0.05 was considered statistically significant.

Results

There was no significant difference between physiological variables, including mean arterial blood pressure, arterial PCO₂, PO₂ and pH from the groups receiving vehicle or PTX (Table 1).

The sham group did not have cortical or striatal infarct volumes, or neurological dysfunction. Relative to the saline group, PTX at 30 and 60 mg/kg significantly lowered cortical and striatal infarct volumes (Figs 1 and 2). However, there were no significant differences between the neurological deficit scores of saline and PTX-treated groups (Fig 3).

Discussion

The physiological parameters such as MAP, arterial blood gases and pH were within normal physiological range, and were not statistically different either in intra or inter-group comparisons, indicating that they did not affect cerebral injuries. Moreover, as was shown previously, sham operation did not induce cerebral infarction or neurological deficits. This indicates that the observed neurological dysfunctions or observed cerebral infarctions were most likely due to occlusion of middle cerebral artery.

Pre-ischemia treatment with PTX at 30 or 60 mg/kg did reduce cortical and striatal infarct

<table>
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<tr>
<th>Variables</th>
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<th>10 min after termination of MCAO</th>
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<tr>
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<td>MAP (mmHg)</td>
<td>Saline (n=4)</td>
<td>PTX-30 (n=4)</td>
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<td></td>
<td>pH</td>
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<td>PTX-30 (n=4)</td>
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<td>PaO₂</td>
<td>78±7</td>
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Fig 1: Values (mean±SEM) cortical infarct volumes of rats receiving saline or pentoxifylline at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60). * denotes significantly different from saline group (p<0.05)

Fig 2: Values (mean±SEM) striatal infarct volumes of rats receiving saline or pentoxifylline at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60). * denotes significantly different from saline group (p<0.05)

Fig 3: Values (mean ± SEM) of neurological deficit score of rats receiving saline or pentoxifylline at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60).
voluems (Figs 1 and 2). Such a finding is in agreement with earlier reports demonstrating that PTX prevented spontaneous brain ischemia in stroke-prone rats, or its administrations before induction of global cerebral ischemia reduced brain injury in global cerebral ischemia in mature and neonate rats. It is tempting to suggest that the effects of PTX might have been enforced through the prevention of TNF-α synthesis or activity during ischemia/reperfusion injuries. In fact, this speculation receive supports from the study of Barone and colleagues which showed that the injection of exogenous TNF-α exacerbated ischemic injuries in transient or permanent MCAO. Furthermore, it receives support from others indicating that the inhibition of TNF-α, by binding proteins, decreased cortical infarct volume in permanent focal cerebral ischemia in mice. However, the findings of this study do not agree with those studies demonstrating that TNF-α induced ischemic tolerance, or transgenic mice lacking TNF-α receptors were more resistant to ischemic injuries. Such differential findings might be due to variations in animal species, experimental protocols or severity of ischemia.

The neuroprotective effects of PTX have been attributed to the inhibition of TNF-α synthesis. Other effects of PTX such as increased cerebral blood flow, inhibition of neutrophil, activation of monocytes/microglia, attenuation of the release of inflammatory mediators such as platelet-activating factors, and prevention of endothelial-leukocyte adhesion, should also be considered.

Despite reducing cortical and striatal infarct volumes, PTX did not improve neurological deficits. However, it was previously shown that administration of PTX, along with the reduced levels of TNF-α, improved neurological function outcomes in brain trauma, and global ischemia. Although, techniques employed in the present study could not explain such a finding, it might be possible to speculate that neurons controlling the action scored in neurological test are located in the ischemic core, which suffered irreversible damages.

Conclusion

The findings of the present study indicate that pentoxifylline might have a neuroprotective role in cerebral ischemia/reperfusion injuries, possibly by reducing the activity of TNF-α, which is believed to have a role in injuries following cerebral ischemia in rat. It also suggests that pentoxifylline might be suitable for clinical trials in cerebral ischemia.

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Reference


Dr. Vakili’s present address is Department of Physiology, Semnan University of Medical Sciences, Semnan, Iran