IJMS
Vol 30, No 4, December 2005

Original Article

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

A.A. Nekooeian[1], A. Vakili[2], G.A. Dehghani[2,3]

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 mm^3 respectively, were significantly lower than that of saline-treated group (208 ±12 mm^3). Similar results were also obtained about the striatal infarct volumes (39±5 and 40±6 vs. 58±5 mm^3). 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.
Iran J Med Sci 2005; 30(4): 169-173.

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

Departments of Pharmacology[1], Physiology[2] and Medicinal & Natural Products Chemistry Research Center[3], 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

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,[4,8] 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 (22-24ºC), 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 petrigopalatine 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±0.5ºC 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 anesthetic, 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^{o}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^{o}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]

Table 1: Values (Mean±SEM) of arterial pressure (MAP), $PaCO_2$, PaO_2 and pH, in sham and experimental groups receiving normal saline or PTX at doses of 30 mg/kg (PTX-30) or 60 mg/kg (PTX-60) at 30 min before MCAO

Variables (mmHg)	Sham (n=5)	10 min before MCAO			Sham (n=5)	10 min after termination of MCAO		
		Saline (n=4)	PTX-30 (n=4)	PTX-60 (n=4)		Saline (n=4)	PTX-30 (n=4)	PTX-60 (n=4)
MAP	92±5	91±7	92±4	80±4	92±10	96±9	93±9	86±4
pH	7.30±0.02	7.34±0.03	77.31±0.01	7.26±0.03	7.28±0.01	7.33±0.04	7.35±0.03	7.29±0.04
P_aCO2	35±7	36±7	38 ± 6	35 ± 4	38±5	34±8	33±8	36±5
PaO2	78±7	82 ±4	76 ± 7	76 ± 5	85±7	78±4	75±4	77±6

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 PCO_2, PO_2 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).

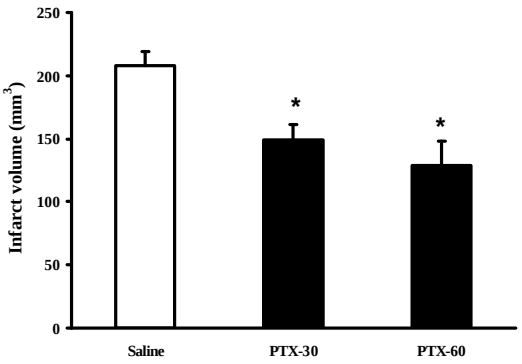

Fig 1: Values (mean±SEM) cortical infarct volumes of rats receiving saline or pentoxifylline at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60). * denotes significantly different from saline group ($p<0.05$)

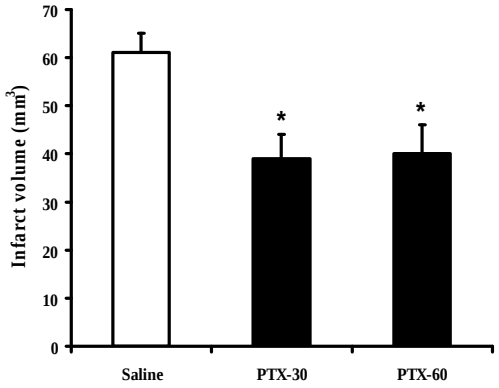

Fig 2: Values (mean±SEM) striatal infarct volumes of rats receiving saline or pentoxifylline at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60). * denotes significantly different from saline group ($p<0.05$)

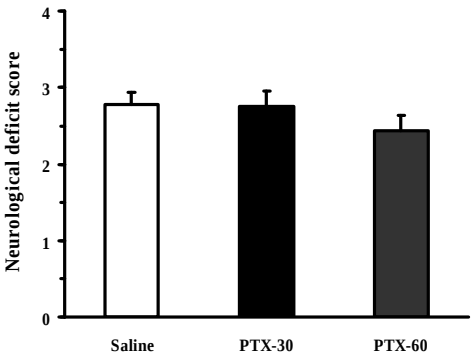

Fig 3: Values (mean ± SEM) of neurological deficit score of rats receiving saline or pentoxifylline at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60).

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,[21] 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

volumes (Figs 1 and 2). Such a finding is in agreement with earlier reports demonstrating that PTX prevented spontaneous brain ischemia in stroke-prone rats,[24] or its administrations before induction of global cerebral ischemia reduced brain injury in global cerebral ischemia in mature and neonate rats.[23,25] 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.[5] 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.[3,4] However, the findings of this study do not agree with those studies demonstrating that TNF-α induced ischemic tolerance,[9,10] or transgenic mice lacking TNF-α receptors were more resistant to ischemic injuries.[27,29] 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.[28] Other effects of PTX such as increased cerebral blood flow, inhibition of neutrophil,[29] activation of monocytes/microglias,[18] attenuation of the release of inflammatory mediators such as platelet-activating factors,[17,30] and prevention of endothelial-leukocyte adhesion,[30] 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,[19] and global ischemia.[20] 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.

Acknowledgments

This work was financially supported in part by a grant (83-2375) from Vice Chancellor for Research, Shiraz University of Medical Sciences.

Reference

1 Feuerstein GZ, Liu T, Barone FC. Cytokines, inflammation and brain injury: the role of tumor necrosis factor-α. *Cerebrovasc Brain Metab Rev* 1994; 6: 341-60.
2 Gregersen R, Lambertsen K, Finsen B. Microglia and macrophages are the major source of tumor necrosis factor in permanent middle cerebral artery occlusion in mice. *J Cereb Blood Flow Metab* 2000; 20: 53-65.
3 Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM. TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. *J Cereb Blood Flow Metab* 1997; 17: 483-90.
4 Nawashiro H, Martin D, Hallenbeck JM. Inhibition of tumor necrosis factor and amelioration of brain infarction in mice. *J Cereb Blood Flow Metab* 1997; 17: 229-32.
5 Barone FC, Arvin B, White RF, et al. Tumor necrosis factor-alpha a mediator of focal ischemic brain injury. *Stroke* 1997; 28: 1233-44.
6 Yang GY, Gong C, Qin Z, et al. Inhibition of TNF-alpha attenuates infarct volume and ICAM-1 expression in ischemic mouse brain. *Neuroreport* 1998; 9: 2131-4.
7 Lavine SD, Hofman FM, Zlokovic BV. Circulating antibody against tumor necrosis factor-alpha protects rat brain from reperfusion injury. *J Cereb Blood Flow Metab* 1998; 18: 52-8.
8 Dawson DA, Martin D, Hallenbeck JM. Inhibition of tumor necrosis factor-alpha reduces focal cerebral ischemic injury in the spontaneously hypertensive rat. *Neurosci Lett* 1996; 218: 41-4.
9 Kim KS, Wass CA, Cross AS, Opal SM. Modulation of blood-brain barrier permeability by tumor necrosis factor and antibody to tumor necrosis factor in the rat. *Lymphokine Cytokine Res* 1992; 11: 293-8.
10 Megyeri P, Abraham CS, Temesvari P, et al. Recombinant human tumor necrosis factor alpha constricts pial arterioles and increases blood-brain barrier permeability in newborn piglets. *Neurosci Lett* 1992; 14: 137-40.
11 Rosenberg GA, Dencoff JE, Correa N Jr, et al. Effect of steroids on CSF matrix metalloproteinases in multiple sclerosis: rela-

tion to blood-brain barrier injury. *Neurology* 1996; 46: 1626-32.
12 Robbins DS, Shirazi Y, Drysdale BE, et al. Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. *J Immunol* 1987; 39: 2593-7.
13 Goldblum SE, Sun WL. Tumor necrosis factor-alpha augments pulmonary arterial transendothelial albumen flux in vitro. *Am J Physiol* 1990, 285: L57-67.
14 Jacoby D, Mohler ER. Drug treatment of intermittent claudication. *Drugs* 2004, 64: 1657-70.
15 Muller R, Schroer R. Cerebrovascular circulatory disorders: new aspects of pathophysiology and therapy. *J Med* 1979; 10: 347-64.
16 Ward A, Clissold SP. Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drugs* 1987; 34: 50-97.
17 Strieter RM, Remick DG, Ward PA, et al. Cellular and molecular regulation of tumor necrosis factor-alpha production by pentoxifylline. *Biochem Biophys Res Commun* 1988; 155: 1230–6
18 Chao CC, Hu S, Close K, et al. Cytokine release from microglia: differential inhibition by pentoxifylline and dexamethasone. *J Infect Dis* 1992; 166: 847–53
19 Shohami E, Bass E, Wallach D, et al. Inhibition of tumor necrosis factor alpha activity in rat brain is associated with cerebroprotection after closed head injury. *J Cereb Blood Flow Metab* 1996; 16: 378-84.
20 Sirin BH, Yilik L, Coskun E, et al. Pentoxifylline reduces injury of the brain in transient ischaemia. *Acta Cardiol* 1998; 53: 89-95.
21 Vakili A, Nekoueian AA, Dehghani GA. L-NAME and 7-nitroindazole reduces brain injuries in transient focal cerebral ischemia in rat. *Iran J Med Sci* 2004; 29: 109-15.
22 Vakili A, Kataoka H, Plesnila N. Role of arginine vasopressin v(1) and v(2) receptors for brain damage after focal cerebral ischemia. *J Cereb Blood Flow Metab* 2005; 25: 1038-43.
23 Eun BL, Liu XH, Barks JD. Pentoxifylline attenuates hypoxic-ischemic brain injury in immature rats. *Pediatr Res* 2000; 47: 73-8.
24 Banfi C, Sironi L, De Simoni G, et al. Pentoxifylline prevents spontaneous brain ischemia in stroke-prone rats. *J Pharmacol Exp Ther* 2004; 310: 890-5.
25 Brint S, Jacewicz M, Kiessling M, et.al. Focal brain ischemia in the rat: methods for reproducible neocortical infarction using tandem occlusion of the distal middle cerebral and ipsilateral common carotid arteries. *J Cereb Blood Flow Metab* 1988; 8: 474-85.
26 Swanson RA, Morton MT, Tsao-Wu G, et al. Semi automated method for measuring brain infarct volume. *Cereb Blood Flow Metab* 1990; 10: 290-3.
27 Shohami E, Ginis I, Hallenbeck JM. Dual role of tumor necrosis factor alpha in brain injury. *Cytokine Growth Factor Rev* 1999; 10: 119-30.
28 Zabel P, Wolter DT, Schonharting MM, Schade UF. Oxpentifylline in endotoxaemia. *Lancet* 1989; 2: 1474-7
29 Bruce AJ, Boling W, Kindy MS, et al. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. *Nature Medicine* 1996; 2: 788-94.
30 Adams JG Jr, Dhar A, Shukla SD, Silver D. Effect of pentoxifylline on tissue injury and platelet-activating factor production during ischemia-reperfusion injury. *J Vasc Surg* 1995; 21: 742-8.

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