Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Testosterone enhances functional recovery after stroke through promotion of antioxidant defenses, BDNF levels and neurogenesis in male rats

Hamed Fanaei[a,*], *Seyed Morteza Karimian*[b], *Hamid Reza Sadeghipour*[b], *Gholamreza Hassanzade*[c,d], *Amir Kasaeian*[e,f], *Fatemeh Attari*[d], *Samira Khayat*[g], *Vahid Ramezani*[h], *Mani Javadimehr*[i]

[a]*Department of Physiology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
[b]*Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[c]*Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[d]*Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[e]*Non-Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
[f]*Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[g]*Nursing and Midwifery Faculty, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[h]*Department of Pharmaceutics, Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[i]*Department of Medical English Language, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*

ARTICLE INFO

Article history:
Accepted 14 February 2014
Available online 22 February 2014

Keywords:
Testosterone
Stroke
Brain-derived neurotrophic factor
Oxidative stress
Neurogenesis

ABSTRACT

It is reported that circulating testosterone levels decrease after cerebral ischemia. The aim of this study was to evaluate the effects of testosterone on oxidative stress, brain-derived neurotrophic factor (BDNF) levels, neurogenesis, histological damage and sensorimotor recovery in a castrated male rat model of focal cerebral ischemia. Animals were divided into four groups. For all animals, castrations were conducted 7 days before transient middle cerebral artery occlusion (MCAO) was done and cerebral ischemia was induced. The first group served as sham. Second was MCAO group and received vehicle only, third was MCAO group that was post-treated with testosterone and the fourth was MCAO group post-treated with testosterone and flutamide. Treatment only with testosterone significantly weakened oxidative stress and increased BDNF levels and sensorimotor recovery during a 10 days period. Rats receiving testosterone demonstrated a significant reduction in infarct volume and a significant increase in neurogenesis on 10th day after focal cerebral ischemia. Our results for the first time showed a potential advantageous effect of testosterone after cerebral ischemia in male rats, which was probably mediated by promoting antioxidant defenses, BDNF levels and neurogenesis.

*Corresponding author. Fax: +98 541 2422888.
E-mail address: fanaei@razi.tums.ac.ir (H. Fanaei).

http://dx.doi.org/10.1016/j.brainres.2014.02.028
0006-8993

1. Introduction

Stroke is one of mainly responsible factors for long-term disability and death throughout the world (Roger et al., 2012; Wang et al., 2012). Epidemiological studies have shown that overall incidence of stroke is higher in men in relation with age-matched women in most countries. A large part of this difference between sexes is attributed to sex steroids (Liu et al., 2009). Previous studies demonstrated that estrogen and progesterone give protection against cerebral ischemia by several mechanisms (Liu et al., 2009; Herson et al., 2009). Human studies suggested male susceptibility to cerebral ischemia is high, because male sex is a stroke risk factor in humans and low testosterone levels have been associated with risk for stroke and worse outcomes after stroke in men (Herson et al., 2009). In rodents, endogenous testosterone removal by castration before cerebral ischemia decreased the histological damage; whereas testosterone replacement increased brain injury (Liu et al., 2009; Herson et al., 2009). In contrast, testosterone replacement after cerebral ischemia in castrated rodents improved histological and behavioral recovery (Liu et al., 2009; Herson et al., 2009; Fanaei et al., 2013; Uchida et al., 2009). It is now well documented through in vivo and in vitro studies that estrogen provides powerful protection against ischemic brain injury through several mechanisms (e.g. reduces inflammatory response and oxidative stress, increases neurogenesis and angiogenesis) (Liu et al., 2009; Herson et al., 2009). But much less is known with regard to mechanisms of neuroprotective effects of testosterone after cerebral ischemia. Accordingly, we aimed at assessing the effects of testosterone replacement after focal cerebral ischemia in male castrate rats on oxidative stress, BDNF levels, infarct volume, neurogenesis, and functional recovery.

2. Results

2.1. Mortality

No animals in the sham group died, whereas 3 in the MCAO group, 2 in the MCAO+T group, and 2 animals in the MCAO+T/F group died within 10 days after MCAO surgery. Data of died animals were excluded from the analysis. However, mortality was not significantly different among groups ($p=0.384$).

2.2. Biochemical analysis of serum

2.2.1. Effect of testosterone post-treatment on Malondialdehyde (MDA) level in serum

Significant differences ($p<0.001$) in MDA level was observed in MCAO group animals when compared to sham group on 1st, 3rd, 7th and 10th days after cerebral ischemia (Fig. 1).

On 7th and 10th days after ischemia, only testosterone post-treated (MCAO+T) group exhibited significant reduction in MDA levels in comparison to MCAO (both $p<0.001$) and MCAO+T/F ($p<0.05$ and $p<0.001$ respectively) groups. Animals of MCAO+T/F group showed no significant differences in MDA level as compared to MCAO group during experiment.

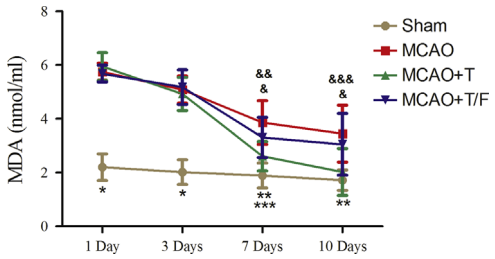

Fig. 1 – Effects of testosterone post-treatment on serum MDA levels on 1st, 3rd, 7th and 10th days after ischemia (AI) (mean ± SD), $n=16$, $^{*}p<0.001$, sham versus all other groups on 1st and 3rd days AI, $^{**}p<0.001$, sham versus MCAO and MCAO+T/F on 7th and 10th days AI, $^{***}p<0.05$, sham versus MCAO+T on 7th day AI, $^{\&}p<0.001$ MCAO+T versus MCAO on 7th and 10th days AI, $^{\&\&}p<0.05$ MCAO+T versus MCAO+T/F on 7th day AI, $^{\&\&\&}p<0.001$ MCAO+T versus MCAO+T/F on 10th day AI.

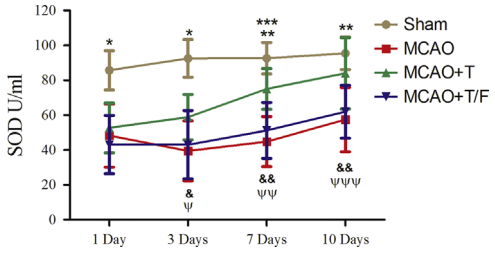

Fig. 2 – Effects of testosterone post-treatment on SOD activity in serum on 1st, 3rd, 7th and 10th days AI (mean ± SD), $n=16$, $^{*}p<0.001$, sham versus all other groups on 1st and 3rd days AI, $^{**}p<0.001$, sham versus MCAO and MCAO+T/F on 7th and 10th days AI, $^{***}p<0.05$, sham versus MCAO+T on 7th day AI, $^{\&}p<0.01$ MCAO+T versus MCAO on 3rd day AI, $^{\Psi}p<0.05$ MCAO+T versus MCAO+T/F on 3rd day AI, $^{\&\&}p<0.001$ MCAO+T versus MCAO on 7th and 10th days AI, $^{\Psi\Psi}p<0.001$ MCAO+T versus MCAO+T/F on 7th day AI, $^{\Psi\Psi\Psi}p<0.01$ MCAO+T versus MCAO+T/F on 10th day AI.

2.2.2. Effect of testosterone post-treatment on superoxide dismutase (SOD) activity in serum

The activity of SOD was significantly reduced ($p<0.001$) in all ischemic groups as compared to sham group on 1st day after cerebral ischemia (Fig. 2). Testosterone post-treatment significantly recovered the activity of SOD in MCAO+T group as compared to MCAO group on 3rd ($p<0.01$), 7th ($p<0.001$) and 10th days ($p<0.001$) and as compared to MCAO+T/F group on 3rd ($p<0.05$), 7th ($p<0.001$) and 10th days ($p<0.01$), respectively. There were no significant differences in the activity of SOD between MCAO+T/F group and MCAO group.

2.2.3. Effect of testosterone post-treatment on catalase (CAT) activity in serum

In the ischemic groups, CAT activities were significantly ($p<0.001$) lower as compared to sham group values during experiment (Fig. 3). CAT activity gradually increased in MCAO+T group and had significant differences with MCAO group on 7th ($p<0.01$) and on 10th ($p<0.001$) days and with MCAO+T/F group on 3rd ($p<0.05$) and on 10th ($p<0.001$) days