Tumor Necrosis Factor-α in Rats Following Transient Focal Cerebral Ischemia Reperfusion and Its Relation to Oxidative Stress

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Received October 27, 2014; Revised November 03, 2014; Accepted November 06, 2014

Abstract Background: The role of TNF-α in ischemic/reperfusion (I/R) is still controversial. The aim of this study was to assess TNF-α in rats subjected to transient cerebral I/R and to correlate their levels with the resulting neurological deficits and oxidative stress biomarkers malondialdehyde and total antioxidant capacity (TAC).

Material and Method: Experimental procedures were performed on 30 adult male Wistar rats. Divided into two groups fifteen rats in each, test group subjected to transient focal cerebral I/R by occlusion of the left common carotid artery (CCA) for 30 minutes followed by reperfusion for 24-hours. A control group underwent the surgery at the same neck region without occlusion of the CCA. Neurobehavioral assessments were evaluated. TNF-α was measured using ELISA method. Malondialdehyde and TAC were estimated colorimetry.

Results: In the test group TNF-α and Malondialdehyde concentration in both serum and brain tissue were significantly higher than control group (P =0.000). In contrast, the serum and brain tissue levels of TAC in the test group was significantly lower compared to the sham operated rats (P = 0.000). The brain tissue and serum level of TNF-α were correlated negatively with neurological deficit and TAC and positively with Malondialdehyde (P = 0.000).

Conclusion: The present study revealed a potential injurious role of TNF-α in rats subjected to cerebral I/R and demonstrated a direct relationship between TNF-α and oxidative stress biomarkers and the consequent neurological deficits.

Keywords: cerebral, ischemia/reperfusion, TNF-α, malondialdehyde and total antioxidant capacity


1. Introduction

Acute ischemic stroke has serious clinical, social, and economic consequences and necessitates a significant effort from both basic scientists and clinicians to understand the complex underlying pathophysiological mechanisms [1]. However, The pathophysiology regarding ischemia/reperfusion (I/R) injury is still obscure; through involvement of oxidative stress and inflammatory mediators [2]. Reactive oxygen species (ROS), derived from hypoxia and reoxygenation during transient focal cerebral I/R, results in extensive damage to lipids, proteins, DNA and other components of organisms. The role of ROS species has been implicated in the pathogenesis of oxidative stress-related diseases, such as stroke. [3] Brain tissue is not well equipped with antioxidant defenses, so ROS and other free radicals, released by inflammatory cells, threaten tissue viability approximately to the ischemic zone [4].

Oxidative stress induced peroxidation of cell membrane lipids and malondialdehyde (MDA) is frequently used as an indicator of lipid peroxidation and correlated well with the size of ischemic stroke as well as the clinical outcome [5]. Furthermore, estimation of the total antioxidant capacity (TAC) is supposed to be a useful measure of the availability of the antioxidants present to guard against oxidative cell damage [6,7].

An inflammatory cytokine tumor necrosis factor-α (TNF-α) assumed to augment or depress cellular survival through activation of receptor-mediated signal transduction [8]. Both injurious and beneficial roles of TNF-α have been reported in the pathogenesis of cerebral I/R. This explained by diverse molecular switches and dynamic changes in signaling of TNF-α through it receptors [9]. The aim of this work was to evaluate potential role of TNF-α in cerebral ischemia and to determine its relation to oxidative stress in rats subjected to transient cerebral ischemia reperfusion.

2. Materials and Methods

2.1. Animals

The studies were approved by the Ethical Committee of the University of Alexandria, and the investigations
conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). 30 Male Wistar rats, weighing 150-250 g were selected and preserved at a constant temperature of 22±2°C with a fixed 12:12-h light-dark cycle. Nutritionally balanced pellets and water were freely available. The animals were divided into two groups (15 rats in each group): test and control.

2.2. Cerebral Ischemia Induction

The animals were fasted overnight prior to surgery with free access to tap water. Anesthesia was induced by ether inhalation and maintained by thiopental sodium (2.5mg/kg) (10). Body temperature was kept constant at 36.5±0.5°C using warm pad. A longitudinal cervical incision (2cm) was made lateral to the midline and the common carotid artery (CCA) was carefully dissected. In the test group (ischemia/reperfusion) (n=15) ischemia was induced by placing non traumatic microvascular clip on the left CCA just prior to its bifurcation [11]. During ischemia rats were monitored for body temperature and respiration pattern. The vascular occlusion was maintained for 30 minutes, and then the clips were removed to resume blood flow to the ischemic region [12]. The incisions were sutured, and then the animal was allowed to recover from anesthesia, and returned to a warm cage for recuperation during reperfusion period for 24 hours.

In the control group (sham-operation) (n = 15), the rats underwent the surgery at the neck region without occlusion of CCA. The number of animals presented for each group is the number of rats that survived during 24-hour reperfusion period. The collected data of the animals that died during 24 hours reperfusion period were excluded.

2.3. Neurological and Behavioral Evaluation

Neurobehavioral tests of all experimental groups were assessed daily to determine the effect of ischemic injury on them. Neurobehavioral evaluations were performed three times: the day before surgery, one hour after the surgery and before sacrifice day. The neurobehavioral study consisted of the following six tests: spontaneous activity, symmetry in the movement of the four limbs, forepaw outstretching, climbing, body proprioception and response to vibrissae touch. The score given to each rat at the end of the evaluation is the summation of all six individual test scores. The minimum neurological score was 3 and the maximum was 18 [13].

2.4. Laboratory Investigations

At the end of experimental period, the rats were sacrificed by decapitation. Brains were rapidly removed from the skull and washed with cold saline and stored at ~20°C for further analysis. A small part of each brain from the affected hemisphere were dissected in to approximately 1-2 mm pieces and they were homogenized in 7 ml of ice-cold extraction buffer contain: (Triton X-100: 1%, MgSO4: 10 mmol/l, EDTA: 1 mmol/l, Dithiothreitol: 1 mmol/l, NaCl: 0.5 mol/l, Protease inhibitor cocktail: 1%, and 20 mmol/l HEPES (pH 7.5) [14]. The homogenate was centrifuged; the supernatant was taken and stored at ~20°C before used. A modification of the method of Lowry was used for the determination of protein in the brain homogenate [15]. Brain tissue and serum level of TNF-α were measured using ELISA kits [16] and TAC concentrations were measured colorimetrically while Satoh method was used to measure serum and brain homogenate MDA levels [17].

3. Data Analysis

Data were expressed as mean ± S.E.M. Differences among groups were evaluated by independent student t-test and the relationships between TNF-α, MDA, TAC and the resulting neurological deficit of rats subjected to ischemia reperfusion were assessed using bivariate correlations. P<0.05 was selected for acceptance of statistical significance.

4. Results

As shown in Figure 1, rats subjected to ischemia reperfusion demonstrated a significant decreased in the neurological deficit (M±SD= 12.79±6.689) compared to sham operated rats (17.50±0.707, P < 0.001). Rats in test group revealed a significant increase in brain tissue of TNF-α and MDA (110.36±6.178 pg/mg protein, 8.56±0.658 nmol/mg protein respectively) as compared to control group (4.9±0.797 pg/mg protein, 3.24±0.226 nmol/mg protein respectively, P = 0.000). While the brain TAC level of rats exposed to ischemia reperfusion (0.018±0.00373 mmol/mg protein) was significantly lower compared to the sham operated rats (0.070±0.0085 mmol/mg protein, P = 0.000) (Figure 2). In Figure 3, the concentrations of serum TNF-α and MDA in rats subjected to ischemia reperfusion (734.76±108.82 pg/ml, 14.88±1.14 nmol/mL, respectively) were significantly higher compared to sham operated rats (37.18±10.183 pg/ml, 5.43±0.44 nmol/mL, respectively, P = 0.000). In contrast, the level of serum TAC of rats in test group (1.21±0.169 mM/L) was significantly lower compared to the sham operated rats (2.52±0.062 mM/L, P = 0.000).

The relationships between brain tissue and serum of TNF-α, MDA, and TAC levels and neurological deficit of rats subjected to ischemia reperfusion are given in Table 1.

### Table 1. Correlations between TNF and MDA, TAC and Neurological deficit

<table>
<thead>
<tr>
<th>The Biomarker</th>
<th>Correlation Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain tissue of TNF-α</td>
<td>Tissue Level</td>
<td></td>
</tr>
<tr>
<td>MDA(nmol/ml)</td>
<td>0.988</td>
<td>.000</td>
</tr>
<tr>
<td>TAC (mM/L)</td>
<td>-0.973</td>
<td>.000</td>
</tr>
<tr>
<td>Neurological deficit</td>
<td>-0.967</td>
<td>.000</td>
</tr>
<tr>
<td>MDA(nmol/ml)</td>
<td>0.954</td>
<td>.000</td>
</tr>
<tr>
<td>Serum of TNF-α</td>
<td>Serum Level</td>
<td></td>
</tr>
<tr>
<td>TAC (mM/L)</td>
<td>-0.976</td>
<td>.000</td>
</tr>
<tr>
<td>Neurological deficit</td>
<td>-0.933</td>
<td>.000</td>
</tr>
</tbody>
</table>
Figure 1. Neurological deficit in control (sham operated) and test (ischemia reperfusion) rats. (15 rats in each group, data are expressed as mean± SEM)

\* \( p<0.05 \) significant differences between test and control group

Figure 2. Brain tissue level of TNF-α, MDA and TAC in control (sham operated) and test (ischemia reperfusion) rats. (15 rats in each group, data are expressed as mean± SEM)

\* \( p<0.05 \) significant differences between test and control group
Figure 3. Serum level of TNF-α, MDA and TAC in control (sham operated) and test (ischemia reperfusion) rats. (15 rats in each group, data are expressed as mean± SEM)

* p<0.05 significant differences between test and control group

5. Discussion

In the present study, it was observed that rats subjected to cerebral ischemia for 30 min followed by reperfusion for 24 hours had a significantly higher serum and brain tissue levels of TNF-α and MDA compared to sham-operated rats. In contrast, TAC was significantly lower in the test group. Interestingly, the current data showed a highly significant negative correlation between both serum and brain tissue level of TNF-α, neurological deficit and MDA, with one more positive correlation between TNF-α and TAC. These findings further support the injurious role of TNF-α and its association with oxidative stress and contribution to neuronal damage in our focal cerebral ischemic model.

The current data are comparable with previous studies conducted to evaluate the role of TNF-α in rat's transient focal and global cerebral ischemia [18,19]. Nevertheless the role of TNF-α during ischemia/reperfusion has not been fully elucidated, with the potential of both beneficial and/or deleterious outcome [9,20].

In accordance to the current finding, numerous researches demonstrated that oxidative stress biomarkers are elevated with reduced levels of antioxidants in the cerebral vasculature during ischemia/reperfusion with contribution to post-ischemic endothelial dysfunction [21,22,23]. Zhang et al revealed that a significant increased in MDA level and reduced antioxidant enzymes in rats with cerebral ischemia reperfusion [24]. A similar results obtained by Liu et al in different model of cerebral ischemia [25]. Moreover, previous reports repeatedly suggest that a significant attenuation in TAC level take place in cerebral ischemia reperfusion [26].

One of the first indications that TNF-α is an important mediator of stroke is the correlation of its expression with stroke damage. Berti et al were in agreement with our finding that TNF-α is increased 3 h after transient middle cerebral artery occlusion (MCAO) and persists for 24 h in the affected hemisphere, this associated with increased level of interleukin (IL)-1b, IL-6, E-selectin, and intercellular adhesion molecule-1 [27]. Furthermore, Haddad et al gained similar finding in another different model of transient focal cerebral ischemia [28]. These propositions were further supported by Clausen et al, who demonstrated that mice exposed to permanent MCAO exhibited a significantly higher level of TNF-α activity measured at 6, 12 and 24 hours after cerebral ischemia. Moreover, they proved that TNF-α was expressed in largely isolated populations of microglia and macrophages in the ischemic brain [29].

At some point in cerebral ischemia, TNF-α produced by brain parenchymal cells may be beneficial for stroke recovery. Sairanen et al reported that astrocytes show vigorous TNF-α immunoreactivity at day 17–18, lagging behind neuronal expression which peaks 2–3 days after human stroke. This may suggest a role of TNF-α in tissue regeneration [30]. Further, Tarkowski et al proposed that the level of TNF-α in the CSF of stroke patients correlates
with anti-apoptotic (bcl-2) expression, indicating that TNF-α may not be entirely detrimental to recovery [31]. This contradictory finding is largely attributed to diverse activation of TNF-α receptors (TNFR1 or 2) and to its differential downstream pathway activated during cerebral ischemia [29,32].

In conclusion, the present study demonstrated that serum and brain tissue of TNF-α were significantly higher in rats subjected to I/R, with a significant correlation between TNF-α and oxidative stress biomarkers (MDA and TAC) and the consequent neurological deficits. This added further evidence to the potential injurious role of TNF-α in transient focal cerebral ischemia/reperfusion in rats.

Competing Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest

Acknowledgment

During this work we have collaborated with many colleagues in Alexandria University - Egypt, for whom I have great regard, and I wish to extend my sincere thanks to Dr. Ali. M. Kobil.

References