The Effect of TGF-alpha on Neurogenesis in Subventricular Zone of Rat Brain after Ischemia-Reperfusion

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A B S T R A C T

Introduction: Stroke is the third important reason of death in adults and an important cause of adult disability. Previous studies suggest that TGF-alpha can induce neurogenesis after stroke. Here in, we studied neurogenesis effects of the TGF-alpha on subventricular zone following ischemia-reperfusion.

Methods: Male wistar rats (250-300 g) were divided into ischemia and treatment groups. After induction of ischemia-reperfusions, PBS (phosphate buffer salin) and TGF-alpha 50 ng were injected stereotaxicaly in lateral ventricle in ischemia and treatment respectively. After 12 days, the nestin expression in subventricular zone was assessed by immunohistochemical staining method.

Results: Our results showed that nestin expression increased significantly in treatment group in comparison with ischemia group ($p<0.05$).

Discussion: Expression of nestin in SVZ indicates that TGF- α can stimulate the neural stem cells proliferation after ischemia – reperfusion injury.

1. Introduction

Stroke is one of the most important reasons of mortality in the world (Senelick, Rossi, & Dougherty, 1999).Cerebral ischemia results in cerebral blood flow, oxygen and metabolites decrease that lead to increase of free radicals. Free radicals such as hydrogen peroxide and hydroxyl radical ($OH^{-}{}^{\circ}$) are produced as Consequent of Ischemia Reperfusion in targeted tissues. The subventricular zone (SVZ) is a paired brain structure situated throughout the lateral walls of the lateral ventricles and has four distinct layers with variable thickness, cell density as well as different cellular composition (Quiñones Hinojosa et al., 2006).

Neurogenesis occurs in different areas of adult brain, including DG and SVZ (Quiñones-Hinojosa & Chaichana, 2007). Studies show that hippocapus damage such as disease, traumatic ischemia and Traumatic injuries caused to neurogenesis in SVZ (Gage, 2000).

Furthermore increase of stem cells in SVZ of adult brain may leads to morphological and functional improvements following brain trauma, ischemia or primary degenerative injuries (Salazar-Colocho, Lanciego, Del Rio, & Frechilla, 2008) but this internal response is unable to compensate the damage. Therefore, use of factors stimulating neurogenesis in SVZ can be an effective pharmaceutical approach in repair of brain injury following ischemia- reperfusion.

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TGF-alpha is a member of EGF (Epidermal Growth Factors) family that activates the EGFR Trans membrane tyrosine kinas and leads to increase of intracellular calcium levels, glycolysis and expression of some genes such as EGFR gene which ultimately leads to DNA synthesis and cell proliferation. EGF family is involved in growth, differentiation, maintain and repair various tissues such as nervous system and gastrointestinal tract .TGF-alpha is a member of this family that in comparison with other ligands of this family have broader abundance and distribution is in CNS(Xian & Zhou, 1999). TGF-alpha is recognized as the most abundant EGF-R ligands in adults CNS development Especially in striatum, olfactory bulb , hippocampus , SVZ and brain stem(Lazar & Blum, 1992).As regards, TGF-alpha leads to cell proliferation in CNS and because ischemia-reperfusion causes to cell death, in the present study we considered the effects of TGF-alpha on the neurogenesis in subventricular zone of rats following ischemia-reperfusion in rat.

2. Methods

The present study was carried out in accordance with the protocol approved by Tehran University of Medical Sciences.

2.1. Animals

14 adult male wistar rats, weighing 250-300 gr, were obtained from the Iranian Razi Institute in Tehran, Iran. The animals were housed at an ambient temperature 22± C under a daily 12-h light-dark cycle with free access to food and water. The animals were randomly assigned to the following groups: Ischemia (n=7) and treatment(n=7) groups. In ischemia group, animals were subjected to anesthesia, surgical procedure of four vessel occlusions (4VO) and PBS injection of 5µl via stereotaxic in lateral ventricle and in treatment group after anesthesia and occlusion 4VO ,TGF-alpha was injected in lateral ventricle strereotaxily .

2.2. Surgery

The initial surgical procedure was the same for all animals. Animals were anesthetized with an intraperitoneal injection of a mixture of Ketamin (10mg/kg) and Xylasine (10 mg/kg). Rectal temperature was maintained at 37 ±0.5◦C throughout the experiment. Vertebral arteries were permanently occluded by electrocuter through the alar foramina at the first cervical vertebra and after midline cervical incision and dissection of muscles the right and left common carotid arteries (CCA) were exposed while leaving the vagus nerve intact. Transient bilateral ligation of CCA, performed by clamping 30min.

2.3. Perfusion

12 day after stereotaxy, the rats were deeply anesthetized and intracardially perfused with 100 ml of saline followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) and decapitated. The brains were removed from the skull; stored at the fixative for 48 hr. and a 10-mm-thick coronal slice of each brain was prepared, dehydrated, and embedded in paraffin.

2.4. Immunohistochemistry

Immunohistochemistry staining was performed to visualize nestin as neural stem cell specific marker.

Sections cleared and hydrated and endogenous peroxides blocking applied. Then slides were placed in 10% H2O2 solution for 10 minutes in and microwaved for antigen retrieval in Citrate buffer (PH= 6) for 10 minutes. Unspecific antigens were blocked with BSA and then primary antibody applied for 1 hour. Following the washing, goat polyclonal secondary antibody (HRP) (abcam, ca6789) applied for 1 hour. Slides washed again and incubated with DAB substrate kit (CA number: 11718096001) for 10 min. and nissle staining used as counter staining to visualize the cytoplasm.

2.5. Statistical Analysis

The data were presented as the mean ± S.E.M and the results were analyzed by SPSS 16 soft ware and student- t test. The P. value ≤ 0.05 was considered statistically significant.

3.1. Result

3.2. Nestin Immunohistochemistry (IHC)

Neurogenesis in SVZ was assessed using analysis imaging software at a total magnification of 400× with OLYMPUS AX70 microscope and defined as the number of brown nestin-positive cells (Figure1 and 2).Average nestin positive cells was obtained by counting 5 serial coronal sections with 120 µm interval. The results indicate that mean of the number of nestin positive cells in SVZ increased in treatment group in compare to ischemia group that was significant($p<0.05$, figure 3).

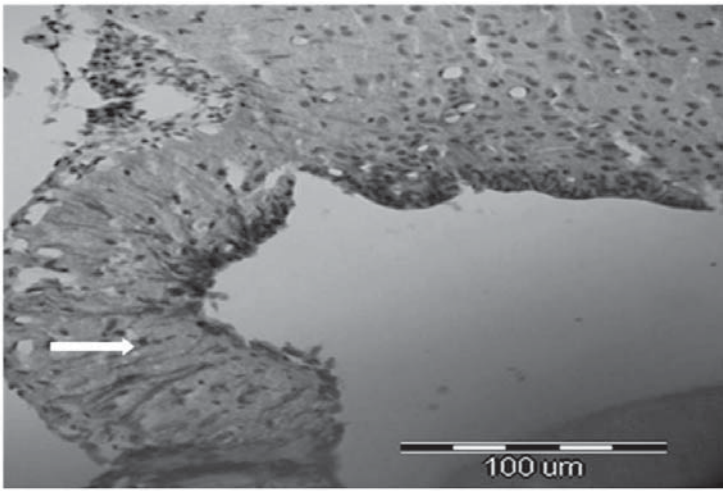

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Figure 1. immunohistochemistry staining in SVZ for detection nestin expression in ischemia group.Nissle staining was used as counter stain. Nestin expression has been detected brown (arrows).

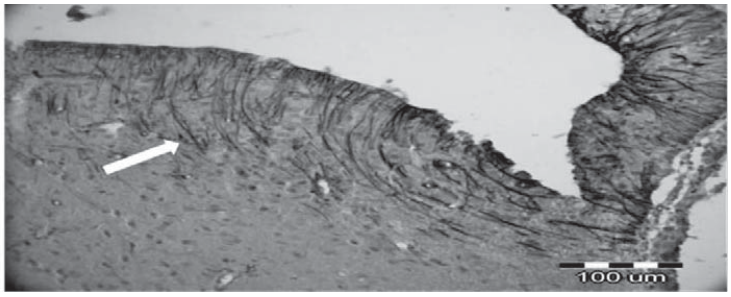

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Figure 2. Immunohistochemistry staining in SVZ for detection nestin expression in treatment group. Nissle staining was used as counter stain. Nestin expression has been detected brown(arrows) .

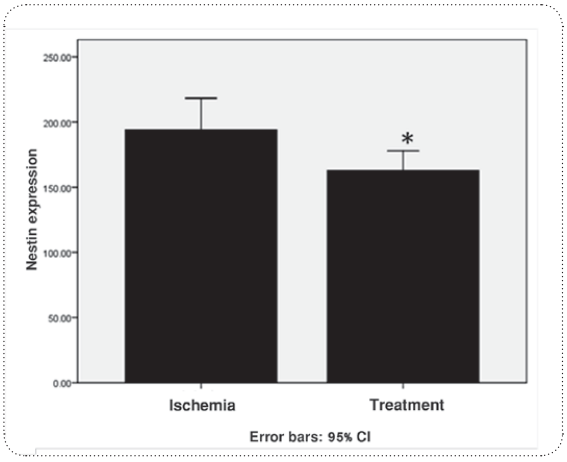

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Figure 3. Quantification of nestin-positive cells in the SVZ. The density of nestin-positive cells in the SVZ was determined stereologically. Nestin positive cells were significantly increased in the SVZ of treatment groups compared with the ischemia group.*p≤0.05.

4. Discussion

In this study we explored the effects of TGF-alpha on the neural stem cells of SVZ with Immunohistochemical stainin. Our study finding showed that TGF-alpha induced neural stem cell proliferation in SVZ. The previous studies showed that neurogenesis occurs in certain areas of the brain including the DG and SVZ throughout the lifetime and also was reported that newly generated neurons play an important role in learning and memory in the adult brain (Altman, 1969). A study showed that new granule cells in hippocampus CA1 are formed in umbilical DG and SVZ (Hastings & Gould, 1999). More extensive efforts of the scientists demonstrated that increase in neurogenesis in SVZ and DG of the adult brain in various mammalian species may leads to morphological and functional improvement after brain ischemia, trauma or primary degenerative injuries (Schmidt & Reymann, 2002). Carles justicia and colleagues found that TGF-alpha reduces infarct volume and this effect is not mediated by change in perfusion of microvessels or cerebral arteries (Justicia, Pérez-Asensio, Burguete, Salom, & Planas, 2001).Leker et al found that TGF-alpha can induce angiogenesis and neurogenesis after stroke (Leker et al., 2009). Guerra crespo and et al concluded that neural stem cells respond to ischemic damage and behavioral recovery increases considerably following TGF-alpha adminstration that represents a treatment approach for chronic stroke and other neurological damages in humans (Guerra-Crespo et al., 2009).

The result of the present study showed that injection of TGF-alpha 50 ng in lateral ventricul after steriotaxic surgery can induce neurogenesis in SVZ that represents a pharmaceutical approach for treatment of chronic stroke and other neurological damages in humans.

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