Pre-Ischemic Oxytocin Treatment Alleviated Neuronal Injury via Suppressing NF-κB, MMP-9, and Apoptosis Regulator Proteins in A Mice Model of Stroke

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Abstract

Objective: This study was designed to determine the effects of pre-ischemic administration of oxytocin (OXT) on neuronal injury and possible molecular mechanisms in a mice model of stroke.

Materials and Methods: In this experimental study, stroke was induced in the mice by middle cerebral artery occlusion (MCAO) for 60 minutes and 24 hours of reperfusion. OXT was given as intranasal daily for 7 consecutive days before ischemic stroke. Neuronal damage, spatial memory, and the expression levels of nuclear factor-kappa B (NF-κB), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), matrix metalloproteinase-9 (MMP-9), brain-derived neurotrophic factor (BDNF) and apoptosis were assessed 24 hours after stroke.

Results: Pre-ischemic treatment with OXT significantly reduced the infarct size (P<0.01); but did not recover the neurological and spatial memory dysfunction (P>0.05). Moreover, OXT treatment considerably decreased the expressions of NF-κB, TNF-α, IL-1β, and MMP-9 (P<0.001) and enhanced the level of BDNF protein. OXT treatment also significantly downregulated Bax expression and overexpressed Bcl-2 proteins.

Conclusion: The finding of this study indicated that administration of OXT before ischemia could limit brain injury by inhibiting MMP-9 expression, apoptosis, inflammatory signaling pathways, and an increase in the BDNF protein level. We suggested that OXT may be potentially useful in the prevention and/or reducing the risk of the cerebral stroke attack, and could be offered as a new prevention option in the clinics.

Keywords: Focal Cerebral Ischemia, Mice, Oxytocin, Pre-Ischemic

Introduction

Cerebral ischemic stroke is a result of interruption or reduction of blood flow to a part of the brain, which is one of the major causes of mortality and disability worldwide. The policies for acute stroke management are mainly focused on the use of neuroprotective agents and intravenous thrombolysis and/or endovascular thrombectomy, but delay in starting the treatment may diminish their effectiveness. However, the prevention approach is always better than the treatment. Today, in addition to managing the modifiable risk factors, antiplatelet or anticoagulant drugs are used to prevent and/or diminish recurrent ischemic stroke (1). Most of the studies have indicated that the use of neuroprotection agents prior to ischemia has better outcomes than post-treatment (2). However, prophylactic treatment is not practicable for a large group of patients with acute stroke. But some patients may be at the risk of ischemic stroke in the short or long term, and for these groups, the prophylactic approach may be appropriate (1). For example, patients with transient ischemic attacks, atrial fibrillation, and asymptomatic carotid stenosis are at high risk of cerebral stroke attacks (1, 3). These patients may need to use suitable prophylactic neuroprotection for a long time. Therefore, a safe and cheap neuroprotective agent would be an interesting treatment option for prophylactic use in patients with high-risk stroke.

It has been shown that matrix metalloproteinase (MMP, as a protease enzyme), nuclear factor-kappa B (NF-κB, as a regulator of pro-inflammatory gene expression), brain-derived neurotrophic factor (BDNF, as a neurotrophic factor) and apoptosis have key roles in the pathogenesis of ischemic stroke (4-6). Oxytocin (OXT) is a peptide hormone, which is conventionally used as a well-known drug for many years to accelerate labor and lactation in humans (7). It has been shown that short-time use of OXT in humans is safe with no significant side effects (8). Currently, OXT has become an attractive topic for research in social behaviors and its potential usage in the treatment of some psychiatric disorders in human beings (9, 10). Moreover, recent animal studies have shown that post-stroke treatment with OXT reduced ischemic injury via suppressing apoptosis and inflammatory pathways (11-13). In addition, some recent studies showed that exogenous OXT can modulate brain BDNF levels and matrix metalloprotease activity under *in vitro* and *in vivo*

conditions (14, 15).

Although previous limited experimental studies offered that OXT is useful in post-ischemic stroke, the effect of OXT as prophylactic neuroprotection remained unclear. Therefore, this study was designed to determine whether seven days of intranasal daily application of OXT before ischemia could reduce brain injury after stroke in mice. Moreover, interfering with OXT with NF-κB, pro-inflammatory cytokines (TNF-α, IL-1β), MMP-9, BDNF, and apoptosis regulator proteins (Bax and Bcl2) were assessed 24 hours after stroke in the brain tissue.

Material and Methods

Animals

In this experimental study, 46 adult male Swiss albino mice (35-40 g, 3-4 months old) were used that obtained from the animal center of Semnan University of Medical Sciences (SUMS). All procedures were performed in accordance with the ethical policy for laboratory animals and approved by the SUMS institutional Committee of Research Ethics (IR.SUMUMS.REC.1396.241).

Intranasal application of oxytocin

OXT (Sigma-Aldrich, O3251, Germany) or saline, as the vehicle (10 µl), was softly injected into the bilateral nostrils (less than 30 seconds) daily for 7 days prior to ischemic stroke, using a catheter PE-10 that was entered into the nasal cavity. The last dose of OXT was injected 30 minutes before the MCAO. To decline stress reaction, mice experienced 1 week of habituation to the holding position every day before the start of the experiment.

Animal model of stroke

To create the stroke model, mice were anesthetized with chloral hydrate (400 mg/kg, IP) and then under microscopic surgery MCA was occluded using a silicone-coated 8–0 monofilament and Laser Doppler Flowmetry (LDF) monitoring. Blood flow in MCA was blocked for 60 minutes and then flow was restored for 24 hours in the brain ischemic tissue. The Body temperature was checked and preserved in the normal range. Buprenorphine (0.05 mg/kg IP, Temad Co. Active Pharmaceutical Ingredients, Iran) was given approximately 30 minutes before the surgery and once again at 8 hours after MCAO to reduce the surgery pain.

Experimental design and protocols

To examine the preventive effect of intranasal OXT (8 IU/ per mouse intranasal) on brain injury and neurological disorder, 21 mice were randomly divided into three equal groups (n=7, each). In group 1, the sham group, surgery was made without ischemia. Group 2, the control group, received saline (10 µl, intranasal) daily for 7 days prior to ischemia. Group 3, the treatment group, received OXT at a dose (8 IU/ per mouse intranasal), daily for 7 days prior to ischemia. We used the dose of 8 IU/ per mouse of OXT as the therapeutic dose, which was obtained

from the data of our previous study (13). At the end of the experiment, neurological impairments and spatial learning and memory were examined, and then infarct size was determined.

For investigating the effect of OXT on the expressions of NF-κB, MMP-9, BDNF, and the level of apoptotic regulator proteins (Bax and Bcl2), 10 mice were divided into 3 groups (Sham=3, saline=4, and OXT=3) with the same interventions as those groups used for brain injury assessment. In all these groups, about 24 hours after ischemia, the animals were euthanized by cervical dislocation, under deep anesthesia, and their brains were isolated and then cut into three equal portions. Then, each part of the brain was used for BDNF measurement using ELISA, NF-κB, and apoptotic regulator proteins (Bax and Bcl2) by western blotting, and MMP-9 using immunohistochemistry methods.

To explore the effect of OXT on the expressions of TNF-α, and IL-1β, 9 mice were randomly divided into 3 equal groups (n=3, each) with the same interventions similar to the groups described above for NF-κB assessment.

Physiological parameters

Physiological parameters were measured in the two separated animal groups 20 minutes before and after MCAO in the mice pretreated with saline (n=3) and/ or OXT (n=3). For the measurement of physiologic parameters, the right common carotid artery was cannulated by a polyethylene catheter (PE-50) to record blood pressure and blood sampling for the analysis of arterial blood gas, pH, hemoglobin, and glucose.

Neurobehavioral test

To assess motor and sensory performance, an adjusted neurological severity score was used (16). The neurological scoring was 10-14 for severe; 5-9 for moderate; and 1-4 for mild injury. An individual who was blinded to the animal groups evaluated neurobehavioral tests.

Spatial learning and memory

Spatial learning and memory were estimated using a Radial Arm Water Maze (RAWM) task with six arms. RAWM trials were performed in three situations, habituation (1 day), training (4 days), and probe (1 day). Habituation: Animals were adapted to the atmosphere of the RAWM for 2-3 minutes. Training: Animals received five trials /day with a 30 seconds inter-trial interval for 4 days. During this period, the animals were given 60 seconds to discover the visible platform. If at this time the animal could not discover the platform, it was assisted to find the platform. On the fifth day (probe test), the platform was removed, and animals were dropped from a similar location and permitted to swim for 60 seconds to find the site of the platform. The time to find the platform position and the period spent in the target location was

verified and analyzed (NoldusEtho Vision XT7, the Netherlands).

Infarct size

Twenty-four hours after ischemia animals were deeply anesthetized and euthanized by cervical dislocation, and then the brain was isolated. Using a brain matrix, five 2-mm-thick slices were prepared with triphenyl tetrazolium chloride (TTC) staining (T8877, Sigma, Germany) and measurement of the infarct area. Data of the infarct area of each section was obtained using an image analyzer software (NIH image analyzer). The volume of infarct size was calculated by multiplying the lesion area in the thickness of each section. Total brain injury was calculated by summing the lesion volume of five slices, and finally, the data was presented as infarct volume (mm³) modified for edema (17).

Western blotting

One week after OXT treatment and 24 hours after ischemia, brain samples were prepared and used to assay the apoptotic regulator proteins (Bax and Bcl2), NF-kB, TNF-α, IL-1β, and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, ab181602, UK) by western blotting technique. Samples were lysed in the RIPA buffer and a common protease inhibitor (20-188; Merck, Germany). Protein concentration was measured by the Bradford method,then tissues were loaded on SDS-polyacrylamide gel electrophoresis (SDS- PAGE), and protein on the gel was transferred to PVDF membranes (Roth) for 80 minutes at 80 V (Bio-Rad). Proteins were then isolated by polyacrylamide gel electrophoresis (Bio-Rad) via 4-20% gradient polyacrylamide gels containing 0.1% sodium dodecyl sulfate for ~2 hours at 95 V. After blocking with 5% non-fat milk in Tris-buffered saline and Tween 20 (pH=7.6) (TBST), the membranes were incubated with primary antibodies against NF-kB (sc-398442; Santa Cruz, USA), Bax (sc-7480; Santa Cruz, USA), Bcl2 (sc-56018; Santa Cruz, USA), TNF-α (sc-133192; Santa Cruz, USA), IL-1β (orb382131; Biorbyt, UK) and GAPDH at 4˚C overnight. Then, the membranes were incubated with horseradish peroxidase-conjugated secondary anti-rabbit antibodies (HRP, 1:5000, ab6721, UK,) for 1 hour at room temperature. Finally, blots were stained by DAB (3, 3′-diaminobenzidine), imaged, and analyzed using the Image J software. The level of protein expression (Bax, Bcl2, NF-kB, TNF-α, IL-1β) was normalized to the GAPDH (ab181602, UK).

Measurement of BDNF

Seven days after OXT treatment and 24 hours after ischemia, brain tissues were homogenized (1:10 w/v) in cold PBS, and then centrifuged at 30,000×g at 4˚C for 20 minutes. The supernatant was used for measuring the BDNF protein level. The quantity of BDNF was estimated by enzyme-linked immunosorbent assay (ELISA) method and a mouse BDNF ELISA kit (orb409268, biorbyt, UK).

Immunohistochemistry

MMP-9 protein expression was measured by immunofluorescent immunohistochemistry staining in the cortex and hippocampus. The samples were post-fixed overnight and then dehydrated in the ascending alcohol series, rinsed with xylene, and after that infiltrated with paraffin. Subsequently, all of the blocks were cut into 5 μm coronal slices. The slices were incubated in 50% formamide and 2x standard sodium citrate buffer for 2 hours at 65˚C and then incubated twice in 100 mM of sodium borate (pH=8.5). The DNA was then denatured by incubating the sections in 2N HCl at 37˚C, rinsed in phosphate-buffered saline (PBS), and finally blocked with 0.4% Triton X-100 in PBS and goat serum (10%, Gibco™ PCN5000 10098792, UK) for 30 minutes. The slices were incubated overnight at 4˚C with primary antibodies for MMP-9 [rabbit anti- MMP-9 (1:100; sc-393859 Santa Cruz, USA)]. The slices were then incubated with secondary antibody FITC anti-rabbit (1:200; ab6785) at 37˚C for 90 minutes in a dark place. Cell nuclei were stained by DAPI (4′, 6-diamidino-2-phenylindole). Tissues were examined under a fluorescence microscope (Olympus, Japan) at 400X magnification. The quantification of the immune-like reactivity of cells was accomplished using Image J software v1.8 (NIH, Wayne Rasband, USA).

Statistical analyses

The normality test was assessed by the Shapiro-Wilk method. Data of infarct size, neurological disorder, spatial learning and memory, apoptosis-related proteins, NF-κB, TNF-α, IL-1β, MMP-9, and BDNF protein were analyzed by one-way ANOVA and Tukey as the posthoc test (Statistical Software, Sigma Stat/ plot 12.3.0,; Jandel Scientific, Erkmiceh, Germany). Data are shown as mean ± SEM. $P<0.05$ was considered statistically significant.

Results

Cerebral blood flow and physiological parameters

Cerebral blood flow (CBF) was monitored by an LDF to assure ischemia. Diminish in local CBF to less than 20% of the basal was a certification for ischemia. In all groups, after ischemia, CBF was reduced to lower than 20% of the initial and preserved during 60 minutes of MCAO (Fig. 1A). There was no significant difference among the groups concerning CBF during 60-minute ischemia and 15 minutes reperfusion ($P>0.05$, Fig.1A).

There is no significant difference among physiological parameters 20 minutes before and after MCAO in animals that were pre-treated with saline and OXT (Table 1).

The effect of pre-ischemic treatment with OXT on the infarct size, neurological function, and spatial learning and memory

The infarct size after 60 minutes of the ischemic event and 24 hours of reperfusion was 150 ± 9 mm³ in the control (saline) group. Intranasal pre-ischemic treatment with OXT for 7 days significantly reduced the infarct size (111 ± 9 mm³) compared to the saline (control) group ($P<0.001$, Fig.1B, C). Moreover, OXT pretreatment did not change the neurological function ($P>0.05$, Fig.1D).

Table 1: Physiological parameters 20 minutes before and 20 minutes after MCAO of animals pre-treated with saline and OXT

Parameters	Before-MCAO		After-MCAO	
	Saline	OXT	Saline	OXT
MABP (mmHg)	72 ± 3	67 ± 3	66 ± 3	64 ± 2
Heart rate (per minute)	367 ± 8	373 ± 6	390 ± 6	393 ± 3
pH	7.28 ± 0.02	7.29 ± 0.01	7.22 ± 0.01	7.23 ± 0.01
Arterial pCO_2 (mm Hg)	44 ± 3	47 ± 0.8	51 ± 2	49 ± 1.8
Arterial pO_2 (mm Hg)	98 ± 2	98 ± 1	97 ± 4	96 ± 4
Blood glucose (mg/dl)	143 ± 9	129 ± 4	152 ± 5	147 ± 10
Hb (g/L)	13.68 ± 1	13.1 ± 0.86	13.34 ± 0.3	12.74 ± 0.14

Values are mean ± SEM. Data of physiological parameters between of two groups were analyzed by t test. MABP; Mean arterial blood pressure, Hb; Hemoglobin concentration, MCAO; Middle cerebral artery occlusion, and OXT; Oxytocin.
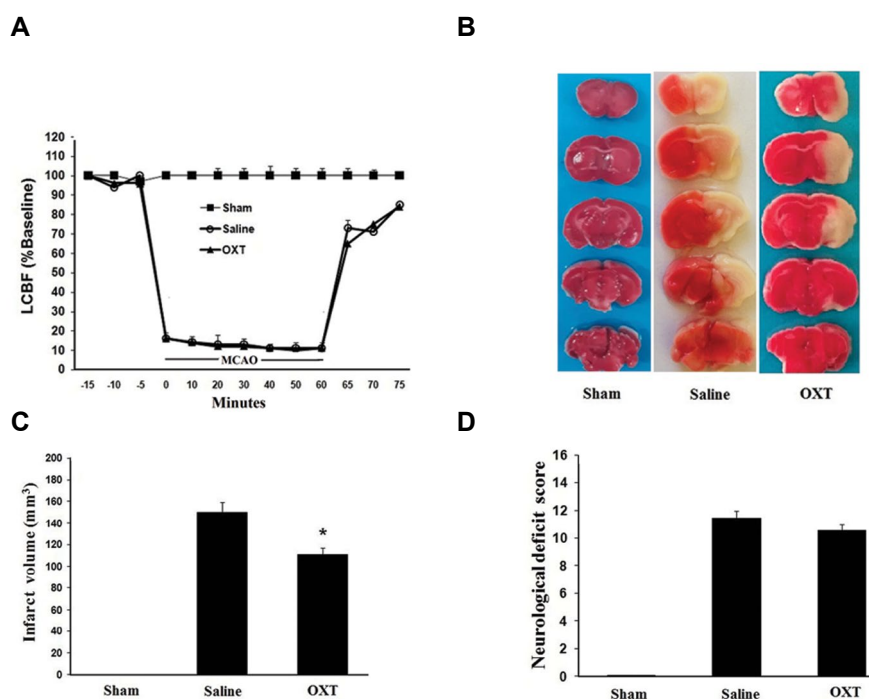

Fig.1: The effect of OXT on the brain injury. **A.** LCBF. **B.** TTC staining image. **C.** Infarct volume. **D.** Neurological deficit scores in the sham operated, saline (control) and OXT groups. White color display damage and red color show normal area. Values are as mean ± SEM (n=7, each). *; P<0.01, compared to the saline (control) group, OXT; Oxytocin, TTC; Triphenyl tetrazolium chloride, and LCBF; Local cerebral blood flow.

The results of spatial memory displayed that in all groups 4 days after training, the time to find the location of the platform (escape latency) was significantly shorter (P<0.01, Fig.2A). After ischemia, the time to find the place of the platform considerably enhanced, and the time spent in the target zone diminished (P<0.01, Fig.2B, C). However, intranasal pre-ischemic administration of OXT for 7 days did not significantly recover these parameters (P>0.05, Fig.2B, C).

The effect of pre-ischemic treatment with oxytocin on the NF-κB, TNF-α, IL-1β, and BDNF proteins

After the interruption of brain blood flow, the level of NF-κB protein considerably increased in the brain compared to the sham groups. OXT pretreatment for 7 days prior to ischemia considerably decreased the

expression of NF-κB protein (P<0.001, Fig.3A).

Twenty-four hours after ischemia, protein levels of TNF-α, and IL-1β were significantly enhanced in the saline (control) group (P<0.001, Fig.3B, C). Pre-treatment with OXT significantly suppressed the synthesis of TNF-α and

IL-1β in the brain tissue (P<0.001, Fig. 3B, C).

The ELISA assessment demonstrated that intranasal administration of OXT for one week before ischemia significantly increased the level of BDNF protein in the brain (P<0.001, Fig.3D).
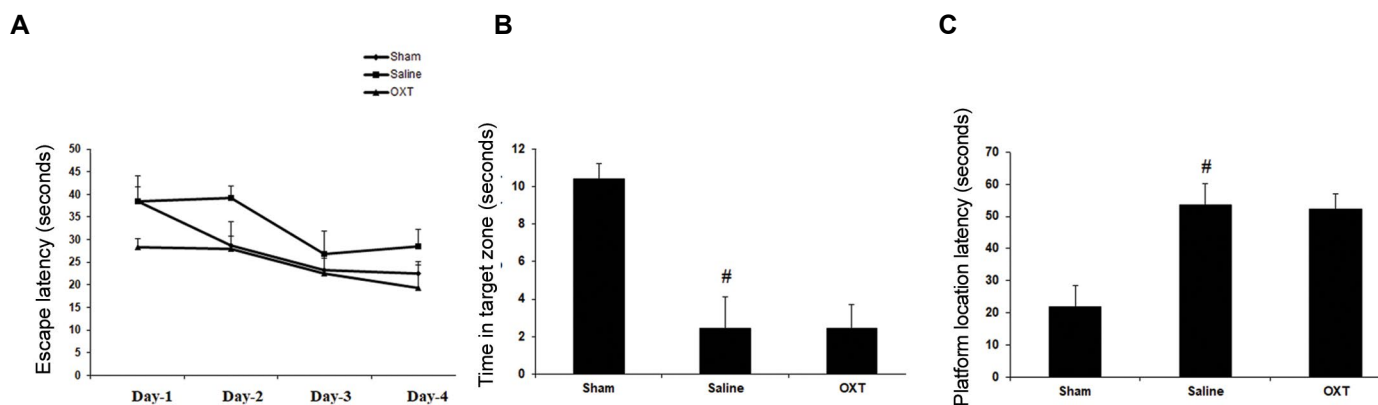

Fig.2: The effect of oxytocin (OXT) on the spatial learning and memory. **A.** Time (second) of 4-days training (escape latency). **B.** Spent in the target zone. **C.** Latency to discovery the platform place in sham-operated, saline (control) and OXT groups. Values are mean ± SEM (n=7, each). #; P<0.001 compared to respective sham-operated group.
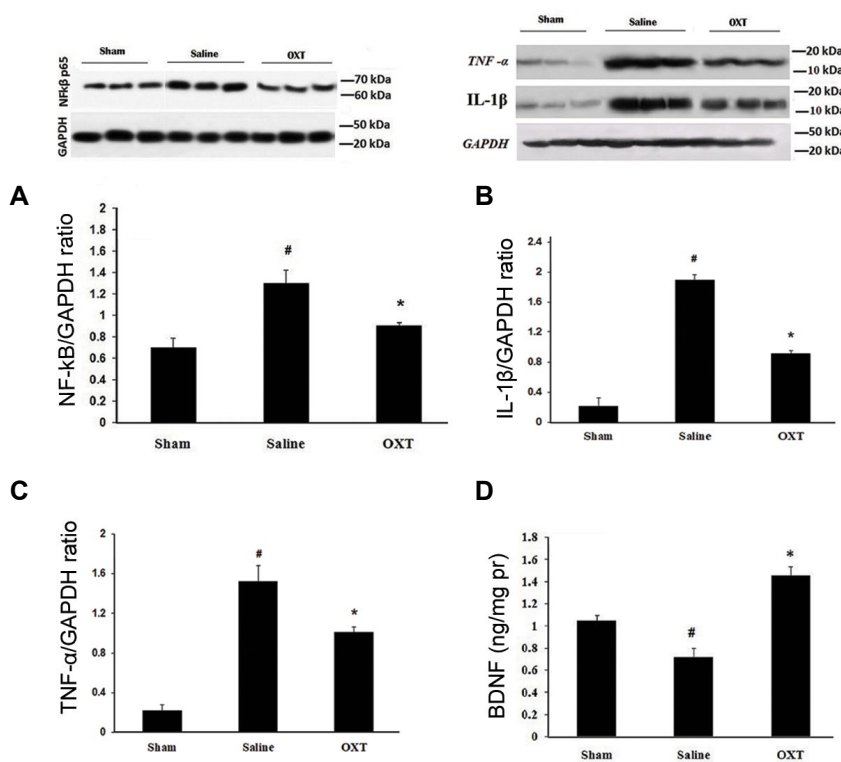

Fig.3: The photograph demonstrates the level of NF-kB, TNF-α, IL-1β and BDNF proteins in the sham-operated and saline (control) and oxytocin (OXT) groups. **A.** The quantitative examination displays as NF-kB/GAPDH. **B.** TNF-α/GAPDH. **C.** IL-1β/GAPDH ratio. **D.** BDNF (ng/mg Pr). Values are mean ± SEM (n=3). *; P<0.001 compared to the saline (control) group and #; P<0.001 compared to respective sham-operated group.

The effect of pretreatment with oxytocin on the expressions of apoptotic regulator proteins

Pro-apoptotic proteins (Bax) significantly increased and anti-apoptotic protein (Bcl2) decreased after an ischemic episode in the brain. Proteins of Bcl-2 were downregulated, and Bcl-2 was significantly upregulated in the group that was pretreated with OXT. The Bcl-2/Bax ratio was also noticeably enhanced in the OXT pre-treated group (P<0.001, Fig.4A-D).

The effect of pre-ischemic treated with oxytocin on the expression of MMP-9

After ischemic stroke, immunohistochemistry analysis showed that the expression of MMP-9 markedly enhanced in the cortex and hippocampus tissues. Intranasal administration of OXT for 7 days prior to ischemia noticeably decreased the expression of MMP-9 protein in these two parts of the brain (P<0.001, Fig.5A, B).
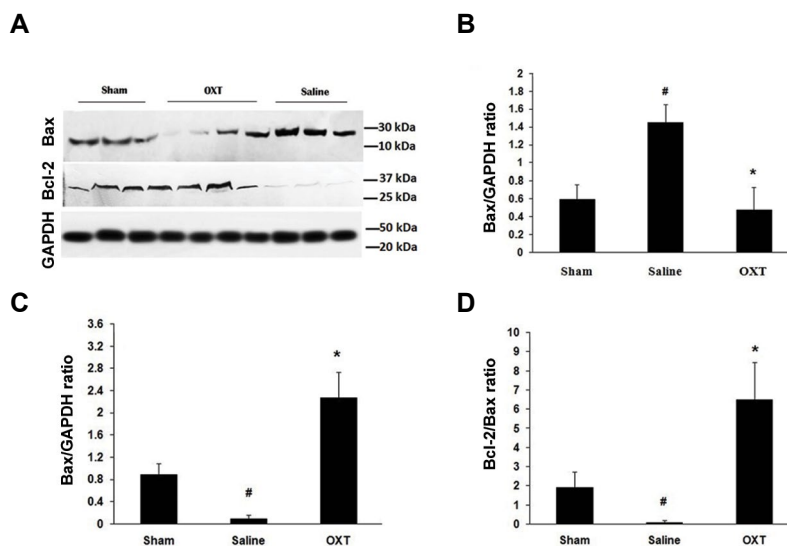

Fig.4: The effect of oxytocin (OXT) on the expressions of apoptotic regulator proteins. **A.** The image demonstrates the levels of Bax and Bcl-2 proteins in the sham-operated and saline (control) and OXT groups as identified by western blotting. **B.** The quantitative analysis illustrated Bax/GAPDH. **C.** Bcl-2/GAPDH. **D.** Bcl-2/Bax ratio in the brain tissue (n=3-4, each). #; P<0.001 from respective sham-operated group and *; P<0.001 compared to the saline (control) group.
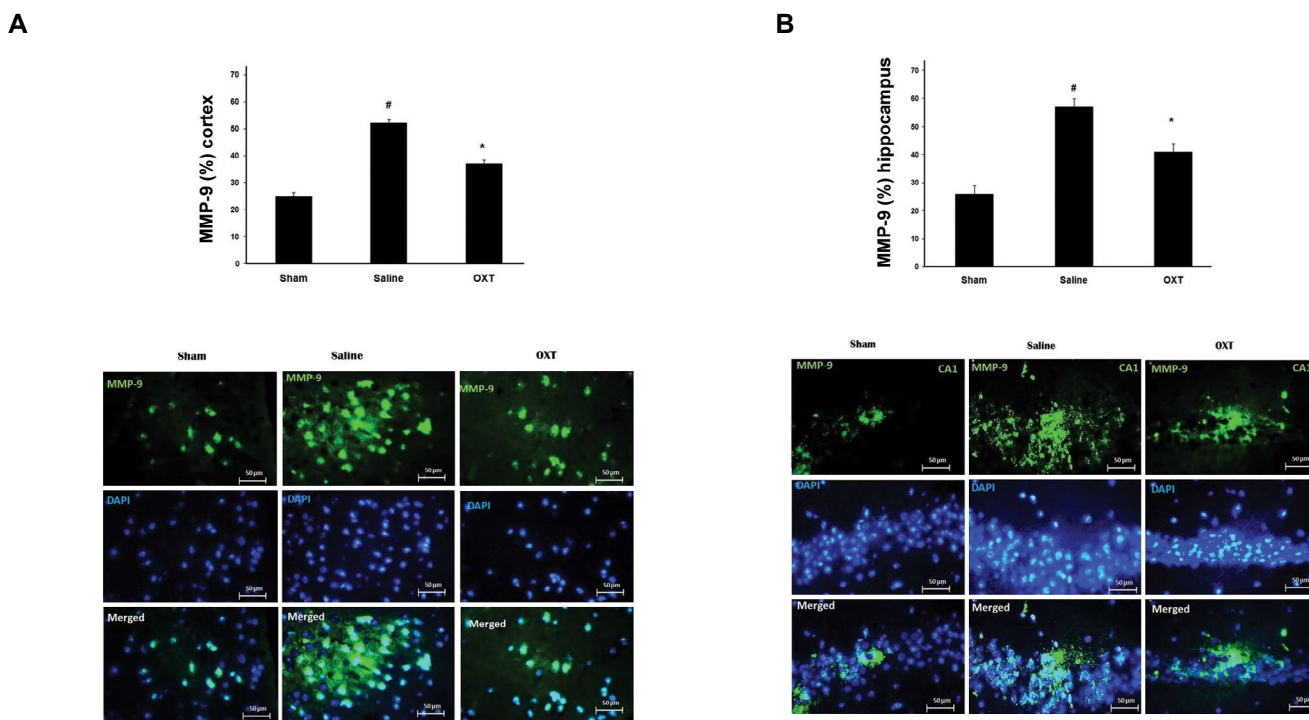

Fig.5: The effect of oxytocin (OXT) on the expression of MMP-9. **A.** Photograph of MMP-9 immune-like reactivity and amount of MMP-9 expression the cortex. **B.** Hippocampus in sham-operated and saline (control) and OXT groups. MMP-9 immune-like reactivity (green) were presented as percentages of the total number of DAPI -stained nuclei (blue) (400× fluorescent microscope). Results presented as mean ± SEM (n=3, each). #; P<0.001 compared to respective sham-operated group and *; P<0.001 compared to the saline (control) group.

Discussion

The principal finding of the current study was that one-week intranasal administration of OXT prior to cerebral ischemia attenuates brain injury by reducing the expressions of NF-κB, pro-inflammatory cytokines (TNF-α, IL-1β), MMP-9, inhibition programmed cell death machinery and enhancing of BDNF protein. Moreover, OXT pretreatment did not correct spatial memory and neurological dysfunction 24 hours after stroke in mice.

Intranasal injection of OXT for 7 days before ischemia declined the infarct size by 26% when compared to the control group; however, it was unable to recover spatial memory and neurological disorders. The reason why OXT pretreatment did not change neurological functions, despite reducing the infarct size, is not clear. According to our previous studies and others (18-20), there is not necessarily a straightforward relationship between the reduction of cerebral damage and improvements in neurological functions. Our previous studies (13, 16, 18) show that in an animal model of stroke improvements in the behavioral disorders mainly occur when an intervention reduces the brain lesion by more than 50%. In the present study, however, our intervention declined brain injury by 25%. This hypothesis is supported by several experimental and clinical studies, which have reported there is not always a direct link between infarct size and neurological outcomes (18-20). However, the possibility of OXT application, as a preventive approach in a high-risk population, needs to be more explored. Therefore, we suggest performing more studies in experimental and clinical trials.

NF-κB is a transcriptional factor, which is activated during the acute phase of cerebral ischemia and plays a detrimental role in stroke pathogenesis by increasing the gene expression of many inflammatory and pro-inflammatory cytokines such as TNF-α and IL-1β (6, 21). It is well established that activation of NF-κB and pro-inflammatory cytokines such as IL-1β and TNF-α in the early stage of stroke could exacerbate brain damage (21, 22). Our findings revealed that pre-treatment with OXT attenuated the expression of pro-inflammatory cytokines (IL-1β and TNF-α) by inhibiting the NF-κB p65 in the brain tissue. This result demonstrates the anti-inflammatory effect of OXT. The anti-inflammatory properties of OXT may be related to the inhibition of nuclear translocation of NF-κB p65 in the microglia or neurons. However, an additional experimental study is required to confirm this hypothesis. Inhibition of NF-κB, TNF-α, and IL-1β by OXT may be responsible for part of its protective effects on cerebral ischemia in the present study. Several in vitro and in vivo studies have shown that OXT has anti-inflammatory activity by inhibiting the expressions of NF-κB and pro-inflammatory cytokines (11, 23, 24), which confirms our finding.

The apoptotic signaling pathway is controlled by many apoptotic-related proteins including Bcl2 family proteins, Bax, Bad, and Bcl-xL (25). Bcl-2 and Bax family proteins are highly expressed between 12-and 24 hours after cerebral ischemia (26). Overexpression of the anti-apoptotic protein Bcl-2 protects the neuronal cell against apoptosis, while the activation of the apoptotic protein Bax triggers apoptosis and neuronal injury in the acute phase of stroke (27). The results of the current study showed that one week of pre-treatment of mice with OXT reduced the apoptosis by down-regulating pro-apoptotic, BAX, and up-regulation anti-apoptotic, BCL2. Our findings are in agreement with Dalia et al.'s study, which showed that pre-treatment with OXT 7 days before myocardial infarction diminished heart injury via reducing Bax and p53 as makers of apoptosis in rats (28). In addition, several studies have reported that OXT has anti-apoptotic effects in in vitro and in vivo situations that confirm our results (28-30).

MMPs belong to the family of protease enzymes, and it has an important contribution to physiology and pathological processes such as extracellular matrix remodeling and cerebral ischemia (31, 32). Data obtained from previous preclinical studies indicated that the activation of MMP-9 in the acute phase of stroke plays a destructive role in the pathogenesis of brain trauma, focal, and global cerebral ischemia may be via interrupting blood-brain barrier, edema formation, and myelin injury (4, 31, 32). A clinical study also showed that there is a link between MMP-9 and the risk of ischemic stroke in humans (33). The finding of our study showed that pretreatment with OXT significantly decreased the expression of MMP-9 in the cortex and the hippocampus following cerebral ischemia in mice. Recently, a study indicated that OXT in a dose-dependent manner inhibits TNF-α induced MMP-1 and MMP-13 expressions at the gene and protein levels in isolated human chondrocyte cells (15), which is somewhat in agreement with our findings. There is some evidence that metalloproteases play a significant role in activating the programmed cell death machinery of apoptosis. For instance, Dang et al. (34) showed that inhibition of MMP2/MMP9 attenuates spinal cord injury via diminishing apoptosis in the mouse model. Therefore, we can conclude that OXT by reducing MMP-9 expression and subsequently inhibiting programmed cell death machinery led to diminishing the infarct size in the present study. Previous studies have reported that various types of cells including neurons, microglia, endothelial cells, and neutrophils can express MMP-9 after cerebral ischemia (35, 36). However, the major cellular source of MMP-9 in ischemic stroke is not exactly clear. Experimental evidence has shown that in the ischemic core, MMP-9 is likely produced by both infiltrating neutrophils and microglia (36). In peri-infarct areas, the MMP-9 is mainly produced by microglial cells (36, 37). Although the cellular source of MMP-9 in the present study is not clear, regarding the size of MMP-9 positive cells (about 15-30 μm wide), it can be concluded that the main source of MMP-9 in microglia cells (38).

Neurotrophic factors such as BDNF, play a key role in

the regulation of neuroplasticity, neurogenesis, and also the recovery of brain damage after strokes (5). The present study showed that one week of daily administration of OXT before ischemia resulted in elevated cerebral BDNF protein levels, which were associated with improving brain injury. There is growing evidence indicating that part of the OXT function was done through interaction with the BDNF in various tissues such as the brain (14, 39, 40). For example, Dayi et al. (14) showed that intranasal administration of OXT increased the levels of BDNF in the brain following chronic stress in rats.

Conclusion

The results of this study indicated that intranasal OXT before ischemia limited stroke-induced brain injury by downregulating NF-κB, pro-inflammatory cytokines (IL-1β, TNF-α), MMP-9, and apoptotic mediators Bax proteins and up-regulating anti-apoptotic Bcl-2 and BDNF protein in mice. We suggest that OXT may be potentially useful in the prevention and/or reducing the risk of cerebral stroke attack and can be offered as a new prevention option in the clinics. However, the possibility of using OXT, as a prophylactic agent to reduce the risk of a stroke attack, needs to be clarified.

Acknowledgments

Authors' Contributions

A.V., M.Z.K.; Contributed to conception and design research. S.M., A.A.V, M.Z.K., A.V; Contributed to all experimental work, data and statistical analysis, and interpretation of data. A.V.; Contributed extensively in the interpretation of the data and the conclusion, responsible for overall supervision, molecular experiments were done in Basic Medical Science Research, Histogenotech Company, Tehran, Iran. All authors read and approved the final version of the manuscript.

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