Physiology and Pharmacology

Physiol Pharmacol 20 (2016) 48-56 **www.phypha.ir/ppj**

Original Article

Inhibitory effects of oxytocin on the inflammatory parameters and vascular endothelial growth factor (VEGF) in the rat air pouch model of inflammation

Elliyeh Ghadrdan[1], Moslem Najafi[2], Sevda Mikaily Mirak[1], Tahereh Eteraf-Oskouei[2]*

1. Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Introduction: The aim of the present study was to evaluate the effect of oxytocin on the angiogenesis and inflammatory parameters in air pouch model of inflammation.

Methods: Inflammation was induced by injection of carrageenan into pouches in male Wistar rats. Oxytocin (4.25, 8.5 and 17 µg) was administered intra pouch at the same time as the carrageenan and then for 2 consecutive days. After 72 h, the pouches fluid was collected to determine exudates volume, interleukin 1-beta (IL-1ß) and vascular endothelial growth factor (VEGF) concentrations. Then, the pouches were dissected out, weighed and the hemoglobin concentration was assessed.

Results: All three doses of oxytocin (4.25, 8.5 and 17 µg) significantly decreased volume of exudates ($P<0.05$, $P<0.01$ and $P<0.001$, respectively) while leukocyte accumulation in the pouch fluid was diminished by 8.5 and 17 µg oxytocin. The granulation tissue weight was also markedly reduced in comparison with the control group. A significant reduction in the angiogenesis rate in oxytocin-treated rats by all doses was seen. Interestingly, there was no significant difference between the effect of oxytocin and diclofenac on the inhibition of angiogenesis, VEGF concentration and inflammatory parameters except leukocyte accumulation. In addition, administration of oxytocin (17 µg/pouch) significantly decreased IL-1ß level (47%) compared to the control group ($P<0.05$).

Conclusion: Oxytocin has an anti-inflammatory effect and inhibits cell influx and exudation to the site of the inflammatory response. The anti-angiogenesis effect of oxytocin may be related to the local inhibition of VEGF levels as similarly shown by diclofenac.

Keywords:
Oxytocin;
Air Pouch;
Inflammation;
VEGF;
Angiogenesis

Received:
14 Sep 2015
Accepted:
24 Feb 2016

***Correspondence to:**
T. Eteraf-Oskouei

Tel: +98 41 33372250
Fax: +98 41 33344798

Email:
eteraf_t@yahoo.com

Introduction

In the recent decades, there have been increasing evidence that a bi-directional communication exists among the immune, endocrine and central nervous systems which plays an important role in maintaining the body's homeostasis (Jiang et al., 1998; Lawrence and Kim, 2000; Maier, 2003; Quan and Banks, 2007; Tian et al., 2012). Oxytocin is a nonapeptide neurotransmitter, hormone produced in hypothalamic nuclei (Szeto et al., 2013). It is mainly involved in uterine contraction during parturition and the milk ejection reflex during lactation (Petersson et al., 2001). Evidence for anti-inflammatory activity of

oxytocin has been found in recent years (Petersson et al., 2001; Iseri et al., 2005b; Jankowski et al., 2010; Ahmed and Elosaily, 2011; Szeto et al., 2013). Kim et al., (2016) showed that oxytocin receptor-antagonist atosiban treatment stimulated inflammatory pathway activation in amnion and increased expression and excretion of the inflammatory cytokines (Kim et al., 2016).

The roles of oxytocin in the cardiovascular system, in the wound healing and experimental sepsis are beginning to be understood. Oxytocin treatment has been shown to modulate immune and anti-inflammatory response (Jankowski et al., 2010). Oxytocin receptors in regulatory T cells have been found (Elands et al., 1990), which may play a role in the blocking of autoimmune process and in termination of the inflammatory response.

Here, we examined oxytocin effects on angiogenesis and inflammatory parameters in the air pouch model. Injection of carrageenan induces inflammation and the pouch provides a localized environment for study of inflammation and cellular response because the pouch has morphology similar to the synovium (Martin et al., 1994; Maleki-Dizaji et al., 2010).

Angiogenesis and inflammation are mutually dependent processes. Inflammation can promote angiogenesis in various ways. For example, macrophages produce several potent angiogenic factors including vascular endothelial growth factor (VEGF), prostaglandins E_2 (PGE_2), interleukin 1 (IL-1), and nitric oxide (NO). Angiogenesis, in turn, contributes to inflammatory pathology, therefore inhibition of angiogenesis is essential to suppress chronic inflammatory diseases (Naldini and Carraro, 2005; Clavel et al., 2006). The key signaling system that regulates angiogenesis is VEGF and is based on its ability to induce vasodilatation, increasing endothelial cell permeability and recruitment of inflammatory cells (Milkiewicz et al., 2005; Karamysheva, 2008). Now, it is accepted that maintaining and promoting the rheumatoid arthritis (RA) is mainly related to angiogenesis so that using of anti-angiogenic agents may suppress or retard arthritis progression (Clavel et al., 2006).

In the present study we also investigated the production level of IL-1ß and VEGF, which are reported to be crucial in up-regulating the angiogenesis responsible for the chronic inflammatory response causing RA (Chakrabarti et al., 2010).

Materials and methods

Experimental design

All experimental protocols and treatments were approved by Tabriz University of Medical Sciences ethical committee for animal research (No. 5.52.137).

Male Wistar rats weighing 200-250 g (Razi Institute, Iran) were used in this study. The animals were kept at conditions of controlled temperature (20±2 °C) and relative humidity (50±10%) in standard polypropylene cage, 6 rats per cage, under 12 h light-dark cycle. They were fed on a standard diet with free access to water. The rats were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the study. The animal care and handling procedures were done in accordance with the Animal Ethics Guidelines at the Tabriz University of Medical Sciences and “Principles of laboratory animal care” (NIH publication No. 86-23, revised 1985).

The rats were divided into 7 groups (at least 6 rats in each group) including control (saline), control (carrageenan), positive control (diclofenac sodium: 1mg/rat) and three oxytocin-treated groups (4.25, 8.5 and 17 µg/pouch). Except saline control, all the animals received 1 ml of carrageenan 1 % as a phlogistic agent. Oxytocin was purchased from Caspian-Tamin, Iran. Stock solutions were diluted with saline and then 1 ml of the diluted solution containing the indicated amount of the drugs were injected into the pouch of each rat at the same time as the carrageenan and then for 2 consecutive days. Exudate volume, the levels of VEGF and IL-1ß in the pouch exudate, white blood cell (WBC) count, granulation tissue weight and angiogenesis were studied after 72 h.

Induction of air pouch type inflammation by carrageenan in rats

To induce an air pouch, the rats were anesthetized with halothane, the backside of rat disinfected with 70% ethanol, and then 20 ml of sterile air was injected on the back of rats subcutaneously. After 2 days, the pouches were reinflated with 10 ml of sterile air. Six days after the initial air injection, inflammation was induced by injection of 1 ml of a 1% w/v

suspension of carrageenan (Sigma, USA) in sterile 0.9% saline into the pouch. Carrageenan suspension had been sterilized by autoclaving at 121 °C for 15 min and adding 0.1 mg of penicillin G potassium and 0.1 mg streptomycin sulfate (Jaber Ebn-e-Hayyan, Iran) per ml of the suspension after cooling to 40–45°C (Martin et al., 1994).

Determination of pouch exudate volume and leukocytes count

On day 9, the rats were sacrificed by halothane overdose. Then 3 ml of phosphate-buffered saline (PBS) were injected into the pouches and they were massaged for 30 seconds subsequently. They were opened and total pouch fluid was collected and its volume was measured. The exudate samples were collected in the EDTA-treated glass tubes, and were refrigerated at 4 °C. The pouch fluid was diluted with PBS and the leukocyte numbers were counted with a hemocytometer.

Determination of granulation tissue weight and angiogenesis

The procedure of air-pouch model of inflammation stimulates the proliferation of cells that cover the surface of the cavity to form a structure similar to a synovium (Edwards et al., 1981). The injection of air over a period of 6 days produced a lining of granulation tissue. Three days after the injection of carrageenan solution, the granulation tissue formed was dissected and weighed. The tissue was washed in PBS, pH=7.4, and cut into small pieces before being homogenized in 2.0 ml of Drabkin reagent (Ziestchem Diagnostics, Iran) using a homogenizer (HO4AP-Edmund Buhler, B. Braun, Germany) for 4 min at scale 40 on an ice bed. The tissue homogenate was centrifuged at 10,000 g in 4 °C for 30 min. The supernatants were filtered through a 0.22 µm filter. The hemoglobin concentration in the supernatant was then determined spectrophotometrically by measuring absorbance at 540 nm using a hemoglobin assay kit (Hemoglobin Colorimetric-method, Ziestchem Diagnostics). The amount of hemoglobin in the granulation tissue was expressed as mg hemoglobin/100 g wet tissue (Ghosh et al., 2002).

Determination of IL-1ß and VEGF concentrations in the pouch fluid

Injection of carrageenan induces inflammation and the pouch serves as a reservoir of mediators that can be easily measured in the fluid that accumulates locally. Three days after the injection of carrageenan solution, the rats were sacrificed by halothane overdose. The pouches were opened with a small incision and the exudates were collected. The pouch fluid was centrifuged at 10,000 g for 10 min to remove infiltrating leukocytes. The levels of IL-1ß and VEGF were measured in supernatants using commercial ELISA kits, (Glory Science Company, USA & Biospes Company, China, respectively) according to the manufacturer's instructions.

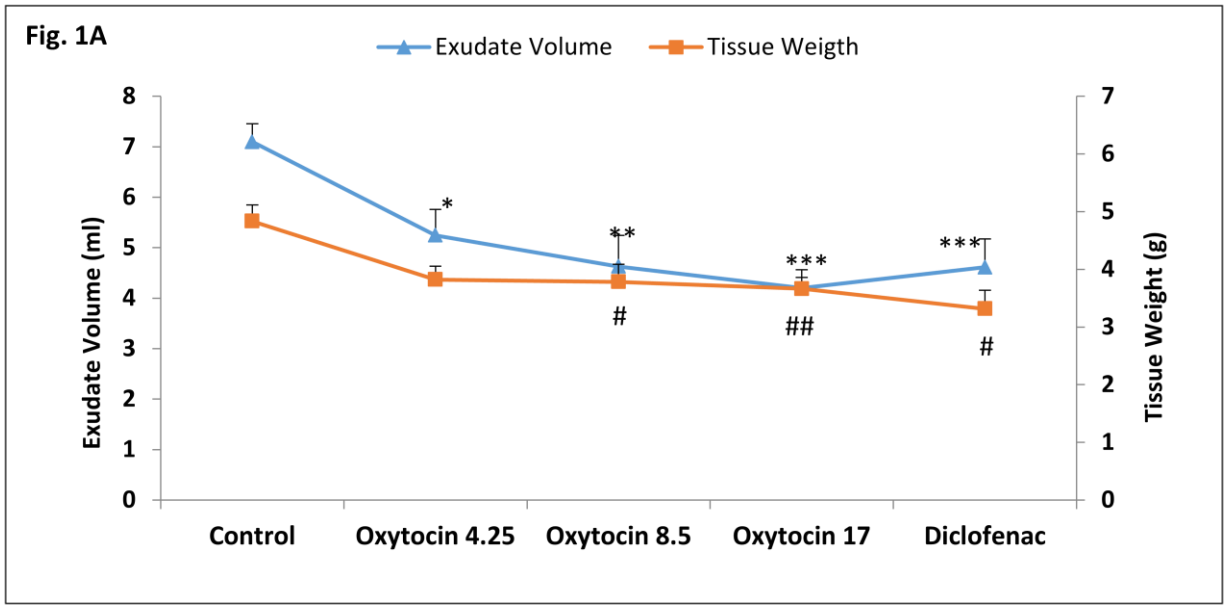
Statistical Analysis

All results were expressed as mean±SEM. The statistical significance of differences between groups was obtained by means of one way analysis of variance (ANOVA) followed by LSD post-test for multiple comparisons. Differences between groups were considered significant at a level of $P<0.05$.

Results

Effects of oxytocin on exudate volume, leukocytes accumulation and granulation tissue weight

In the three days postcarrageenan challenge, as shown in Figure 1A, by intra-pouch injection of oxytocin (4.25, 8.5 and 17 µg/pouch) the volume of exudates recovered from air-pouches decreased dose dependently (5.2±0.5 ml: $P<0.05$, 4.6±0.6 ml: $P<0.01$, and 4.2±0.4 ml: $P<0.001$, respectively) compared to that observed with carrageenan alone (7.1±0.4 ml). Granulation tissue weight was evaluated at 72 h after induction of inflammation by intra-pouch injection of carrageenan. Oxytocin was injected into the pouch immediately before injection of carrageenan and once a day on consecutive days. As indicated in Figure 1A, oxytocin significantly decreased granulation tissue weight (from 4.83±0.3 g in control to 3.78±0.3 g ($P<0.05$), and 3.66±0.2 g ($P<0.01$) in groups treated with 8.5 and 17 µg/pouch oxytocin, respectively). The weight of granulation



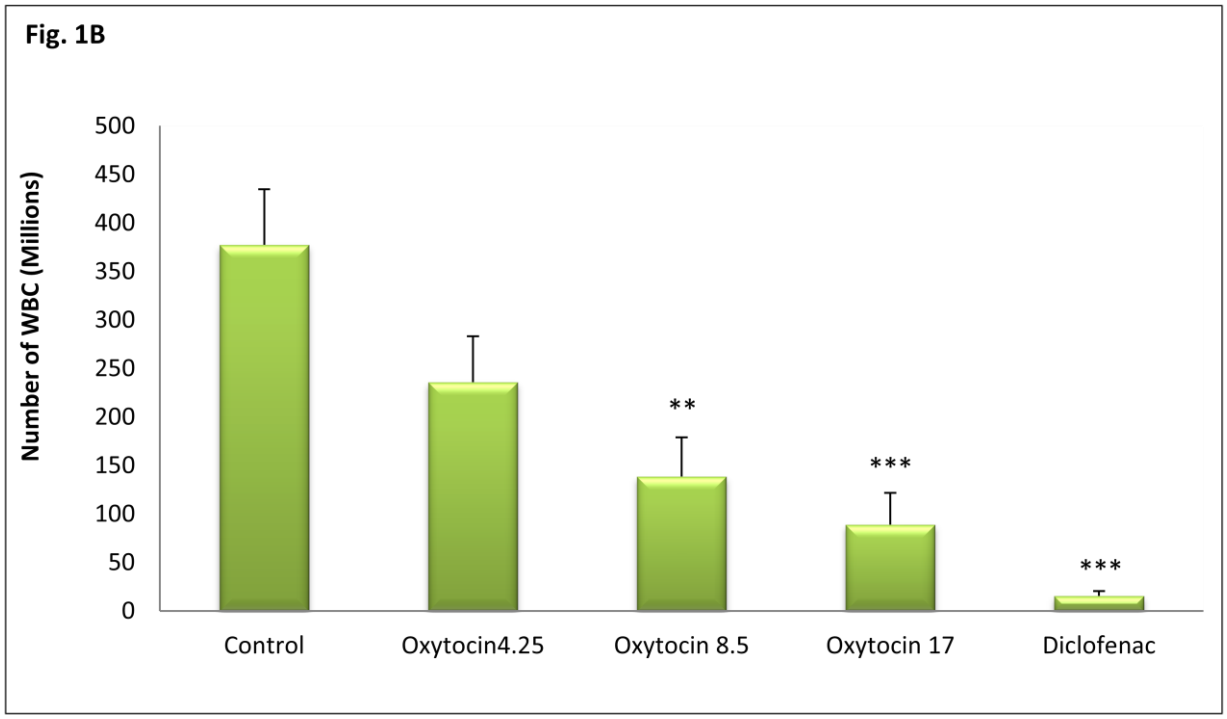


Fig.1. Effects of oxytocin on inflammatory parameters 72 h after carrageenan injection. One ml of a 1% carrageenan solution in saline was injected into the air pouch. Oxytocin by doses of 4.25, 8.5 and 17 µg/pouch, normal saline (control) or diclofenac (positive control) dissolved in 1 ml of saline were injected into the pouch just before carrageenan injection and then once a day on two consecutive days. Exudates volume, granulation tissue weight (A), and leukocytes count (B) were determined 72 h after carrageenan injection. Values are the mean±SEM shown by vertical bars. Asterisks and # indicate significant change from control; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ and $^{\#}P<0.05$, $^{\#\#}P<0.01$.

tissue with 17 µg/pouch of oxytocin was not significantly changed compared to the positive control (3.32±0.3 g).

As shown in Figure 1B, intra-pouch injection of oxytocin resulted in significant decrease in accumulation of leukocytes ($P<0.05$ and $P<0.001$ for doses of 8.5 and 17 µg/pouch, respectively) compared to that observed with carrageenan alone (377.34×10^{6}).

As expected, intra pouch administration of diclofenac (1.0 mg/kg) as positive control caused significant inhibition of leukocytes and exudation.

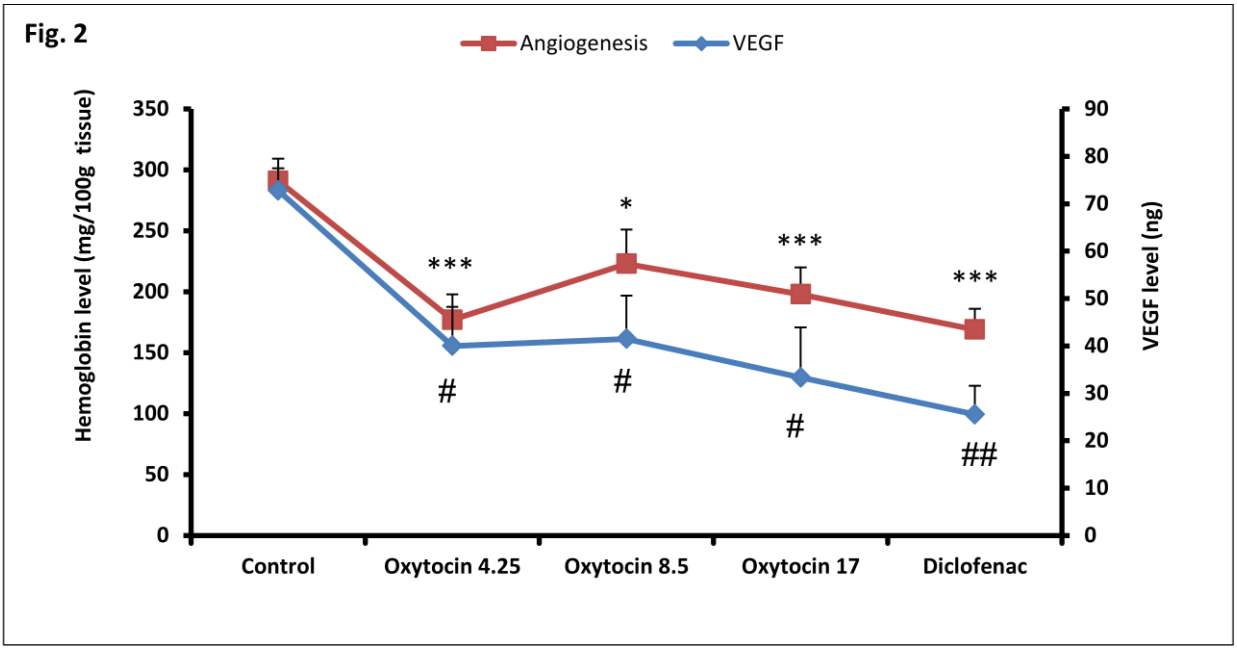


Fig.2. Effects of oxytocin on hemoglobin level as a marker of angiogenesis and VEGF level 3 days after carrageenan injection. One ml of a 1% (w/v) carrageenan solution in saline was injected into the air pouch. Oxytocin by doses of 4.25, 8.5 and 17 µg/pouch, normal saline (control) and diclofenac sodium (positive control) were injected into the pouch just before carrageenan injection and then for 2 consecutive days. Three days after carrageenan injection, VEGF level of exudate fluid and hemoglobin level in granulation tissue were determined. Values are the mean±SEM shown by vertical bars. Asterisks and # indicate significant change from control; *P<0.05, ***P<0.001, $^{\#}$P<0.05, $^{\#\#}$P<0.01.

Effects of oxytocin on angiogenesis in granulation tissue

Seventy-two hours after carrageenan injection, angiogenesis was measured by evaluating the hemoglobin concentration in the vascularized granulation tissue. As shown in Table 1, there was a significant decrease in hemoglobin content in oxytocin-treated rats when compared with the control rats by all three used doses (from 291±10.5 mg/100 g wet tissue in control to 177±21 mg: P<0.001, 223±28mg: P<0.05 and 198±22 mg: P<0.01 in the groups treated with 4.25, 8.5 and 17 µg/pouch of oxytocin, respectively). There was no significant difference between the effects of 4.25 µg/pouch oxytocin and diclofenac sodium on the hemoglobin contents of granulation tissue.

Detection of VEGF and IL-1ß in the pouch fluid, and effects of oxytocin

To investigate the modulatory effect of oxytocin on the inflammatory cytokines, the production of VEGF and IL-1ß were determined. The animals were treated with oxytocin or vehicle just before carrageenan injection and then once a day on two consecutive days. Then, VEGF and IL-1ß levels were measured in the inflammatory exudates extracted from air-pouches 72 h after induction of inflammation. Oxytocin showed inhibitory activity against VEGF in the inflammatory exudate with all doses (P<0.05) and this effect was correlated to the hemoglobin level in the granulomatous tissue reduction (Figure 2).

In addition, oxytocin (17 µg/pouch) decreased IL-1ß level (47%) in comparison with the control group (from 185.8±12.2 ng in the control group to 98.9±21.8 ng in the treatment group; P<0.05). Moreover, diclofenac sodium showed inhibitory effect (88.7±18.5 ng, P<0.01) on the IL-1ß level (Table 2). There was no significant difference between the effects of oxytocin and diclofenac sodium on the IL-1ß contents of inflammatory exudate (P>0.05).

Discussion

The present study was designed to examine the effects of oxytocin on the inflammatory parameters in air pouch model. The obtained results showed that intra pouch administrations of oxytocin with doses of 4.5, 8.5 and 17 µg/pouch decreased the levels of total

Table1: Effect of oxytocin on angiogenesis in granulation tissue versus control (Carrageenan)

	Carrageenan	Carrageenan + Diclofanac	Carrageenan + oxytocin 4.25	Carrageenan + oxytocin 8.5	Carrageenan + oxytocin 17
Angiogenesis (mg/100 g tissue)	291±10.5	169±17***	177.5±22***	223.3±28*	198.2±21***
VEGF (ng)	72.8±6.8	25.6±6**	40±8.3*	41.5±9.1*	33.4±10.5*

Data represented as Mean±SEM. Asterisks indicate significant change from control (Carrageenan); * P<0.05, ** P<0.01, *** P<0.001.

Table2: Effect of oxytocin on inflammatory mediators in exudate of pouch versus control (Carrageenan)

	Carrageenan	Carrageenan + Diclofanac	Carrageenan + oxytocin 17
IL1b (ng)	185.8±12.2	88.7±18.5**	98.9±21.8*
VEGF (ng)	72.8±6.8	25.6±6**	33.4±10.5*

Data represented as Mean±SEM. Asterisks indicate significant change from control (Carrageenan); * P<0.05, ** P<0.01.

leukocyte. In addition, oxytocin significantly reduced the volume of exudates in the pouch fluid.

Recent research has demonstrated a new role for oxytocin in regulating angiogenesis responses, but there is no agreement on how and where oxytocin is exerts its effect in this condition (Ross et al., 2013). Oxytocin may probably affect the number of immune cells and their migration into the tissue or the kinds of activating signals expressed (Croll et al., 2004).

In this study, our data illustrated that oxytocin can inhibit inflammatory mediators such as IL-1ß level. In consistent with our results, other researchers showed that oxytocin decreased the plasma levels of IL-6, IL-1β and TNF-α (Iseri et al., 2005b; Clodi et al., 2008).

The present study was also aimed to determine the potential anti-angiogenic effect of oxytocin in an *in vivo* inflammatory model. Oxytocin effect on the VEGF level reduction was coincident with decreasing in angiogenesis (Pearson Correlation Coefficient: p=0.049, r= 0.5). Therefore, one of the possible mechanisms that could support the inhibitory effect of oxytocin on the angiogenesis is the VEGF suppression in the inflammatory exudate (Yeniel et al., 2014). VEGF, the most potent angiogenic growth factor, stimulates proliferation, migration, and tube formation in endothelial cells (Chakrabarti et al., 2010). In addition, reports by Croll et al demonstrated that VEGF may act as a potent pro-inflammatory cytokine. Indeed, VEGF elevates leukocyte infiltration mediated by the increase in adhesion protein ICAM-1. The interaction of leukocytes with ICAM-1 appears necessary for the induction or augmentation of inflammation (Croll et al., 2004). Yeniel et al showed that oxytocin can decrease inflammatory cytokine levels in plasma and peritoneal fluid and VEGF level in a rat endometriosis model (Yeniel et al., 2014). Various factors are involved in the regulation of VEGF and consequently they influence the angiogenesis. Many of inflammatory mediators especially PGE_2, NO, TNF-α, IL-6, and IL-1 can stimulate both the VEGF mRNA and protein levels (Jackson et al., 1997; Wang et al., 2009). In our data, the inhibitory effect of oxytocin on IL-1β was observed. On the other hand and in contrast to our results, some studies have shown that oxytocin has a pro-angiogenic role during the embryonic development of the hypothalamos-neurohypophyseal system (Cattaneo et al., 2008; Gutnick et al., 2011). One possible explanation for these contradictory results may be the differences in model or dose of oxytocin used.

Reactive oxygen species (ROS), one of the angiogenic factors, has a significant role in the VEGF-mediated angiogenesis process (Chakrabarti et al., 2010). Activated neutrophils and macrophages are ROS producer that trigger the inflammatory

reactions (Iseri et al., 2005a). Activated macrophages induce neovascularization by secreting angiogenic products (Maleki-Dizaji et al., 2010). Nevertheless, oxytocin can attenuate angiogenesis through inhibition of these secretory products (Croll et al., 2004).

Monocytes, macrophages and polymorphonuclear leukocytes express some G-protein-coupled receptors for chemoattractants and chemokines (Sun and Ye, 2012). The presence of oxytocin and its receptor in the immune system implies that oxytocin not only possesses paracrine function but also is an autocrine factor that can regulate the function of immune cells. Oxytocin may also exert an alternate role by regulating and maintaining a balance of anti-inflammatory and pro-inflammatory cytokines (Jankowski et al., 2000; Ndiaye et al., 2008). Some researchers showed that adhesion molecules increase migration of endothelial cells during angiogenesis (Jackson et al., 1997), but oxytocin prevents angiogenesis by blocking NO-mediated cell adhesion (Dal Secco et al., 2003; Dal Secco et al., 2006). Despite the above mentioned mechanisms that support our finding, oxytocin is also shown to elevate NO (Iseri et al., 2008) which is known as an angiogenic agent (Kaczmarek et al., 2008). It can be postulated that the intensity of oxytocin effect on each of these factors probably determines whether it is angiogenic or anti-angiogenic agent. However, further investigation on the mechanism of action of oxytocin has to be conducted to confirm both findings.

In the current study, we also showed that administration of oxytocin decreased WBC in pouch fluid. As evidenced, recent studies have shown that oxytocin is able to suppress TNFα and IL-1, which play pivotal role in the inflammatory processes. These cytokines activate inflammatory cells such as neutrophils, macrophages or monocytes, platelets, and mastocytes (Tugtepe et al., 2007; Iseri et al., 2008). Many reports suggest that inflammatory diseases may be affected by oxidative stress (Pavlick et al., 2002; Biyikli et al., 2006). Oxytocin is shown to reduce oxidative stress and subsequent inflammation through several mechanisms. Activated neutrophils as important markers of inflammation, produce reactive oxygen metabolites and cytotoxic proteins, and also trigger the inflammatory reactions and tissue injury; all can be hampered by oxytocin (Tugtepe et al., 2007).

Biyikli et al found that oxytocin treatment displays anti-inflammatory and antifibrotic action on various tissues with inflammatory challenges (Biyikli et al., 2006). In the present study, our results clearly showed that treatment with oxytocin reduced granulation tissue formation. Fibroblast accumulation, synthesis of collagen, and angiogenesis are important elements in granulation tissue formation, and macrophages are known to play essential roles by producing various cytokines such as IL-1. In the present study, we showed that oxytocin administration inhibited WBC accumulation, IL-1 level and angiogenesis which are essential in the granulation tissue formation induced by carrageenan (Sato et al., 2005).

Conclusion

The present study is the first to demonstrate the inhibitory effects of oxytocin on the angiogenesis in an *in vivo* inflammatory model. Here, we showed that oxytocin significantly reduced angiogenesis, IL-1β and VEGF levels. We also highlight evidences for the promising anti-angiogenic and anti-inflammatory effects of oxytocin that could be mediated by different mechanisms such as attenuation of leukocyte migration, modulation of IL-1β and VEGF production. Further studies such as histopathological examinations are required to clarify the precise underlying mechanisms.

Acknowledgment

The results described in this paper were part of a Pharm. D student thesis. The authors would like to thank Drug Applied Research Center and Student Research Committee, Tabriz University of Medical Sciences for their technical and financial supports.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Ahmed MA, Elosaily GM. Role of oxytocin in deceleration of early atherosclerotic inflammatory processes in adult male rats. Int J Clin Exp Med 2011; 4: 169-78.

Biyikli NK, Tugtepe H, Sener G, Velioglu-Ogunc A, Cetinel S, Midillioglu S, et al. Oxytocin alleviates oxidative renal

injury in pyelonephritic rats via a neutrophil-dependent mechanism. Peptides 2006; 27: 2249-57.

Cattaneo MG, Chini B, Vicentini LM. Oxytocin stimulates migration and invasion in human endothelial cells. Br J Pharmacol 2008; 153: 728-36.

Chakrabarti S, Rizvi M, Morin K, Garg R, Freedman JE. The role of cd40l and vegf in the modulation of angiogenesis and inflammation. Vascul Pharmacol 2010; 53: 130-7.

Clavel G, Valvason C, Yamaoka K, Lemeiter D, Laroche L, Boissier MC, et al. Relationship between angiogenesis and inflammation in experimental arthritis. Eur Cytokine Netw 2006; 17: 202-10.

Clodi M, Vila G, Geyeregger R, Riedl M, Stulnig TM, Struck J, et al. Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men. Am J Physiol Endocrinol Metab 2008; 295: E686-91.

Croll SD, Ransohoff RM, Cai N, Zhang Q, Martin FJ, Wei T, et al. Vegf-mediated inflammation precedes angiogenesis in adult brain. Exp Neurol 2004; 187: 388-402.

Dal Secco D, Moreira AP, Freitas A, Silva JS, Rossi MA, Ferreira SH, et al. Nitric oxide inhibits neutrophil migration by a mechanism dependent on icam-1: Role of soluble guanylate cyclase. Nitric Oxide 2006; 15: 77-86.

Dal Secco D, Paron JA, de Oliveira SH, Ferreira SH, Silva JS, Cunha Fde Q. Neutrophil migration in inflammation: Nitric oxide inhibits rolling, adhesion and induces apoptosis. Nitric Oxide 2003; 9: 153-64.

Edwards JC, Sedgwick AD, Willoughby DA. The formation of a structure with the features of synovial lining by subcutaneous injection of air: An in vivo tissue culture system. J Pathol 1981; 134: 147-56.

Elands J, Resink A, De Kloet ER. Neurohypophyseal hormone receptors in the rat thymus, spleen, and lymphocytes. Endocrinology 1990; 126: 2703-10.

Ghosh AK, Hirasawa N, Ohtsu H, Watanabe T, Ohuchi K. Defective angiogenesis in the inflammatory granulation tissue in histidine decarboxylase-deficient mice but not in mast cell-deficient mice. J Exp Med 2002; 195: 973-82.

Gutnick A, Blechman J, Kaslin J, Herwig L, Belting HG, Affolter M, et al. The hypothalamic neuropeptide oxytocin is required for formation of the neurovascular interface of the pituitary. Dev Cell 2011; 21: 642-54.

Iseri SO, Gedik IE, Erzik C, Uslu B, Arbak S, Gedik N, et al. Oxytocin ameliorates skin damage and oxidant gastric injury in rats with thermal trauma. Burns 2008; 34: 361-9.

Iseri SO, Sener G, Saglam B, Gedik N, Ercan F, Yegen BC. Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. Peptides 2005a; 26: 483-91.

Iseri SO, Sener G, Saglam B, Gedik N, Ercan F, Yegen BC. Oxytocin protects against sepsis-induced multiple organ damage: Role of neutrophils. J Surg Res 2005b; 126: 73-81.

Jackson JR, Seed MP, Kircher CH, Willoughby DA, Winkler JD. The codependence of angiogenesis and chronic inflammation. FASEB J 1997; 11: 457-65.

Jankowski M, Bissonauth V, Gao L, Gangal M, Wang D, Danalache B, et al. Anti-inflammatory effect of oxytocin in rat myocardial infarction. Basic Res Cardiol 2010; 105: 205-18.

Jankowski M, Wang D, Hajjar F, Mukaddam-Daher S, McCann SM, Gutkowska J. Oxytocin and its receptors are synthesized in the rat vasculature. Proc Natl Acad Sci U S A 2000; 97: 6207-11.

Jiang CL, Lu CL, Liu XY. The molecular basis for bidirectional communication between the immune and neuroendocrine systems. Domest Anim Endocrinol 1998; 15: 363-9.

Kaczmarek MM, Blitek A, Kaminska K, Bodek G, Zygmunt M, Schams D, et al. Assessment of vegf-receptor system expression in the porcine endometrial stromal cells in response to insulin-like growth factor-i, relaxin, oxytocin and prostaglandin e2. Mol Cell Endocrinol 2008; 291: 33-41.

Karamysheva AF. Mechanisms of angiogenesis. Biochemistry (Mosc) 2008; 73: 751-62.

Kim SH, MacIntyre DA, Hanyaloglu AC, Blanks AM, Thornton S, Bennett PR, et al. The oxytocin receptor antagonist, atosiban, activates pro-inflammatory pathways in human amnion via gαi signalling. Mol Cell Endocrinol 2016; 420: 11-23.

Lawrence DA, Kim D. Central/peripheral nervous system and immune responses. Toxicology 2000; 142: 189-201.

Maier SF. Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition. Brain Behav Immun 2003; 17: 69-85.

Maleki-Dizaji N, Eteraf-Oskouei T, Fakhrjou A, Maljaie SH, Garjani A. The effects of 5ht3 receptor antagonist granisetron on inflammatory parameters and angiogenesis in the air-pouch model of inflammation. Int Immunopharmacol 2010; 10: 1010-6.

Martin SW, Stevens AJ, Brennan BS, Davies D, Rowland M, Houston JB. The six-day-old rat air pouch model of inflammation: Characterization of the inflammatory response to carrageenan. J Pharmacol Toxicol Methods 1994; 32: 139-47.

Milkiewicz M, Hudlicka O, Brown MD, Silgram H. Nitric oxide, vegf, and vegfr-2: Interactions in activity-induced angiogenesis in rat skeletal muscle. Am J Physiol Heart Circ Physiol 2005; 289: H336-43.

Naldini A, Carraro F. Role of inflammatory mediators in angiogenesis. Curr Drug Targets Inflamm Allergy 2005; 4: 3-8.

Ndiaye K, Poole DH, Pate JL. Expression and regulation of functional oxytocin receptors in bovine t lymphocytes. Biol Reprod 2008; 78: 786-93.

Pavlick KP, Laroux FS, Fuseler J, Wolf RE, Gray L, Hoffman J, et al. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. Free Radic Biol Med 2002; 33: 311-22.

Petersson M, Wiberg U, Lundeberg T, Uvnas-Moberg K. Oxytocin decreases carrageenan induced inflammation

in rats. Peptides 2001; 22: 1479-84.

Quan N, Banks WA. Brain-immune communication pathways. Brain Behav Immun 2007; 21: 727-35.

Ross KM, McDonald-Jones G, Miller GE. Oxytocin does not attenuate the ex vivo production of inflammatory cytokines by lipopolysaccharide-activated monocytes and macrophages from healthy male and female donors. Neuroimmunomodulation 2013; 20: 285-93.

Sato K, Komatsu N, Higashi N, Imai Y, Irimura T. Granulation tissue formation by nonspecific inflammatory agent occurs independently of macrophage galactose-type c-type lectin-1. Clin Immunol 2005; 115: 47-50.

Sun L, Ye RD. Role of g protein-coupled receptors in inflammation. Acta Pharmacol Sin 2012; 33: 342-350.

Szeto A, Rossetti MA, Mendez AJ, Noller CM, Herderick EE, Gonzales JA, et al. Oxytocin administration attenuates atherosclerosis and inflammation in watanabe heritable hyperlipidemic rabbits. Psychoneuroendocrinology 2013; 38: 685-93.

Tian L, Ma L, Kaarela T, Li Z. Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. J Neuroinflammation 2012; 9: 155.

Tugtepe H, Sener G, Biyikli NK, Yuksel M, Cetinel S, Gedik N, et al. The protective effect of oxytocin on renal ischemia/reperfusion injury in rats. Regul Pept 2007; 140: 101-8.

Wang C, Duan H, He L. Inhibitory effect of atractylenolide i on angiogenesis in chronic inflammation in vivo and in vitro. Eur J Pharmacol 2009; 612: 143-52.

Yeniel AO, Erbas O, Ergenoglu AM, Aktug H, Taskiran D, Yildirim N, et al. Effect of oxytocin treatment on explant size, plasma and peritoneal levels of mcp-1, vegf, tnf-alpha and histopathological parameters in a rat endometriosis model. Eur J Obstet Gynecol Reprod Biol 2014; 175: 134-9.