Contribution to A New Potential Role of Oxytocin in Combating Atherogenic Events in Hyperlipidemic Castrated Male Rats

MAGDA M. EL-HAMZAWY, M.D. 1; HEBA M. SHAWKY, M.D. 1; HANY EL-SEBAIE, M.D. 1; HEBA S. SHOUKRY, M.Sc. 1; LAILA A. RASHED, M.D. 2; MAHA B. ZICKRI, M.D. 3 and HALA GABR, M.D. 4

The Departments of Physiology 1, Medical Biochemistry 2, Histology 3 and Clinical Pathology 4, Faculty of Medicine, Cairo University

Abstract

Oxytocin was determined in many previous studies to be involved in mechanisms related to attenuation of cardiovascular disorders. We aimed in this study to investigate the role of oxytocin in weakening the atherosclerotic events. To achieve this, the present experiment involuted the main group of hyperlipidemic male rats subjected to high-fat diet regimen for 8 weeks. The second group was subjected to surgical bilateral orchiectomy. Blood samples were withdrown to measure the serum lipid profiles, high sensitive C-reactive protein (CRP), IL-6 and adiponectin levels. Biochemical analysis of gene expressed levels of IL-6, ER-alpha and adiponectin receptors 1&2 (ADIPR1, ADIPR2) in the adipose as well as vascular tissue were assessed, in addition to histopathological and morphometric estimation of associated signs of inflammatory cell infiltration in these tissues. After 8 weeks, Oxytocin therapy in our thesis displayed a potent role in the improvement of atherosclerosis through the highly significant reduction in the level of atherosclerotic markers, body weight and visceral fat in oxytocin-treated group in comparison to castrated-saline treated group. Moreover, there was a significant reduction in the inflammatory markers and Il-6 gene expression. Our results displayed a significant increase in the level of serum adiponectin, ER-α and adiponectin receptors 1&2 gene expressed levels in oxytocin-treated group in comparison to castrated-saline treated and sham-operated groups, a significant improvement in the histopathological results of the aortic and adipose tissue after administration of oxytocin, through a significant reduction in the level of infiltrating inflammatory cells and reduction in the level of apoptotic nuclei. As regards the adipose tissue, there was also a significant reduction in the infiltrating cells as well as significant reduction in the size of adipocytes and the vessels congestion.

In Conclusion: We provide evidence that oxytocin could prevent the stress that induce the atherosclerotic events thus attenuating its pathogenesis as an anti-stressor. Moreover, peripheral oxytocin administration was shown to act as an anti-inflammatory, anti-oxidant and anti-fibrotic agent.

Key Words: Atherosclerosis – Oxytocin – Aortic tissues – Adipose tissues – Adiponectin.

Introduction

RECENT work on cholesterol-fed animals exposed to social isolation or chronic stressful conditions, lack the ability to clear cholesterol from their circulation. This subsequently results in elevated levels of lipid profiles in their plasma and development of extensive atherosclerosis throughout the arterial wall [1].

Inspite of the numerous studies on the possible mechanisms responsible for the effects of atherosclerosis, yet these mechanisms are not yet fully clarified.

The over-recruitment and activation of leucocytes characteristic of early atherosclerosis is considered the driving force behind atheroma development and is regulated by the concerted activities of several cytokines, chemokines and adhesion molecules expressed by endothelial cells and surround a lipid core [2].

Furthermore, in addition to the infiltrating monocytes and macrophages to the vessel wall and their expression of cytokines (e.g. TNF-α and interleukins) (IL-1β and IL-6), T-lymphocytes infiltrate the lesion site and secret γ-interferon [3]. The latter reduces the synthesis of connective tissue and smooth muscle proliferation [4]. Nevertheless, the release of these harmful cytokines, on the other hand is associated with the release of anti-inflammatory cytokines as IL-4 & IL-10 [5], in addition to increased adiponectin synthesis from adipose tissue [6].

The balance of the stimulatory and the inhibitory cytokines is crucial to the stability of the atheroma plaque and subsequently to the slowing of the pathogenic events.
In recent years adiponectin has been acknowledged as a possible target therapeutic agent. It is an adipokine secreted mostly from adipose tissue. Adiponectin through binding with its receptors (1 & 2) has attracted much attention because of its multiple actions especially as an anti-inflammatory [6] and anti-atherogenic agent [7]. Recent studies have revealed novel links between adipose tissue inflammation, adipokines and atherosclerosis [8]. In-vitro studies reported that adiponectin inhibits monocyte attraction to the vascular bed by attenuating TNF alpha induced expression of adhesion molecules [9]. Moreover, a potential link between adiponectin and reproductive related hormones has been identified [10].

Oxytocin (OT) which is a nonapeptide hormone produced in the hypothalamus and known for its reproductive-related function [11], it plays a potential role in mediating beneficial effects in cardiovascular disorders. It has been investigated that OT plays a role in atherosclerosis. The oxytocin receptor (OTR) is present in several major cell types as vascular smooth muscle cells, endothelial cells, macrophages and adipocytes [12]. Studies conducted in vitro have revealed that OT modulates these processes that are critical to early lesion formation within vascular and immune tissues. Specifically, OT was demonstrated to have antioxidant effects on vascular smooth muscle cells, aortic endothelial cells and macrophages through attenuation of NADPH-oxidase-dependent superoxide production [13].

Increased visceral adiposity has been independently associated with increased risk of cardiovascular disease, moreover, these fat depots may be particularly susceptible to infiltration by macrophages in a pro-inflammatory state [14].

Although oxytocin receptors are present on adipocytes and circulating monocytes and macrophages but to date only one study had examined the potential benefits of OT administration on atherosclerosis in an in vivo model.

The current study sought to test for the first time if peripheral administration of exogenous OT could have a possible role in reducing tissue inflammation and slowing the progression of atherosclerosis in hyperlipidemic castrated animals prone to developing the disease.

Thus, the present study aimed to elucidate the possible role of oxytocin involved in these events through an in-vivo animal model of experimental stress atherosclerosis applied in castrated rats. To achieve this, the levels of the serum lipid profiles, CRP, IL-6 and adiponectin were assessed. Since inflammation of adipose tissue is a common observation in the events of atherosclerosis, thus, the pathogenesis of the disease and the effect of hormonal treatment in combating its events were further explored through evaluation of the gene expressed levels of IL-6, ER-alpha and adiponectin receptors 1&2 (ADIPR1, ADIPR2) in the adipose as well as vascular tissue, in addition to histopathological and morphometric estimation of associated signs of inflammatory cell infiltration in these tissues.

Material and Methods

Experimental animals:

This study was performed using 40 adult male albino rats aging 11-12 weeks and of approximate body weights ranging from 180-200 grams. The animals were purchased from the Animal House of Faculty of Medicine, Cairo University. Experimental procedures and follow-up management of the animals were accomplished in the laboratories of the Physiology, Biochemistry, Clinical Pathology and Histology departments, Faculty of Medicine, Cairo University. All procedures were carried out in compliance with the guide for care and use of laboratory animals published by the US National Institutes of Health (NIH publication 85-23 revised 1985) and in compliance with the Local Animal Ethics Committee of Kasr Al Aini, Faculty of Medicine, Cairo University.

The rats were placed under ordinary living conditions for acclimatization (i.e. room temperature, humidity and dark/light cycle) for 7days before initiation of the experimental procedures. During this period of adaptation and the following 8weeks of the study duration, the animals had free access to food and water.

After one week of purchase, the animals were randomized into:

Group I (n=10): Control group of non-atherosclerotic uncastrated rats which received ordinary diet of animal chow throughout the experimental period of the study.

In the remaining rats, atherosclerosis was induced experimentally using a high fat diet regimen for 8 weeks. To further participate the pathogenic events, groups of 5 rats each were housed under stressful social conditions in confined cages.

The rats were then divided into:

Group II (n=10): Atherosclerotic non-castrated group in which the animals were subjected to sham operation.
Group III (n=20): Atherosclerotic castrated rats in which castration was achieved by bilateral orchiectomy performed 5 days before starting the atherosclerotic regimen. These animals were then included in the following subgroups.

- Group III A (n=10): Atherosclerotic castrated rats treated with isotonic saline as a vehicle in an intraperitonial dose of 1ml, 6 days per week for comparison with the oxytocin-treated animals [15].

- Group III B (n=10): Atherosclerotic castrated oxytocin-treated group. They received a single intraperitonial injection of lypholized synthetic oxytocin powder (Sigma Company, USA) daily for 6 days per week in a dose of 5 IU/Kg dissolved in isotonic saline [15].

**Experimental induction of atherosclerosis:**

The rats for a duration of 8-9 weeks were kept on a feeding regimen of high fat diet documented and applied in previous animal studies. The diet comprised an animal chow containing atherogenic high-fat content in the form of 60% fat in each 100 gram [16].

**Surgical procedure of bilateral orchiectomy:**

The rats were anaesthetized with 40mg/Kg body weight of sodium phenobarbital intraperitoneally. Surgical castration was performed via a midline scrotal incision allowing bilateral access to the hemiscrotal contents. The spermatic cord was ligated then the testicle was removed. The skin was closed with silk sutures, and a local antibiotic skin ointment (terramycin) was then applied [17].

**Sham operation:**

The 2nd group of atherosclerotic rats was subjected to a sham operation in which a scrotal incision was made and then the wound was closed with silk sutures.

**Mortality rate:**

Throughout the period of the study, 5 rats which were afterwards replaced, died either from a wrong surgical procedure (3 rats) or from failure to adapt to the high-fat regimen and drug supplementation (2 rats).

**Body weight:**

The animals were weighed initially before starting the experimental procedures, then body weights were recorded weekly through the study duration. The weight was used as a marker of general health and follow-up status, in addition to its indication of the animal response to the high-fat diet regimen and drug treatment.

**Blood sampling:**

Rats in all experimental groups were allowed to fast for 7-8 hours before blood sampling. Using heparinized capillary tubes, samples were withdrawn retroorbitally in 10ml eppendorf tubes. At the end of study period, blood withdrawal was achieved from the thoracic aorta immediately after sacrifice of the animals for assessment of serum lipids (i.e serum total cholesterol (TC) and serum triglycerides (TG), serum levels of testosterone, circulating inflammatory markers (high sensitive C-reactive protein (CRP) and IL-6) and serum adiponectin levels. Tissue collection:

On the day of sacrifice, after cervical dislocation of the animals and collection of blood samples, a median incision was performed and visceral fat and aortic strips were extracted for histopathological, morphometric and biochemical studies for assessment of the signs of inflammation which included the rate of macrophage infiltration and foam cells formation, and estimation of the gene expressed levels of Interleukin (II-6), estrogen receptor alpha (ER-\(\alpha\)) and adiponectin receptor (1&2).

The visceral fat was separated, dissected and was left to dry on filter paper. It was weighed [18], then preserved in foil paper. After removing the heart and thoracic aorta, the latter was dissected free. It was then fixed to a piece of paper on a cork board and the surface area was determined. The aortic tissue was stripped of adventitia and was cut longitudinally for enface preparation. Tissues were stored in 10% buffered formalin for later staining and quantification of atherosclerotic disease [19].

The prostate was dissected from the surrounding connective tissue, the bladder (caudally) and from the seminal vesicles (cranially). The prostatic weight was then used as a marker of the gland response to present androgenic effect.

**Histopathological and morphometric study of atherosclerosis and visceral fat inflammation:**

After dissection and separation of the aortic and adipose tissues, specimens were fixed in 10% formol saline for 24 hours, paraffin blocks were prepared and 5 micrometer thick sections were subjected to histopathological study by haematoxylin and eosin-stained sections [20] photographed using Olympus Microscope computer-assisted digital camera. Morphometric study was carried out using Leica Qwin 500 LTD computer-assisted image-analysis system.
Diameter of adipocytes, thickness of the media, count of apoptotic nuclei and the area of foam cells were estimated in aortic and adipose tissues. The measurements were done in 7 low power fields (LPF) using interactive measurements menu. A reference aorta template was created from the average size and shape of all the aortas and was laid onto each aortic image. Present lesion areas were then calculated.

**Biochemical study:**

Plasma total cholesterol and triglycerides were measured by quantitive-Enzymatic-Colorimetric determination by commercial available kits [21].

High sensitive C-Reactive Protein was estimated by immunoenzymometric assay by (Immunobiological Laboratories, Inc. Minneapolis, USA) according to manufacturers instruction [19].

Measurement of Adiponectin and IL-6 in the blood was achieved using ELISA kit from (B-Bridge International, Inc. USA) according to manufacturers instruction [22,23].

Gene expression levels of IL-6, estrogen receptor alpha and Adiponectin receptors 1,2 were detected in adipose and aortic tissues using Real Time-Polymerase Chain reaction (RT-PCR) [24].

Total RNA was extracted from aorta and adipose tissue tissue homogenate using RNeasy purification reagent (Qiagen, Valencia, CA). The cDNA was generated from 5µg of total RNA extracted with 1 µl (20pmol) antisense primer and 0.8µl superscript AMV reverse transcriptase for 60min at 37°C. The relative abundance of mRNA species was assessed using the SYBR® Green method on an ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA). PCR primers were designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from GenBank (Table 1). All primer sets had a calculated annealing temperature of 60°C. Quantitative RT-PCR was performed in duplicate in a 25-µl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer and 2-3µl of cDNA. Amplification conditions were 2min at 50°C, 10min at 95°C and 40 cycles of denaturation for 15s and annealing/extension at 60°C for 10min. Data from real-time were calculated using the v1 ·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of VEGF,TNF alpha and IL 10mRNA was calculated using the comparative Ct method. All values were normalized to the beta actin genes.

<table>
<thead>
<tr>
<th>Primer sequence</th>
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<tr>
<td>Adiponectin receptor 1</td>
<td>GATTITTCATGTCCTGGTGGA</td>
</tr>
<tr>
<td>Adiponectin receptor 2</td>
<td>GCATGGGAACGAATGGAGATT</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>TGGAGTCACAGAAGGATGGCTA AG</td>
</tr>
<tr>
<td>Estrogen-receptor alpha</td>
<td>AGTACAGCTGTCGGCCTCACC AC</td>
</tr>
<tr>
<td>Beta actin</td>
<td>TGTTGTCCTGTATGCTTCT-3'</td>
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**Statistical analysis:**

Data was first coded and entered using the statistical package SPSS version 15, then summarized using mean, SD and range for the quantitative variable. While analysis of varies groups (ANOVA) was used for multiple comparisons. Post HOC test was applied for normally distributed quantitative variable, while non-parametrical cross Kruskal Wallis test and mean-Whitney tests were used for quantitative variables which were not normally distributed. p-values less than or equal to 0.05 were considered statistically significant.

**Results**

**Behavioral analysis:**

In response to oxytocin administration the atherosclerotic castrated rats were observed even despite their stressful social conditions due to their confinement in narrow cages to acquire a positive affiliative social behavior. Furthermore, most of the animals were acquiescent and subdued.

**Effect of oxytocin administration on the serum levels of testosterone:**

The level of serum testosterone in group III B (oxytocin-treated) showed insignificant variation with a mean value of 0.29ng/ml in comparison to group III A (saline-treated) with mean value of 0.3ng/ml.
**Effect of oxytocin administration on the body, visceral fat and prostate weights in castrated-atherosclerotic rats**

**Body weight:**

The impact of oxytocin administration in group III B was reflected on the body weight, in which our results showed a significant reduction of body weight with mean value of 210±8gm in comparison to group III A (saline-treated) and group II (sham-operated) with mean value of 340±11gm and 260±12gm respectively \( (p<0.01) \).

**Visceral fat:**

As regards the visceral fat, oxytocin treated group showed a highly significant reduction in visceral fat weight with a mean value of 4.25±2.2gm in comparison to group III A with a mean value of 15.6±2.4gm and group II with a mean value of 7.86±1.1gm \( (p<0.01) \).

**Prostate weight:**

As regards the prostatic weight there was a significant increase in group III B (oxytocin-treated) with a mean value of 42±13mg in comparison to group III A (saline-treated) with a mean value of 23±7mg \( (p<0.05) \). On the other hand there is no significant difference in comparison to group II with mean value of 36±7mg \( (p>0.05) \).

**Effect of oxytocin administration on the serum levels of lipid profile:**

Our results showed a significant reduction in the level of cholesterol and the level of triglycerides in group III B (oxytocin-treated) with mean values of 50.21±5.24mg/dl and 111.45±8.32mg/dl respectively in comparison to group III A (saline-treated) with mean value reaching 88.27±8.81mg/dl and 189±10.39mg/dl respectively \( (p<0.001) \).

**Effect of oxytocin administration on the serum expression levels of IL-6, estrogen receptor alpha and adiponectin receptors 1&2:**

**IL-6 gene expression level:**

As regards IL-6 gene expression in group III B (oxytocin-treated), there is a significant decrease with a mean value of 0.63±0.125 A.U in comparison to group III A (saline-treated) with a mean value of 2.79±0.763 A.U and group II (sham-operated) with a mean value of 2.18±0.64 A.U \( (p<0.05) \) (Fig. 2A).

**Estrogen receptor alpha**

As shown in (Fig. 2A), our results showed a significant elevation in ER-\( \alpha \) gene expression in group III B (oxytocin-treated) with a mean value of 0.75±0.24 A.U in comparison to group III A (saline-treated) with a mean value of 0.49±0.12 \( (p<0.05) \) but as regards to group II (sham-operated) with mean value of 0.72±0.135 A.U the level is insignificantly different \( (p>0.05) \).

**Adiponectin receptor 1&2:**

On administration of oxytocin to castrated rats on high fat diet in group III B there was a significant increase in the mean values of adiponectin receptor 1 with a mean value of 1.04±0.13 A.U in comparison to group III A (saline-treated) and group II (sham-operated) with mean values of 0.50±0.234 and 0.84±0.132 A.U respectively (Fig. 2A).

As regard adiponectin receptor 2 there is a significant augmentation in group IIIIB with a mean value of 0.51±0.125 A.U in comparison to group II, IIIA with mean values of 0.38±0.218 and 0.36±0.24 A.U respectively \( (p<0.05) \) (Fig. 2A).
A.U in group III B (oxytocin-treated) in comparison to group III A and II with mean values of 2.83±0.15 and 2.17±0.52 A.U respectively (p<0.01).

**Estrogen receptor alpha**

As regard to ER-a there was a highly significant elevation in group III B (oxytocin-treated) with a mean value of 0.88±0.23 A.U in comparison to group III A (saline-treated) with a mean value of 0.32±0.12 A.U and in comparison to group II (sham-operated) with a mean value of 0.62±0.16 A.U (p<0.01) (Fig. 2B).

**Adiponectin receptor 1 & 2**

As shown in (Fig. 2B), adiponectin receptor 1 gene expression levels in response to oxytocin administration in group III B were significantly elevated with mean value of 0.65±0.19 A.U in comparison to groups III A (saline-treated) and group II (sham-operated) with mean values of 0.25±0.15 A.U and 0.43±0.12 respectively (p<0.05).

Adiponectin receptor 2 also showed significant increase in group III B (p<0.05) showing a mean value of 0.74±0.15 A.U in comparison to group III A (saline-treated) with a mean value of 0.53±0.27 and in comparison to group II (sham-operated) with mean value of 0.56±0.12 A.U (p<0.05) (Fig. 2B).

**Histological results:**

**Morphological changes in the aorta**

**Group II: Sham atherosclerotic group**

Intima showed partial lining with rounded nuclei and mononuclear infiltrate in expanded subendothelial connective tissue. The media revealed some cells with foamy cytoplasm and oval nuclei, few dark nuclei of smooth muscle cells appeared (S.M.Cs) (Fig. 3).

**Group III A: Atherosclerotic castrated saline-treated group**

As shown in (Fig. 4), the intima showed rounded nuclei. The media revealed cells with foamy cytoplasm either exhibiting oval or rounded nucleus. Dark nuclei of S.M.Cs appeared.

**Group III B: Atherosclerotic castrated oxytocin-treated**

In group III B (oxytocin-treated), the media showed few foam cells and few dark nuclei of smooth muscle cells as seen in (Fig. 5).

**Morphological changes in the adipose tissue**

**Group II: Sham-operated atherosclerotic group**

Microscopic picture of the adipose tissue showed congested vessels. Minimal infiltrating cells were evident, some adipocytes appeared distended (Fig. 6).

**Group III A: Atherosclerotic castrated saline-treated group**

Adipocytes were seen distended, with congested vessels and infiltrating cells were detected (Fig. 7).

**Group III B: Atherosclerotic castrated oxytocin-treated**

Multiple adipocytes appeared non distended with no obvious congestion, and no cellular infiltrate as seen in (Fig. 8).
Histological changes in the aorta:

**Fig. (3):** Photomicrograph of a section in the aorta of a rat in group II: Showing partial intimal lining with rounded nuclei (arrow) and mononuclear infiltrate (arrowhead), foam cells (*) and few dark nuclei (d) of smooth muscle cells are seen (H & E, x 400).

**Fig. (4):** Photomicrograph of a section in the aorta in group III A: Showing rounded nuclei (arrow). Foam cells (*) and dark nuclei (d) of SMCs are seen (H & E, x 400).

Histological changes in the Visceral Fat:

**Fig. (5):** Photomicrograph of a section in the aorta of a rat in group III B: Showing few foam cells (*) and few dark nuclei (d) of SMCs in the media (H & E, x 400).

**Fig. (6):** Photomicrograph of a section in the visceral fat of a rat in group II: Showing some distended adipocytes (a). Note congested vessels (c) with minimal infiltrating cells (arrow head) (H & E, x 200).
Discussion

Atherosclerosis or "arteriosclerotic disease" is a progressive disease that involves multiple major cell components, including endothelial cells, leucocytes and intimal smooth muscle cells. The artery wall thickens as a result of a buildup of fatty materials, mainly cholesterol [25].

Since oxytocin (OT) is a neuropeptide which has long been identified to be involved in both affiliative social behavior and cardiovascular homeostasis, hence, it was suggested that oxytocin may have a role in mediating the benefits of positive social interactions on atherosclerosis [12].

Animal studies investigating the effects of peripheral OT administration in models of inflammatory diseases have provided evidence for the existence of potentially cardioprotective pathways involving OT. It has also been shown that OT decreases myeloperoxidase (MPO) activity which contributes to the production of reactive oxygen species (ROS) leading to lipid peroxidation, activation and recruitment of macrophages [26].

The current study is one of very few studies that emphasized the effect of chronic peripheral OT administration on slowing and reducing the progress of atherosclerosis and the markers of adipose inflammation and vascular tissue in an in vivo animal model [12,13]. In fact the findings of the present research are considered the first to attempt to emphasize the role that peripheral oxytocin administration might confer in several protective mechanisms which may slow the progression of the atherosclerotic process in hyperlipidemic castrated rats.

During the course of the experiments, we observed that even under the chronic stressful confined conditions created in this study, the rats treated with OT were quiescent and acquired a subdued behavior compared to the vehicle-treated. This finding was earlier explained by Uvnas-Moberg in a study (1998) [27] in which peripheral administration of a high dose of OT was observed to cross the blood brain barrier and acts via activation of serotonin receptors on the medial amygdala to produce sedation.

On observing the general physical activity and social behavior, differences were recorded among the OT treated animals in the form of lesser aggression and positive affiliative social bonding suggesting a positive role of peripheral oxytocin on CNS related centers [28]. These findings are partly controversial with those by Nation et al., (2010) [12] who mentioned that physical activity was not affected in oxytocin-treated animals.

The present results regarding the modulating impact of OT on hypercholesterolemia in castrated animals, implicate a subsequent attenuation of the atherosclerotic events in the adipose tissue as well as the vascular wall. This was primarily initiated through significant reduction in the parameters of the lipid profiles which was also reflected by the significant decrease in the mean values of the body and visceral fat weights compared to those of the vehicle-treated castrated animals. These observations give support to recent studies that concluded a delayed onset of obesity negatively correlates with OT treatment [29,30].

As regards the increased prostatic weight which associated the peripheral OT administration, in
this study, previous reports support our findings that proposed a hyperplastic and proliferative OT effect on the prostatic tissue [31].

Furthermore, the current study has confirmed the results established by Ondrejcakova et al., (2010) [32] who demonstrated a negative correlation between the circulating OT concentrations and the size of adipocytes during intensive stress situations. On the other hand, the present results are conflicting with those of a recent study which revealed that OT failed to induce any changes in either the food intake or the body weight [33].

Since adipose tissue with a higher number of small adipocytes has a greater ability to accumulate triacylglycerols and supports a redistribution of lipids from ectopic sites to adipose tissue [34] thus a significant concept was further proposed that OT treatment exerts positive effects on white adipose tissue growth without increasing the adiposity by mediating specific activation of eukaryotic elongation factor 2. Thus OT treatment decreases the diameter of adipocytes and increase the adipose tissue protein content without changing the adipose tissue mass [33].

Oxytocin is further proposed to stimulate increased metabolism by activating sympathetic nervous tone. Through this OT effect, brown adipose tissue containing low lipid droplets is able to activate cold-induced thermogenesis. Accordingly, OTR deficient male mice exhibited early onset-obesity associated with increases in abdominal fat pads and fasting plasma triglycerides [35,36], thus concluding that OT may play a role in the regulation of energy homeostasis.

The current study showed that peripheral OT treatment also significantly reduced the inflammatory markers in the hyperlipidemic castrated rats (CRP, IL-6), as well as the IL-6 expressed from the affected tissues. Furthermore, all signs of tissue inflammation were recorded to be significantly decreased, in our study thus revealing the modulating role that OT can play as an anti-inflammatory agent in the processes that are critical to early atherosclerotic lesion initiation and progression.

Importantly, our results emphasize that the observed improvement in the inflammation markers (CRP & IL-6) after OT administration to be a result of changes in the activity levels, since OT may have participated in the reduction of the hypothalamic-pituitary axis activation [27]. In this issue, numerous studies demonstrated that hyperlipidemic animals housed in a positive social environment develop less extensive atherosclerosis than those housed in isolation or in a stressful social environment through activating anti-inflammatory processes [37,38].

Our results further confirm and support cardiovascular studies conducted in vitro which stated that OT exerts antioxidant effects on the cultured vascular smooth muscle cells, aortic endothelial cells and macrophages by attenuating NADH oxidase-dependent superoxide production [39,40].

Additional in-vitro findings indicated that OT was reported to inhibit lipopolysaccharide-induced IL-6 production from endothelial cells and macrophages [13].

Furthermore, these in-vitro studies concluded that OT is capable of reducing pro-inflammatory cytokine release from macrophages, as early lesions are characterized by intravasation of activated macrophages in the pro-inflammatory state [42,42]. Further research work however, is still needed to evaluate the effect of OT on the later stages of the disease.

Simewise, the significant reduction in the number of apoptotic cells in the aorta and the decrease in aortic thickness of OT-treated group confirms previous studies that assessed the role of the peripheral OT as an anti-oxidant agent [43].

The fact that tissue collagen decreases in OT-treated animals shows that OT may also have an anti-fibrotic effect which may influence fibrinolysis and prevent platelet aggregation by increased production of prostacyclin [12]. This is based on the fact that collagen accumulation which is an important indicator of extracellular matrix formation is a marker for atherosclerosis plaque severity and contributes to ingoing lipid peroxidation and macrophage recruitment [44].

Although replication and future studies are still needed, these present findings suggest that increasing the positive social contact in individuals at risk of developing CVS disease, may be crucial in slowing the progression of atherosclerosis through increasing the levels of the circulating oxytocin [45].

Conflicting results were revealed regarding the impact of castration on OTR binding. Early studies showed that OTR binding and mRNA levels decreased in castration, whereas others earlier suggested that castration and inhibition of aromatase activity reduce testosterone and estradiol but increase OT binding [46]. This may admirably explain the dramatic beneficial effects of OT treatment to
the castrated rats in the current study, since the OT through its strong binding with OTRs had a potent impact on all the studied parameters.

The fact that OT was also able to slow the initial development of these lesions in a region of high-lesion prevalence, suggests that it may be working through mechanisms important during the atherosclerotic lesion initiation.

Interestingly, Nation et al., (2010) [12] found that OT treatment led to diminished atherosclerotic lesion in the thoracic aorta in Apo-E-male mice, but no effect was found in the aortic arch. Different atherosclerosis-prone regions have been described in literature on murine models of hyperlipidemia that frequently vary in their responses to treatment and development over time [47].

This is proved in the current findings which aimed to assess the early stage of the disease in which few lesions were observed below the thoracic aorta. Recently, it has also been shown that differential gene expression profiles exist through the aorta and that these differences can convey varying susceptibility to specific atherogenic mechanisms. Site-specific differences in hemodynamics, including low shear stress, cyclic reversal of flow direction, stretch stress, and turbulence, could also be responsible for differential treatment effects at various locations within the aortic tree [48].

Oxytocin in the present results stimulated a significant increase in the levels of gene expressed ER-α in the vascular and adipose tissue. The present findings in this issue could focus more attention on the participating OT role which could be exerted through a mediated impact on estrogen production. These findings also indicate and support the concept that OT can have organizational effects on the expression of estrogen-receptor alpha [49].

Furthermore, the current results depicting the significant elevation of ER-α levels in adipose and vascular tissue in response to OT administration focuses an extra significant benefit of OT in the events of atherosclerotic pathogenesis.

The fact that we observed that estrogen receptor alpha levels increase as a function of OT treatment in the male species, however partly disagrees in this issue with the findings of Pournajafi-Nazarloo et al., (2007) [50], who hypothesized that this OT-mediated increase in ER-α occurs only in females.

On the other hand, the present results are conflicting with the work performed by Cassoni et al., (2002) [51]. Through their work on neoplastic cells, they deduced that OT modulates the expression of ER-β both at mRNA and protein levels. However, this modulation in the number of ER-β was paralleled in their study with a temporary increase in the binding affinity of these receptors as well as significant increase in the induced transcription activity.

We can explain this confliction by the fact that the effects of OT manipulation are age-dependent, sexually dimorphic and site-specific [82]. Also, the complexity in these OT effects may be related to the fact that OT can influence the physiological and endocrinological functions differently at various anatomical sites receiving OTergic innervations. Therefore depending on the type of species, route of administration, dose given, period of observation and other experimental factors, variable effects in response to OT may be observed.

To our knowledge, the present study is the first research work so far that attempted to investigate whether the influence of OT treatment in attenuating atherosclerosis events could be possibly mediated via an increase in the adiponectin production in castrated atherosclerotic animals.

Our results propose a significant potential role for peripheral OT in increasing adiponectin secretion from adipocytes. Furthermore, this stimulatory impact of OT extended to produce a highly significant increase in the expressed levels of both adiponectin receptors 1&2 in vascular as well as adipose tissue. We tend to believe that this enhanced adiponectin secretion may be partly one of the mechanisms involved in the atheroprotection action mediated by oxytocin, although we cannot explain the exact mechanisms involved by OT to cause this marked increase in adiponectin release and the up-regulation of its receptors. However, we hypothesize that OT exerted a potent inhibitory impact on the inflammatory markers which in other conditions would have had a suppressing effect on adiponectin secretion and inhibition of the gene expression of its receptors.

In conclusion, we provide evidence that oxytocin could prevent the stress that induce the atherosclerotic events thus attenuating its pathogenesis as an anti-stressor. Moreover, peripheral oxytocin administration was shown to act as an anti-inflammatory, anti-oxidant and anti-fibrotic agent. Although the hormonal replacement therapy may have adverse effects and the possible beneficial impact is still poorly understood, yet, further studies are still needed to help guide the evolution of treatment recommendations. In particular, these
studies may contribute to setting research priorities for clinical, translational and basic scientists to further explore the area of hormone therapy in cardiovascular diseases, and especially in the events of atherosclerosis.

References


8- KARDIOLOGIIA: Adipose tissue inflammation and atherosclerosis, 49 (12): 80-6, 2009.


