Oxytocin Administration Attenuates Atherosclerosis and Inflammation in Watanabe Heritable Hyperlipidemic Rabbits

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Abstract

Oxytocin (OT) is a neurohypophyseal peptide traditionally associated with female reproductive functioning, and more recently with prosocial behavior. OT and its receptor are also expressed in the heart and vascular tissue and play a role in cardiovascular homeostasis. *In vitro*, it has been demonstrated that OT decreases NADPH-dependent superoxide production and pro-inflammatory cytokine release from vascular endothelial cells and macrophages, suggesting that OT may attenuate pathophysiological processes involved with atherosclerotic lesion formation. The present study sought to determine the effect of chronic exogenous OT administration on inflammation and atherosclerosis in an animal model of dyslipidemia and atherosclerosis, the Watanabe Heritable Hyperlipidemic (WHHL) rabbit. Twenty-two, 3-month-old WHHLs were surgically implanted with osmotic mini-pumps containing OT (n=11) or vehicle (n=11), and then were individually housed for the entire study. Blood and 24-hour urine samples were taken at baseline and after 8 (midpoint) and 16 (endpoint) weeks of treatment. At endpoint, the aortas and visceral fat samples were dissected and stored for analyses. There were no group differences in body weight, serum lipids, plasma/urinary measures of oxidative stress, plasma cortisol or urinary catecholamines over

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Contributors
Angela Szeto took primary responsibility for the conduct of the study, and was involved in the analysis of all biomarkers, and writing of the study.
Maria Rossetti assisted Dr. Szeto with the conduct of the study.
Armando Mendez oversaw the hormonal, lipid, inflammatory and other biomarker measurements, and was involved in the design, analysis and writing of the study.
Crystal Noller assisted Dr. Szeto with the conduct of the study.
Edward Herderick carried out the histological examination of the vascular tissue and the quantification of atherosclerosis.
Julie Gonzales oversaw the animal husbandry and care, and assisted with the conduct of the study.
Neil Schneiderman participated in the design, analysis and writing of the study.
Philip McCabe oversaw all aspects of the study, including design, conduct, analysis and writing.

Conflict of Interest Statement
None of the authors have any conflict of interest that could inappropriately influence or be perceived to influence this work.

the 16-week treatment. OT-treated animals exhibited significantly lower plasma C-reactive protein levels at midpoint and endpoint and developed significantly less atherosclerosis in the thoracic aorta relative to vehicle control animals at endpoint (p<0.05). Cytokine gene expression from visceral adipose tissue samples suggested that there was a decrease in adipose tissue inflammation in the OT-treated group compared to the vehicle control group, however these differences were not statistically significant. These results suggest that chronic peripheral OT administration can inhibit inflammation and atherosclerotic lesion development.

Keywords

Oxytocin; atherosclerosis; inflammation; WHHL rabbit

Introduction

It is well established that the neuropeptide, oxytocin (OT), acts centrally to facilitate a variety of prosocial behaviors (Carter et al., 2008; Ross and Young, 2009). In addition, affiliative social behaviors and warm contact stimuli are associated with elevations in plasma OT (Grewen et al., 2005; Light et al., 2005; Holt-Lunstad et al., 2008). It has been suggested that OT may serve as an impetus for the organism to seek out social support and prosocial behavior (Taylor et al., 2006; Taylor et al., 2010). In a separate line of research, it has also been demonstrated that OT works centrally to attenuate peripheral stress responses (Petersson et al., 1999; Neumann et al., 2000; Petersson et al., 2005). These observations are also consistent with the notion that OT may play a role in the beneficial effects of affiliative social interactions on stressful behavior and stress-related disease (Knox and Uvnas-Moberg, 1998; Uvnäs-Moberg, 1998; Smith and Wang, 2012).

In addition to its CNS effects, OT and its receptor (OTR) are expressed in a variety of peripheral tissues, including the heart and blood vessels (Jankowski et al., 2000; Gimpl and Fahrenholz, 2001; Kiss and Mikkelsen, 2005). It has been suggested that this cardiovascular OT system is involved in the maintenance of normal homeostatic functions (Gutkowska et al., 1997; Jankowski et al., 1998; Jankowski et al., 2000), however, it has also been proposed that OT may work directly on vascular cells to slow the progression of pathophysiological processes involved in disease (Paredes et al., 2006; Szeto et al., 2008; Nation et al., 2010). In our laboratory, we demonstrated that OT may also have antiatherogenic properties by inhibiting oxidative stress and inflammation in cultured vascular endothelial and smooth muscle cells, monocytes, and macrophages (Szeto et al., 2008). In vivo, chronic OT infusions in an animal model of hyperlipidemia and atherosclerosis, the ApoE −/− knockout mouse, attenuated aortic atherosclerosis in a site-specific manner and inhibited the ex vivo secretion of IL-6 from visceral adipose tissue (Nation et al., 2010).

A major pathophysiological process underlying atherosclerosis and coronary artery disease (CAD) is chronic inflammation ((Ross, 1999; Mullenix et al., 2005). Systemic and local inflammatory events, driven largely by pro-inflammatory cytokines and oxidative stress, mediate all phases of atherogenesis. The pro-inflammatory cytokines involved in the development and progression of atherosclerosis include interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 among others (Ait-Oufella et al., 2011). Of notable importance in CAD is IL-6, which has been shown to up regulate the production of C-reactive protein (CRP), and affect lipid metabolism and the acute phase response (Yudkin et al., 2000). CRP, a molecule found in plasma and produced by hepatocytes, is elevated in inflammatory disorders and is used as a systemic marker of inflammation (Agrawal et al., 2010).

An additional source of circulating proinflammatory cytokines (Yudkin, 2003) is adipose tissue, and increased adiposity has been shown to be a risk factor for a variety of inflammatory disorders, including CAD (Allende-Vigo, 2010; Reaven, 2011). Increased adiposity results in the modification of adipocytes leading to physiological dysfunction, characterized by changes in production and secretion of adipokines (adipose tissue derived cytokines). Altered adipokine concentration mediate functions that are associated with CAD such as fibrinolysis, coagulation, blood pressure, inflammation, insulin resistance, and the development of atherosclerosis (DeClercq et al., 2008; Hajer et al., 2008). Interestingly, it has been demonstrated that the major cellular components of adipose tissue, adipocytes and macrophages, both express OTRs (Bonne and Cohen, 1975; Szeto et al., 2008).

In view of the potential role of OT in the attenuation of inflammatory processes and disease, the current study examined the potential anti-atherogenic effects of *in vivo* chronic OT infusion in the Watanabe Heritable Hyperlipidemic (WHHL) rabbit. Due to a spontaneous genetic defect in cholesterol clearance, WHHLs are extremely dyslipidemic from birth and develop severe atherosclerosis in a compressed time frame (Buja et al., 1983). It has been demonstrated that WHHL rabbits exposed to a stable social environment, characterized by increased affiliative social behavior, exhibited 50% less aortic atherosclerotic lesions than WHHLs subjected to an unstable social environment or in a social isolation (McCabe et al., 2002). It was also demonstrated that social environment influences inflammatory cytokines and vascular oxidative stress in WHHLs (Nation et al., 2008). Although these studies suggest that social environment and behavior play an important role in disease progression (even in animals with strong genetic determinants), the mechanisms by which social/ emotional behaviors influence the course of disease are not clear. The current study further examines the hypothesis that OT, which is linked to social affiliation and other prosocial behaviors, attenuates disease. In addition to the extent of aortic atherosclerosis, we also assessed the effect of OT on metabolic, stress and inflammatory biomarkers, as well as the expression of adipose tissue derived adipokines.

Methods

Experimental Animals

Twenty-two male WHHL rabbits (2.5 months old, 2.9–3.5 kg) were obtained from Brown Family Enterprises (Odenville, AL). Previously, we have shown that social environment can influence the progression of atherosclerosis in WHHL rabbits (McCabe et al., 2002). In order to assess the role of OT independent of differences in social environment, all animals in the current study were maintained in individually-caged conditions. Animals were housed in individual cages (6 sq ft) exposed to 12-hour light and dark conditions (lights on at 0700h) and fed standard rabbit chow (Purina; 2.5% total fat, 0% cholesterol) and water *ad libitum*. Rabbits were acclimated for seven days before initiation of experiments. The rabbits were weighed weekly. All procedures were approved by the Animal Care and Use Committee of the University of Miami.

Blood Draws and Urine Collection

Rabbits were fasted overnight at treatment baseline (collected 1 day prior to implantation of osmotic mini-pumps), midpoint (week 8), and endpoint (week 16) for measurement of serum cholesterol, triglyceride, insulin, glucose, plasma cortisol, C-reactive protein (CRP) and oxidized LDL. Samples for plasma oxytocin measurement were taken at various times during the study (indicated in Figure 1). Using loose restraint, blood was sampled from the marginal ear vein. Serum or plasma was obtained by centrifugation at $1000 \times g$ for 15 minutes at 4°C and stored in aliquots at −80°C until assay.

Animals were placed in metabolic cages for 24-hour urine collection at baseline, midpoint and endpoint for the measurement of epinephrine and norepinephrine. Urine samples were collected on ice and acidified to 5.0 mmol/l HCl, aliquotted prior to storage at −80°C.

Osmotic Minipumps and Surgeries

Osmotic minipumps (Alzet model 2ML4, infusion rates of 2.5 μl/hr, DURECT Corporation, Cupertino, CA) were filled following manufacturer's guidelines with 2 ml of 300 μg/ml OT for an infusion rate of 250ng/kg/hr (Bachem, Torrance, CA) or vehicle (50 mM sodium citrate, pH 4.0) solutions. Osmotic minipumps were used to deliver the peptide in order to obtain a chronic, constant elevation of OT over the entire study period. OT has a half-life of less than 10 minutes, suggesting that single injections would have only short, acute effects. The infusion rate was established in dose response pilot studies and the dose chosen gave on average an 8-fold increase in plasma OT steady state levels compared to vehicle infusions. The pumps were then primed in a sterile saline bath at 37°C for 6 hours. Rabbits were anesthetized with isofluorane (1–3% in 100% oxygen) and scrubbed with 70% isopropanol and betadine solutions before surgery. A small subcutaneous incision was made in the midscapular region, the pumps were inserted and the wound was sutured. Animals were allowed to recover in a 37°C incubator before returning to their home cages. Pump-exchange surgeries were performed every 4 weeks over 4 months.

Tissue Collection

After 16 weeks of treatment, rabbits were euthanized and the entire aorta and heart were removed via a midline thoracic and abdominal incision, and the tissue was placed in a 10% solution of buffered formalin for later staining and quantification of atherosclerotic disease. The epididymal fat were weighed and a portion stored in RNA Qiazol lysis buffer (Qiagen).

Biochemical Assays

Serum cholesterol, triglyceride, insulin, glucose, plasma cortisol and urinary creatinine were measured by automated analyzer (Roche Diagnostics, Indianapolis, IN). Plasma oxidized LDL (Mercodia, Winston Salem, NC), urinary isoprostane (Neogen Corp., Lexington, KY) and urinary catecholamines (Alpco Diagnostics, Salem, NH) were measured by commercially available reagents and following the manufacturer's instructions. For CRP assay, samples were diluted 1:400 in assay buffer prior to analysis with a commercially available high sensitivity ELISA kit (Immunology Consultants Laboratory, Inc., Newburg, OR). Oyxtocin was measured after extraction of 1 ml of plasma as previously described in detail (Szeto et al., 2011) and quantified using a commercially available OT kit (ADI-900-153; Enzo Life Sciences, Plymouth Meeting, PA). For HPLC analysis plasma was pooled from three animals in each group in order to have sufficient material for analysis and extracted under identical conditions as used for assay. HPLC separation was performed using equipment and conditions as described previously (Szeto et al., 2011).

Quantitation of Atherosclerosis

All histomorphometric procedures were performed in a blind fashion. The method for preparation of mice aortas and quantification of disease was performed as described previously (Karra et al. 2005) by oil-red-O staining of atherosclerotic lesions with digital data analysis. Percent lesion area was calculated from the proportional area of pixels stained with oil-red-O for a given aortic section.

RT-PCR analysis

Total RNA (optical density ratio of 260/280 nm, >1.8) was isolated from approximately 50 mg of adipose tissue using the RNAeasy kit (Qiagen, Valencia, CA) and treated with

DNAse I (Qiagen, Valencia, CA). cDNA was prepared using the reverse transcriptase reaction (Applied Biosystems by Life Technologies, Carlsbad, CA) following manufacturer's protocol. Inventoried rabbit primers for polymerase chain reactions (PCR) were from Applied Biosystems; IL-6 (Cat #: Oc03822686_s1), MCP-1 (Cat #: Oc03823307_s1), adiponectin (Cat #: Oc03823307_s1), IL-10 (Cat #: Oc03396942_m1), TNF-α (Cat #: Oc03397715_m1), and 18S (Cat #: Hs03928990_g1).

Quantitative gene expression of the primers with the use of real-time PCR was performed with the TaqMan gene expression assay (Applied Biosystems by Life Technologies). Twenty micrograms of cDNA were amplified with TaqMan Universal PCR Master Mix and reactions run using universal cycling conditions on an Applied Biosystems Step-One Plus RT-PCR System. Samples were analyzed in triplicate and were normalized to the housekeeping gene, 18S. To analyze relative quantitation (RQ), the Comparative Ct method ΔΔCT (threshold cycle, (Pfaffl, 2001)) was used.

Statistical Analyses

Analysis of variance with repeated measures was used to examine treatment effects (OT group, Vehicle group) on dependent measures across three time points (baseline, midpoint, endpoint). Student's t-tests were used to compare groups on percent lesion area and epididymal fat mRNA expression. A significance level of $p < 0.05$ was required for all tests.

Results

Validation of OT infusion

To ensure OT was being delivered and elevating peripheral concentrations in the treated group, plasma levels were measured in all rabbits over the course of the study (Figure 1A). In vehicle treated animals we observed that plasma OT values were initially elevated and then reached a steady state level for the remainder of the study ($F_{(1, 20)} = 4.86$, p=0.004). OT levels in animals receiving OT infusions were increased by an average of 135 pg/ml relative to the vehicle group, which represented an 8-fold increase on average. This elevation in OT was sustained throughout the entire study ($p < 0.0001$). To confirm that the increase in OT measured in the ELISA was due to intact OT and not other immunoreactive products (Szeto et al., 2011), samples from the final blood collection were subjected to HPLC separation and fractions collected to assess the distribution of oxytocin immunoreactivity (Figure 1B and C). In the sample from the control group, we noted that in addition to a peak of immunoreactivity coincident with authentic OT (eluting at ~8 min), several other peaks of immunoreactivity were observed that account for the majority (60%)of the immunoreactivity in this sample, similar to what we described previously for human plasma samples (Szeto et al., 2011). When the sample from the OT-treated group were similarly analyzed, the increase in immunoreactivity was accounted for almost entirely by intact OT, however, other peaks of immunoreactivity similar to those seen in the control group were still observed, but apparently not altered by OT-infusion suggesting that they represent endogenous OT-immunoreactive substances. These data show that the use of osmotic pumps for delivery of OT results in sustained high levels of intact OT in the peripheral circulation.

Atherosclerosis

Atherosclerosis was evaluated in all animals at endpoint by *en face* quantification of Oil Red O stained atherosclerotic lesions. All animals exhibited evidence of atherosclerosis, with the greatest extent of disease at the aortic arch and far less disease in the abdominal aorta (Figure 2). Although there were no significant group differences in disease in the aortic arch (segments 1 & 2), OT-treated animals exhibited less atherosclerosis in the thoracic aorta

(segments 3, 4 and 5) compared to vehicle control animals (p < 0.05 for segments 3 & 4, p = 0.088 for segment 5). Although mean values for disease were also less in the abdominal aorta (segments 6–9) for the OT group, these differences were not statistically significant. Therefore, these data suggest that chronic OT infusion decreases aortic atherosclerosis in a site-specific manner.

Metabolic, Inflammatory, and Stress Biomarkers

Prior to implantation of the pumps, baseline measure of metabolic, inflammatory and stress biomarkers were comparable between the two groups (Tables 1 and 2). OT infusion had no significant influence on body weight or plasma lipids at midpoint or endpoint (Table 1). Fasting glucose levels were also similar between the groups at each time point, however OT infusion increased fasting insulin levels at midpoint (t (20) = −2.20, p=0.038; Table 1). This finding is consistent with prior research (Bobbioni-Harsch et al., 1995) that suggests OT powerfully stimulates insulin secretion *in vitro*.

Serum cortisol and urinary epinephrine and norepinephrine were not statistically different between groups at midpoint or endpoint, suggesting that OT infusion did not affect these stress biomarkers (Table 2). Oxidative stress was assessed by measuring levels of plasma oxidized LDL and urinary isoprostane. Again, no differences in these measures were observed between the two groups at midpoint or endpoint (Table 2). OT-treated rabbits had significantly exhibited lower levels of systemic inflammation as measured by plasma CRP at midpoint and endpoint compared to vehicle controls (p < 0.05; Table 2).

Adipose Tissue Adipokine Expression

We evaluated the effect of OT infusion on adipokine mRNA expression in epididymal fat, a visceral fat depot (Figure 3). Although there were no significant group differences in adipokine expression, there were several changes suggestive of a decrease in adipose tissue inflammation. For example, the OT-treated group exhibited lower mean expression for the proinflammatory adipokines, MCP-1 and IL-6, and an increase in the anti-inflammatory adipokine, adiponectin. While these changes are in the predicted direction, this study was not sufficiently powered to detect differences in mRNA expression levels in this tissue.

Discussion

The major finding of this study is that chronic OT treatment slows the progression of atherosclerosis in an animal model of disease with strong genetic determinants. Prior work from our laboratory demonstrated that OT inhibits inflammation and oxidative stress in cultured vascular cells and macrophages (Szeto et al., 2008). In the present study, *in vivo* infusion of OT significantly reduced atherosclerosis and was associated with decreased systemic inflammation, as reflected by circulating CRP. This is consistent with a recent study (Ahmed & Elosaily, 2011) in which OT injections in a methionine-fed rat model of atherosclerosis resulted in a decrease in systemic inflammation and disease. Although not statistically significant, expression of adipokines from visceral fat following chronic exposure to OT was indicative of decreased adipose tissue inflammation. In previous work from our lab, chronic OT infusion significantly reduced the secretion of IL-6 from epididymal fat *ex vivo* in ApoE (−/−) mice (Nation et al., 2010). It has been demonstrated that transplantation of epididymal fat pads into ApoE (−/−) mice increased inflammation and accelerated the development of atherosclerosis relative to sham operated ApoE (−/−) mice (Ohman et al., 2008). Taken together, these findings suggest that peripheral OT may work directly on vascular tissue, as well as visceral fat, to reduce inflammation and the pathophysiological sequelae leading to disease.

The OT-related attenuation of disease occurred in the aorta below the level of the aortic arch. These findings in the WHHL rabbit are consistent with a prior study from our laboratory that evaluated the effects of chronic OT administration on atherosclerosis in the ApoE−/− knockout mouse, an animal model with a phenotype similar to the WHHL rabbit (Nation el al., 2010). Regional differences in aortic atherosclerosis development over time and in response to treatment have been described (Nakashima et al., 1994; VanderLaan et al., 2004). Lesions develop first in the aortic arch, then the thoracic region, and later in the renal and iliac bifurcations. This is consistent with the current study in which we found progressively more disease from the proximal aorta to the distal aorta. It has also been shown that differential gene expression profiles exist throughout the aorta and may account for the regional differences in disease susceptibility (Karra et al., 2005). Site-specific differences in hemodynamics can also be responsible for differential disease severity at various locations within the aorta (VanderLaan et al., 2004). OT has a partial affinity for the vasopressin V1 receptor at high concentrations (Gimpl and Fahrenholz, 2001) raising the possibility that OT infusion could increase blood pressure by activating the V1 receptor. However, studies have shown that acute subcutaneous administration of OT can transiently reduce blood pressure (Petersson et al., 1996; Maier et al., 1998). Pilot studies from our lab using New Zealand White rabbits infused with OT at a higher (500ng/kg/hr) and lower (125 ng/kg/hr) dose of OT than used in the current study (250 ng/kg/hr) showed no differences in blood pressure or heart rate compared to vehicle controls over a two week period. This suggests that alteration of blood pressure and heart rate did not modulate the OT effect on disease in the current study.

It is interesting that chronic OT infusion slowed the progression of disease without affecting other traditional risks factors for disease or stress hormones. OT administration did not differentially affect body weight, plasma glucose, serum cholesterol, triglycerides, free fatty acids, or plasma and urinary measures of oxidative stress. Similarly, plasma cortisol and urinary catecholamine values were similar between the OT-treated and vehicle control groups, and as mentioned above, at the dose used in this study OT did not significantly alter blood pressure in pilot work. Since OT does not appear to influence these risk factors but does reduce inflammation, these data support the argument that the OT-related attenuation of disease may be through the suppression of local vascular and systemic inflammation. Also, at physiological concentrations such as those in the current study, OT does not cross the blood-brain-barrier (Churchland and Winkielman, 2012), and therefore the results in the current study are due to OT's peripheral effects.

A relatively unexpected finding was the significant increase of plasma insulin levels at midpoint in the OT-treated rabbits. In fact, previous studies have shown that OT increases glucose uptake in rat cardiomyocytes (Florian et al., 2010) and increases glucose oxidation resembling insulin-like activity in rat epididymal fat pads (Pittman et al., 1961). OT also has been shown to stimulate insulin release from beta cells in the rat pancreas independent of glycemic control (Bobbioni-Harsch et al., 1995; Lee et al., 1995). Therefore, OT has potent insulinogenic properties, whether the increased circulating insulin levels are associated with impaired glucose tolerance or insulin resistance is not known and warrants further study.

Although the measurement of adipokine gene expression from visceral fat was in the predicted direction, none of the differences were statistically significant. Adipokine gene expression is stimulated in the presence of chronic low-grade inflammation due to obesity (Fantuzzi, 2005; Ohman et al., 2008; Zhou et al., 2011). In the current study, although the WHHLs developed atherosclerosis, they were not obese or overweight, and therefore, adipokine expression may not have been elevated, making it more difficult to observe an OT-related attenuation of expression. Another consideration is that in the current study, epididymal fat samples were taken from random regions of this visceral fat depot. A study

published subsequently revealed that epididymal adipose tissue may not have uniform metabolic activity across its proximal and distal (relative to the testes) segments (Altintas et al., 2011), which may explain the variability of gene expression in our samples. Given these concerns, it is likely that the present study was not sufficiently powered to detect significant differences in adipokine gene expression.

It is well established that stressful behavior and social isolation are associated with negative disease outcomes (Kaplan et al., 1982; Clarkson et al., 1987; Manuck et al., 1995; Berkman and Orth-Gomer, 1996; Schneiderman and Skyler, 1996; Syme, 1996; Kaplan et al., 2009). Conversely, there is also accumulating evidence that prosocial behavior is associated with the attenuation of disease, and that social affiliation may impart some degree of protection from pathophysiological processes (Knox and Uvnas-Moberg, 1998; Uvnäs-Moberg, 1998; Paredes et al., 2006; Tom and Assinder, 2010; Fekete et al., 2011; McCall and Singer, 2012). Several studies have shown that hyperlipidemic animals exposed to prosocial environments develop less atherosclerotic lesions than animals housed in socially stressful or isolated environments (Shively et al., 1989; McCabe et al., 2002; Paredes et al., 2006; Bernberg et al., 2008). Positive social interactions have also been shown to increase peripheral OT (Uvnas-Moberg, 1997; Uvnäs-Moberg, 1998; Grewen et al., 2005; Light et al., 2005; Holt-Lunstad et al., 2008). Taken together with the findings from the current study, these studies suggest that the beneficial effects of a prosocial environment on disease progression may be, in part, due to elevated levels of peripheral OT associated with positive social behavior. The fact that OT influences both macrophages and adipose tissue, which are not specific to the cardiovascular system, suggests that peripheral OT may have widespread effects on inflammation and disease in other tissues. For example, OT infusion in models of myocardial infarction improves function in the injured heart through reduction of inflammation (Jankowski et al., 2010; Kobayashi et al., 2009). There is also a growing literature examining the influence of OT on cancer cells and neoplastic processes (Cassoni et al., 2004; Strunecka et al., 2009). This being the case, social environment and peripheral OT could represent important targets for therapeutic intervention in disease.

Acknowledgments

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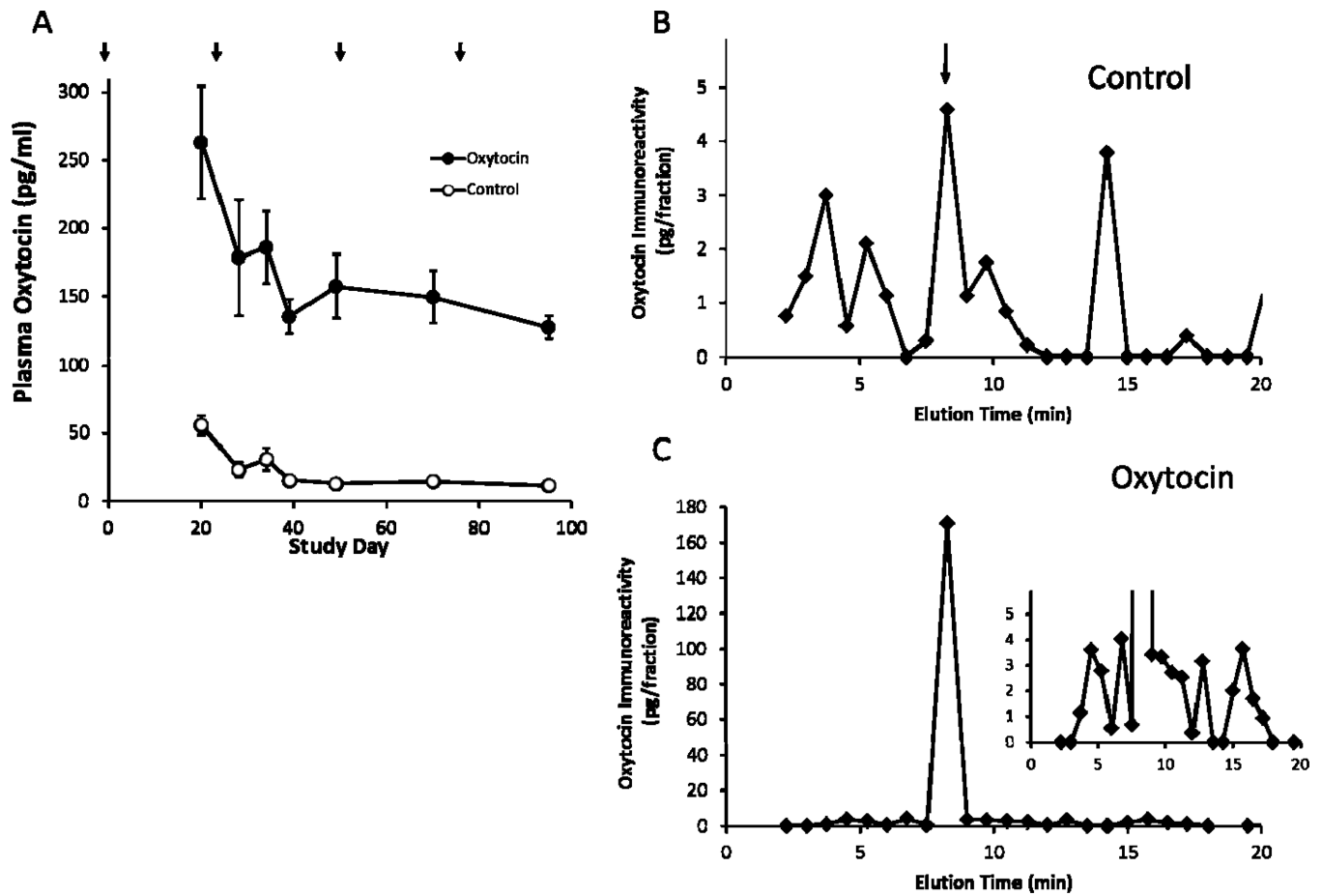
Figure 1.
Plasma OT Levels in control and OT-treated WHHL rabbits and the HPLC profile of oxytocin immunoreactivity of extracted plasmas. Panel A. Plasma OT levels in vehicle (Control) or OT-Treated WHHL animals during the study period. Arrows indicate times that osmotic pumps were implanted or replaced. $p < 0.05$ between groups at all time points. Panels B and C. Plasma from three animals in the Control (B) or Oxytocin-treated (C) group were pooled and extracted as described under Methods and the extract from 3 ml plasma subjected to HPLC separation and the collected fractions were used to measure oxytocin immunoreactivity. Arrow in panel B indicates the elution time of authentic oxytocin standard. Inset in panel C has a reduced scale in order to visualize the minor peaks that were present. Plasma levels of oxytocin in the pooled samples were 7.8 and 70 pg/ml for the control and OT-treated rabbits, respectively.
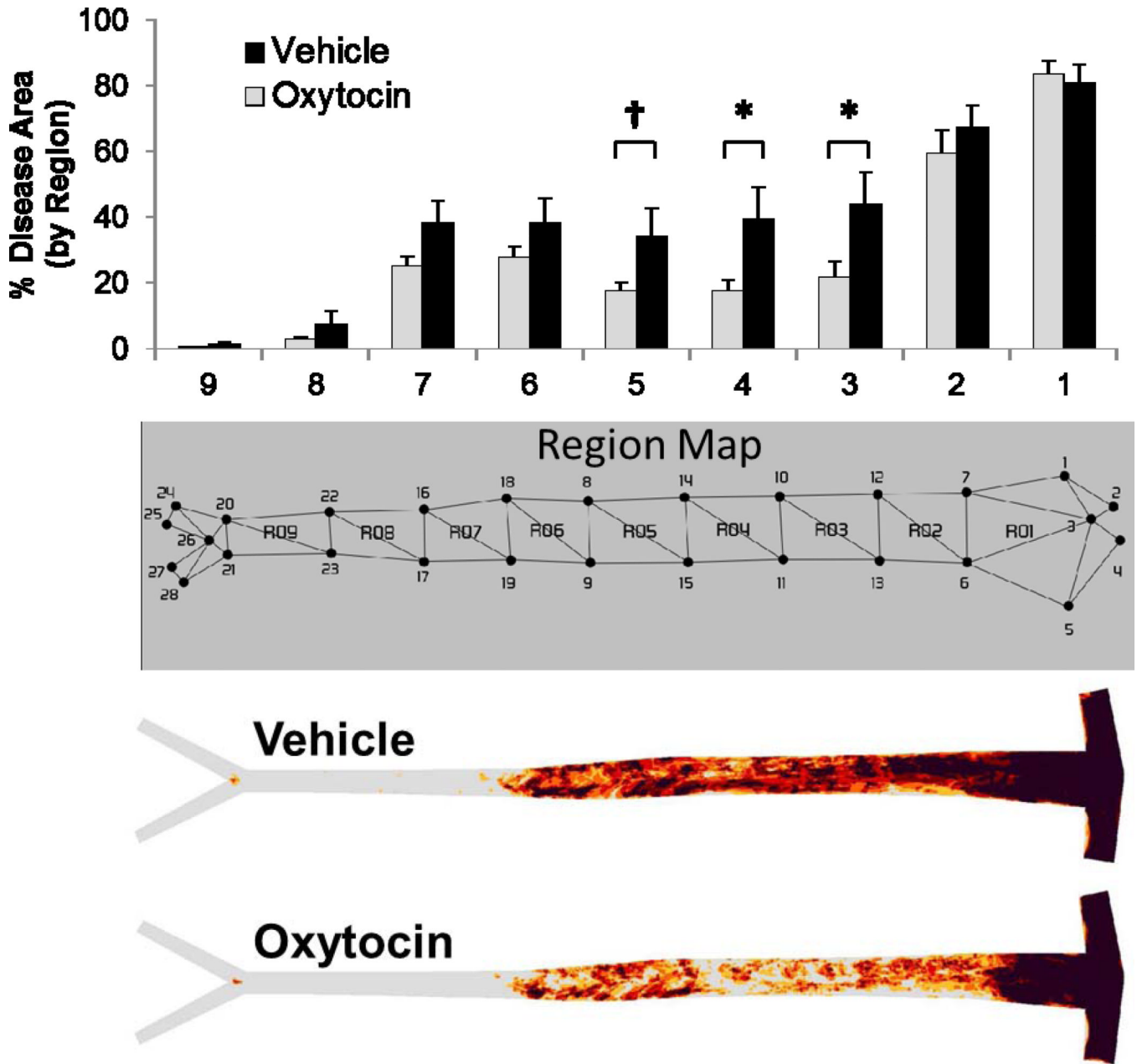
Figure 2.
Aortic atherosclerosis lesion prevalence map and bar graph showing quantitative lesional area within assigned aortic regions in WHHL rabbits chronically infused with OT or saline for 16 weeks. There was a significant decrease in atherosclerosis in the thoracic aorta identified as regions 3, 4 and 5 and trends toward lower extent of disease in the upper abdominal aorta (regions 6 and 7). No differences were observed in the aortic arch (regions 1 and 2). *p 0.05 between Groups, † $p = 0.088$.
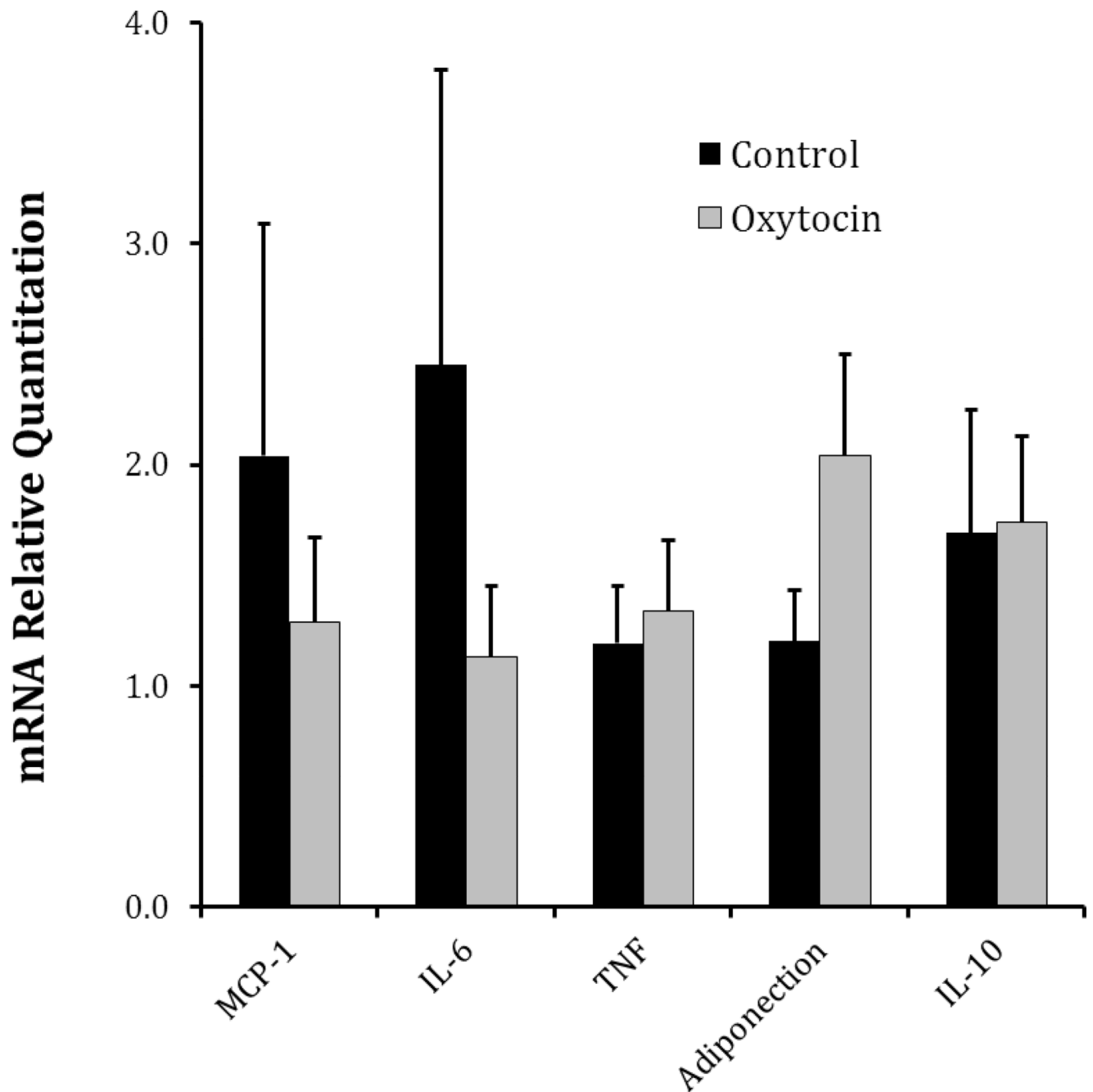
Figure 3.
mRNA expression levels of adipose tissue adipokines in control and OT treated WHHL rabbits. Adipose tissue mRNA of the indicated genes was measured by quantitative rt-PCR. Data represent mean ± S.E.M. Although there were no statistically significant differences between groups, OT treated animals exhibit an adipokine expression pattern consistent with reduced inflammation.

Table 1

Mean Values of Body Weight, Serum Lipids, Insulin, and Glucose Across Time

	Vehicle (n=11)	OT (n=11)
Weight (kg)		
Baseline	3.02 ± 0.09	2.87 ± 0.10
Midpoint	3.26 ± 0.07	3.16 ± 0.09
Endpoint	3.54 ± 0.07	3.45 ± 0.12
Cholesterol (mg/dl)		
Baseline	610.5 ± 55.1	596.7 ± 39.1
Midpoint	601.3 ± 43.2	621.2 ± 36.7
Endpoint	541.5 ± 44.3	535.8 ± 36.0
Triglyceride (mg/dl)		
Baseline	316.5 ± 28.7	313.8 ± 38.3
Midpoint	253.7 ± 44.2	211.7 ± 35.6
Endpoint	227.8 ± 36.5	195.8 ± 35.7
Non-Esterified Fatty Acids (μM)		
Baseline	488.2 ± 23.9	545.8 ± 39.7
Midpoint	593.4 ± 27.6	565.4 ± 48.7
Endpoint	893.6 ± 56.8	785.6 ± 20.4
Glucose (mg/dl)		
Baseline	118.7 ± 1.9	120.1 ± 2.7
Midpoint	121.0 ± 1.7	115.5 ± 2.5
Endpoint	112.9 ± 3.4	109.3 ± 1.7
Insulin (ng/ml)		
Baseline	0.88 ± 0.16	0.73 ± 0.15
Midpoint	**0.59 ± 0.11**	**1.15 ± 0.23** [*]
Endpoint	1.08 ± 0.32	2.43 ± 0.63

Values are mean ± standard error of the mean. Baseline is pretreatment. Midpoint is after 8 weeks of treatment. Endpoint is after 16 weeks of treatment. Bold numbers represent significance differences between Groups.

[*] $p < 0.05$ between Groups

Table 2

Mean Values of Biomarkers Across Time

	Vehicle (n=11)	OT (n=11)
Cortisol (μg/dl)		
Baseline	0.16 ± 0.11	0.16 ± 0.05
Midpoint	0.11 ± 0.03	0.36 ± 0.17
Endpoint	0.20 ± 0.04	0.18 ± 0.05
Urinary Epinephrine (ng/mg creatinine)		
Baseline	2.0 ± 1.0	1.3 ± 0.8
Midpoint	1.0 ± 0.1	1.0 ± 0.2
Endpoint	1.3 ± 0.1	1.5 ± 0.2
Urinary Norepinephrine (ng/mg creatinine)		
Baseline	25.3 ± 4.6	24.5 ± 11.7
Midpoint	17.7 ± 2.6	16.4 ± 2.0
Endpoint	26.7 ± 3.4	23.4 ± 3.9
Oxidized LDL (U/l)		
Baseline	33.3 ± 1.4	32.4 ± 1.3
Midpoint	33.3 ± 1.8	30.3 ± 1.8
Endpoint	27.2 ± 1.1	27.2 ± 1.4
Urinary Isoprostane (ng/mg creatinine)		
Baseline	16.4 ± 9.3	16.0 ± 9.4
Midpoint	6.9 ± 0.7	7.9 ± 1.2
Endpoint	12.2 ± 0.8	12.8 ± 1.4
C-Reactive Protein (CRP; μg/ml)		
Baseline	35.2 ± 6.6	57.6 ± 18.0
Midpoint	**25.8 ± 3.4**	**16.6 ± 2.7** [*]
Endpoint	**44.9 ± 6.8**	**28.5 ± 4.2** [*]

Values are mean ± standard error of the mean. Baseline is pretreatment. Midpoint is after 8 weeks of treatment. End point is after 16 weeks of treatment. Bold numbers represent significance differences between Groups.

[*] $p = 0.05$ between Groups