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MATERNAL-FETAL MEDICINE

Elevated cardiac oxidative stress in newborn rats from mothers treated with atosiban

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Abstract
Purpose The purpose of this study was to evaluate the cardiac and cerebral oxidative stress in the offspings of pregnant rats treated with oxytocin antagonist atosiban.
Methods Experimentally naive, adult female Wistar-albino rats (200–250 g) were mated with adult male rats for copulation. After confirming pregnancy, eight gravid rats were then randomly assigned into two equal groups. The animals were treated from days 15 to 20 of gestation. One group acted as a control group, and received intraperitoneal (i.p.) injections of saline in a daily dose volume of 6 mg/kg/day. The second group received 6 mg/kg/day i.p. atosiban. On day 21 of gestation, pups were delivered by cesarean. The heart and brain tissues of the newborn rats were dissected and sent for the measurement of total oxidant status, total antioxitant status and oxidative stress index.
Results There was no significant difference in birthweight or in the number of pups between two groups. Newborns from atosiban-treated mothers showed significantly increased oxidative stress in the plasma and heart tissue than that of controls which was confirmed by histological examination ($P < 0.05$). Oxidative stress parameters and histopathological results of the brain tissues of newborns were similar between two groups ($P > 0.05$).
Conclusion Oxytocin receptor blockage for the treatment of premature delivery may be associated with increased fetal morbidity and mortality secondary to the elevated oxidative stress in the heart of the newborns.

Keywords Obstetric labor · Premature · Atosiban · Oxidative stress · Oxytocin

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Introduction

Preterm labor is defined as being before 37 completed weeks and complicates 10–15% of all pregnancies [1]. It is the most important single determinant of adverse infant outcome, in terms of both survival and quality of life. Treatment of preterm labor is important, not as an end in itself, but as a means of reducing adverse events for the infants. Delaying delivery by 48 h allows obstetricians to administer a full course of glucocorticoids to the expectant mother, which can reduce the incidence of neonatal morbidity and mortality in the preterm neonate [2, 3]. A wide variety of agents have been advocated as suppressing uterine contractions in patients with preterm labor. Those in current use include beta-agonists, calcium channel blockers, prostaglandin synthetase inhibitors, nitric oxide donors and oxytocin (OT) receptor antagonists [4].

Atosiban, a peptide antagonist of OT and vasopressin V1a receptors, has been approved for use in Europe. Numerous studies have demonstrated the efficacy of atosiban as a tocolytic in animals and humans. In previous multicentric, randomized clinical trials, however, atosiban

failed to improve relevant neonatal outcomes and was linked with significant fetal and neonatal morbidity and mortality [5, 6]. These reports raised concerns about its effects of perinatal safety and atosiban is not recommended for such use in several countries. The main reason of atosiban-related fetal–neonatal mortality was considered to be related with non-balanced randomization in the relevant studies, in which higher percentage of patients in labor before 26 weeks' gestation was included in the atosiban group, however, the topic has not been extensively studied [6–8].

Oxytocin, which was originally considered as a maternal hormone regulating reproductive functions and complex maternal behavior, has gained an extensive interest with its effects on stress-induced behaviors and physiological functions shown in both animal and human research [9–11]. Transplacental passage of oxytocin and atosiban from mother to fetus was previously shown in either human or animal models [12–14]. Furthermore, it has been previously shown that OT alleviates dermal, gastric, hepatic, cerebral and colonic injury by reducing oxidant stress in different models of inflammation and these protective effects can be reversed via OT receptor blockage by atosiban [15–21]. In a recent trial, cardioprotective effect of OT on ischemia/reperfusion injury in an in vivo rat model has been presented [22].

On the basis of this background, using oxidative stress index (OSI) values, we aimed to investigate the effects of oxytocin receptor blockage on the heart and brain tissues of newborn rats exposed atosiban during late prenatal period in an experimental model.

Materials and methods

Care of animals and treatment

This study was carried out in the Experimental Research Laboratory of the Faculty of Medicine, complying with the approval of the ethic committee and the guidelines for care and use of experimental animals. Eight adult female Wistar rats each weighing between 200 and 250 g were purchased from Animal Laboratory. All rats were examined by a veterinarian and determined to be in good health. The rats were housed in plastic cages and they were kept under standard conditions: 12-h light and 12-h dark periods, 20°C constant temperatures and a humidity range between 40 and 60%. The rats had free access to standard dry pellets ad libitum and tap water until the end of the study. The female rats at proestrous, determined by vaginal lavage were caged overnight with sexually matured males of the same strain. The following morning, vaginal smear were obtained and observed under the light microscope and presence of sperm indicated zero day of pregnancy. The pregnant female rats were divided into two groups of four animals. The animals were treated from days 15 to 20 of gestation. One group acted as a control group, and received intraperitoneally (i.p.) injections of saline in a daily dose volume of 6 mg/kg. The second group received 6 mg/kg/day i.p. atosiban. Atosiban (Ferring Pharmaceuticals, Malmö, Sweden) was purchased from a local pharmacy shop. All animals were examined at least once daily for drug-related clinical signs of toxicity.

Parturition

On day 21 of gestation, pregnant Wistar rats were euthanized and pups were delivered by cesarean section. Fetuses were removed individually, weighed and examined for external, visceral, and skeletal alterations and were found to be normal. The heart and brain tissues of the newborns were completely dissected under a microscope.

Tissue preparations for analysis

After removal, the heart and brain tissues were stored in a deep freeze (−80°C) until processing. All tissues were homogenized in ice-cold 140 mM KCI at 16,000 rpm for 2 min using a homogenizer (IKA Ultra-Turrax T25 basic homogenizer, Germany). The homogenate was centrifuged at 3,000 rpm for 5 min at +4°C. The supernatant was used to measure the total antioxitant status (TAS) and total oxidant status (TOS) activities.

Measurement of TAS

Plasma and tissue TAS levels were determined using a commercially available kit developed by Erel [23, 24] (REL assay diagnostics, Mega Tip, Gaziantep, Turkey). In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing of ferrous ion solution and hydrogen peroxide solution. In the most recently developed assay by Erel [23], same reaction is used. In the assay, ferrous ion solution, which is present in the reagent 1, is mixed by hydrogen peroxide, which is present in the reagent 2. The sequentially produced radicals such as brown-colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values, which are lower than 3%. The results are expressed as millimoles of Trolox (Sigma-Aldrich Chemical Co., Deutschland, Germany) equivalent per liter.

Measurement of TOS

Plasma and tissue TOS levels were determined using a commercially available kit, developed by Erel [23, 24] (REL assay diagnostics, Mega Tip, Gaziantep, Turkey). In this method, oxidants present in the sample oxidize the ferrous ion-odianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L). Hydrogen peroxide and other derivatives of peroxides, produced physiologically in organisms and occurring in higher concentrations in the tissues under some pathologic conditions, and diffuse into plasma. The level of total peroxide was measured and expressed as TOS in this study.

Measurement of OSI

The ratio of TOS to TAS was accepted as the OSI. For calculation, the resulting unit of TAS was converted to μmol/L, and the OSI value was calculated according to the following formula : OSI (arbitrary unit) = TOS (μmol H_2O_2 equiv/L)/TAC (μmol Trolox equiv/L) [23–25].

Histologic examination

All the specimens were also sent for histologic investigation in order to confirm the tissue diagnosis. For light microscopic evaluation, neonatal heart and brain samples were fixed in phosphate buffered 10% formalin and prepared for routine paraffin embedding. Sections of tissues were cut at 5 μm, mounted on slides, stained with hematoxylin and eosin (H&E) and examined by a Leica DFC280 light microscope and Leica Q Win Image Analyses System (Leica Microsystems Imaging Solutions, Cambridge, UK). Tissue sections were then examined microscopically by a pathologist blind to clinical data and experimental procedure. The major histopathological findings of cellular damage, vacuolation, necrosis, vascularity and leucocyte infiltration were evaluated. The histological score of the organ was calculated as the sum of the scores (0–3) given for each criterion, using the semiquantitative scale. Histological scorings were made at magnification of 40× from 20 random fields per section from each specimen.

Statistical analysis

The comparisons of baseline charactheristics of newborn rats were performed with independent sample t test by using the Statistical Package for Social Sciences (SPSS 11.5, SPSS Inc., Chicago, IL, USA). Since the results showed a non-normal distribution, Mann–Whitney *U* test was used for detection of TAS, TOS and OSI value differences between the groups.

Kruskal–Wallis test was conducted on histological variables to compare all groups together. For detecting inter group differences, the Bonferroni Mann–Whitney *U* test was used permutatively. All data were expressed as mean ± SD. A *P* value of less than 0.05 was accepted as statistically significant.

Results

Maternal treatment with atosiban had no effect on the number of offspring born or antropomethric measurements. No fetal death or resorption was found in any of the dams. The mean body weight of the pups in study group and control group were 5.04 ± 0.610 and 5.22 ± 0.72 g, respectively ($P = 0{,}372$). The mean length of the pups of the study and control group were 18.8 ± 1.71 mm, 19.2 ± 1.29 mm, respectively ($P = 0.124$).

Assessment of TAS, TOS and OSI

A marked increase in plasma TOS levels in atosiban-treated group was determined when compared to control group ($P = 0.021$). The mean TAS, TOS and OSI values determined in plasma and tissue samples of the newborns are presented in Tables 1, 2 and 3. The mean cardiac TOS and OSI values of the Atosiban-treated group was significantly higher than that of control cases ($P = 0.025$ and $P < 0.001$, respectively) (Fig. 1). Oxidative stress parameters in the brain tissue of the dams were similar between the groups.

Table 1 Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress ındex (OSI) in the brain tissues of newborn rats

Parameters	Atosiban group	Control group	*P* value
TAS (mmolTrolox Equiv/L)	0.712 ± 0.354	0.659 ± 0.259	0.882
TOS (μmol H_2O_2 Equiv/L)	5.306 ± 1.989	6.018 ± 1.08	0.119
OSI (ratio)	0.927 ± 0.449	1.231 ± 0.851	0.07

Data are expressed as mean ± SD

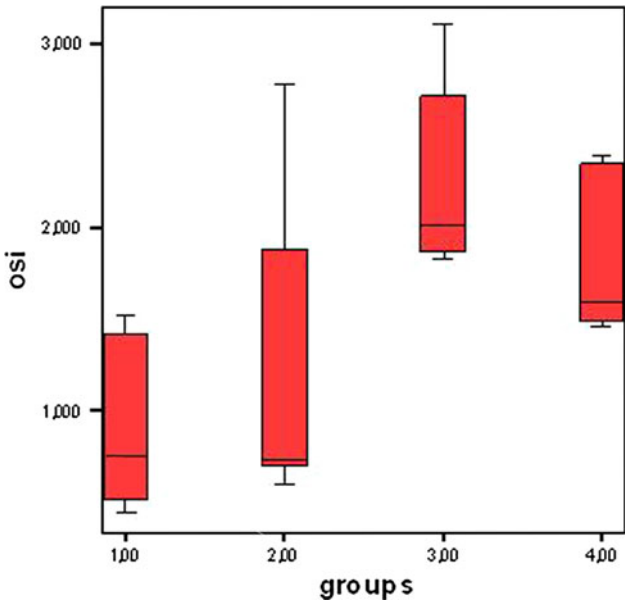

Fig. 1 Comparison of oxidative stress index (OSI) values between the groups [*1* Study group: brain; *2* Control group: brain ($P = 0.07$); *3* study group: heart; *4* Control group: heart ($P < 0.01$)]

Table 2 Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress ındex (OSI) in the heart tissues of newborn rats

Parameters	Atosiban group	Control group	*P* value
TAS (mmolTrolox Equiv./L)	0.325 ± 0.277	0.413 ± 0.286	0.136
TOS (μmol H_2O_2 Equiv./L)	7.245 ± 4.762	6.861 ± 4.253	*0.025*
OSI (ratio)	2.266 ± 0.485	1.807 ± 0.401	*<0.001*

Data are expressed as mean ± SD

Values in italics indicate that the difference is statistically significant

Table 3 Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress ındex (OSI) in the plasma of newborn rats

Parameters	Atosiban group	Control group	*P* value
TAS (mmolTrolox Equiv./L)	0.912 ± 0.442	0.704 ± 0.314	0.09
TOS (μmol H_2O_2 Equiv./L)	8.306 ± 1.115	6.018 ± 0.920	*0.021*
OSI (ratio)	1.932 ± 0.557	1.441 ± 0.951	0.061

Data are expressed as mean ± SD

Value in italics indicates that the difference is statistically significant

Assessment of histopathological examination

The histologic appearances of cardiac and cerebral specimens obtained from the siblings at postnatal day 0 are shown in Fig. 2. Histopathological scores were significantly higher in all the cardiac tissues from the rats exposed to Asotsiban, demonstrating structural degeneration accompanied by vasocongestion, and diffuse edema ($P < 0.01$). Inflammatory cell infiltration were not different between the groups (Table 4).

There was no any significant histopathological changes in brain tissues, neither in study group nor in control group.

Discussion

In the present study, it has been clearly shown that intraperitoneal administration of Atosiban to pregnant rats at a dosage level of 6 mg/kg in late pregnancy, caused oxidative damage in the heart tissues of newborns as demonstrated by increased total oxidative parameters together with the histological findings.

Despite being the first peptide hormone to be characterized and synthesized, the effects of OT were considered to be restricted to the reproductive system. It was believed that OT is released from hypothalamic nerve terminals of the posterior pituitary into the circulation where it stimulates uterine contractions during parturition and milk ejection during lactation. However, OT receptor is expressed in many other organ systems and the hormone may have the potential role to affect the normal cellular functioning in these tissues (17–20). Previously, it was shown that the heart is a site of OT synthesis and release . The presence of OT was detected in all four chambers of the rat heart, and its gene expression was determined by the presence of specific OT mRNA after PCR analyses [26]. It has been suggested that intrinsic OT system may play an important physiological role in regulation of vascular tone, as well as control of cardiac function [27, 28]. Oxytocin protects oxidative damage by acting as an antioxidant agent and its protective effect in the brain, colon, liver and kidney appears to be dependent on its inhibitory effect on the production of reactive oxygen species [17, 18, 29].

In a recently published experimental model of ischemia/reperfusion injury, Houshmand et al. [30] demonstrated the blockage of the cardioprotective effect of OT by atosiban. Another possibility to consider is that OT may release atrial natriuretic peptide, which is a vasodilator and has antioxidative properties [31]. In view of the emerging significance of OT on cardiovascular health, we investigated, for the first time, the effects of atosiban, a choice of treatment for premature delivery, on the cardiac oxidative status of newborns.

In the human and animal studies, it was suggested that there was an increase in the number of stillbirths in pregnants exposing atosiban during late pregnancy [6, 32]. In their large-scale multicentric, randomized controlled study, Romero et al. have reported that the fetal-infant mortality rate was significantly higher in the pregnants receiving atosiban than that of placebo group (4.5 vs. 1.7%; $P < 0.05$). These data, together with our results, may provide an explanation of

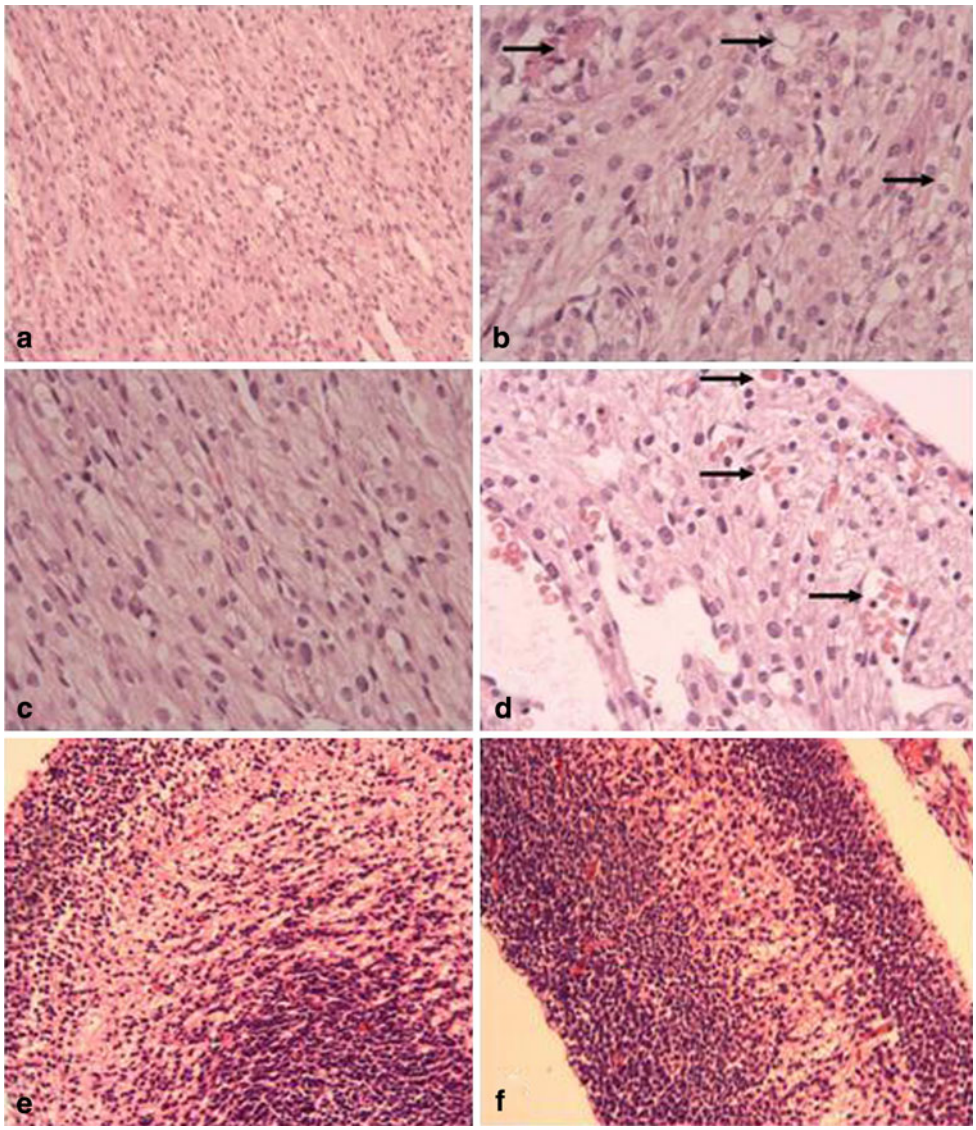

Fig. 2 The histopathological appearance of cardiac samples of the pups from control (**a**–**c**) and Atosiban (**b**–**d**) groups (H&E ×40). *Arrows* indicate necrotic and hydropic changes in cardiomyocytes (**b**) and increased vascular congestion (**d**) in the heart. Histopathological changes of the brain tissue were not different between the Atosiban (**e**) and control groups (f)

Table 4 Mean histological scores of the cardiac tissues of the Atosiban and control groups

Histological changes	Groups		*P* value
	Atosiban group	Control group	
Necrosis	1.20 ± 0.71	0.50 ± 0.57	*<0.01*
Hydropic vacuolization	2.06 ± 0.58	0.46 ± 0.30	*<0.01*
Vascular congestion	2.03 ± 0.92	0.77 ± 0.46	*<0.01*
Leucocyte infiltration	0.30 ± 0.46	0.20 ± 0.40	0.380

Data are expressed as mean ± SD

Values in italics indicate that the difference is statistically significant

increased fetal mortality in patients treated with atosiban in order to prevent premature delivery.

Although, there are different opinion on the subject, in humans, it has been shown that maternal oxytocin concentrations rise gradually with advancing gestation [33, 34]. However, little transport of oxytocin across the placenta has been shown an in vivo study [34].

Pharmacokinetics, plasma kinetics and placental transfer of atosiban has been investigated both with single i.v. bolus and different infusion schemes in pregnant women. Valenzuela et al. [12] demonstrated the transplacental passage of atosiban in pregnant women. Following an infusion rate of 300 μg/min in healthy pregnant women at term, the fetal/maternal atosiban concentration ratio was found 0.12. In experimental rat models, the fetal-to-maternal concentration ratio of the drug has been found 0.01–0.02. Although such a low placental transfer rate, our results must raise concerns about the fetal and neonatal safety of OT receptor blockage for the treatment of premature labor.

Recently, it has become clear that oxytocin also exerts numerous actions within the brain to modify behavior in several species, including humans [35, 36]. It was shown that 1–2% of peripherally given dose of OT passes the

blood–brain barrier. Furthermore, in humans, fetal oxytocin concentrations are higher in the umbilical artery than in the umbilical vein at term, suggesting that the fetus itself is an active producer of the hormone [37, 38]. Tyzio et al. [39] have reported a novel role for oxytocin within the rat brain, as a neuroprotective agent that protects the fetal hippocampus from hypoxic or hypoglycemic insult during delivery. They showed that treatment of fetuses with atosiban, advanced the onset of anoxic depolarization in the hippocampus of term rats in vitro. The neuroprotective effects of oxytocin have been suggested to be associated with the triggering an inhibitory switch in GABA (γ-aminobutyric acid) signaling which prolongs the time to anoxic depolarization in neural cells. However, in our study, the mean brain TAS, TOS and OSI values after treatment were not siginifcantly different between the atosiban and control groups. Furthermore, there was no evidence of cellular damage in the brain tiusse sections and the tissue arcitecture was appeared histologically normal in all cases from both groups. This may be related to the small numbers of litters per experimental group, selected dose of the atosiban and/or the short duration of treatment in the present study.

In this study, we have used novel measurement methods to evaluate the extent of oxidative stress in the heart and brain tissues of newborn rats. It provides a useful method for the rapid evaluation of the TAS and TOS, which are valuable in the diagnosis of oxidative stress [23–25].

There are several limitations to this study. This is an experimental study with small numbers of pregnant rats per groups. This was due to our ethical concern regarding to conform of 'principle of reduction' in animal experiments; however, much larger numbers would be needed to find smaller histological and biochemical differences between the groups. The second limitation was using a sigle-dose Atosiban in the present study design, instead of evaluation the biochemical and histological changes in different doses. Therefore we were unable to reach a 'dose-response curve', which would certainly be more valuable to analyse the possible detrimental effects of Atosiban on heart and brain tissues of newborns and to make a clear conclusion.

In conclusion, the discovery of protective effects of OT on various tissues including heart and brain, provides us another point of view for the treatment of premature delivery. We are introducing a new research trend based on a novel concept. Evidence linking oxytocin receptor blockage and increased cardiac oxidative stress in our study could provide insights into the atosiban-associated fetal–neonatal mortality which was previously reported. According to the best of our knowledge, this is the first time such an observation has been reported in an experimental model. For mentioned reasons above, to prevent the possible deleterious effects of the atosiban associated-oxidative stress in the newborns, the addition of antioxidant therapy may be considered in women with a diagnosis of premature delivery, and treated with oxytocin antagonists.

Conflict of interest None.

References

1. Ananth CV, Vintzileos AM (2006) Epidemiology of preterm birth and its clinical subtypes. J Matern Fetal Neonatal Med 19:773–782
2. Sehdev HM, Abbasi S, Robertson P, Fisher L, Marchiano DA, Gerdes JS, Ludmir J (2004) The effects of the time interval from antenatal corticosteroid exposure to delivery on neonatal outcome of very low birth weight infants. Am J Obstet Gynecol 191:1409–1413
3. Purdy IB, Wiley DJ (2004) Perinatal corticosteroids: a review of research. Part I: antenatal administration. Neonatal Netw 23:15–30
4. Blumenfeld YJ, Lyell DJ (2009) Prematurity prevention: the role of acute tocolysis. Curr Opin Obstet Gynecol 21:136–141
5. Moutquin JM, Sherman D, Cohen H, Mohide PT, Hochner-Celnikier D, Fejgin M, Liston RM, Dansereau J, Mazor M, Shalev E, Boucher M, Glezerman M, Zimmer EZ, Rabinovici J (2000) Double-blind, randomized, controlled trial of atosiban and ritodrine in the treatment of preterm labor: a multicenter effectiveness and safety study. Am J Obstet Gynecol 183:1191–1199
6. Romero R, Sibai BM, Sanchez-Ramos L, Valenzuela GJ, Veille JC, Tabor B, Perry KG, Varner M, Goodwin TM, Lane R, Smith J, Shangold G, Creasy GW (2000) An oxytocin receptor antagonist (atosiban) in the treatment of preterm labor: a randomized, double-blind, placebo-controlled trial with tocolytic rescue. Am J Obstet Gynecol 182:1173–1183
7. Valenzuela GJ, Sanchez-Ramos L, Romero R, Silver HM, Koltun WD, Millar L, Hobbins J, Rayburn W, Shangold G, Wang J, Smith J, Creasy GW (2000) Maintenance treatment of preterm labor with the oxytocin antagonist atosiban. The Atosiban PTL-098 Study Group. Am J Obstet Gynecol 182:1184–1190
8. French/Australian Atosiban Investigators Group (2001) Treatment of preterm labor with the oxytocin antagonist atosiban: a double-blind, randomized, controlled comparison with salbutamol. Eur J Obstet Gynecol Reprod Biol 98:177–185
9. Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008) Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. Neuron 58:639–650
10. Yoshida M, Takayanagi Y, Inoue K, Kimura T, Young LJ, Onaka T, Nishimori K (2009) Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. J Neurosci 29:2259–2271
11. Slattery DA, Neumann ID (2008) No stress please! Mechanisms of stress hyporesponsiveness of the maternal brain. J Physiol 586:377–385
12. Valenzuela GJ, Craig J, Bernhardt MD, Holland ML (1995) Placental passage of the oxytocin antagonist atosiban. Am J Obstet Gynecol 172:1304–1306
13. Malek A, Blann E, Mattison DR (2006) Human placental transport of oxytocin. J Matern Fetal Med 5:245–255
14. Zellers JE, Davis CC, Oldham JW, Mackey MJ, Szot RJ (1996) Subcutaneous developmental toxicity study of RWJ-22164-021 (atosiban) in female Crl:CD®B.R., VAF/Plus® rats. Internal report no. 382520:1-DS-94307. RW Johnson Pharmaceutical Research Institute, Raritan, NJ
15. Moosman B, Behl C (2002) Secretory peptide hormones are biochemical antioxidants: structure–activity relationship. Mol Pharmacol 61:260–268

16. Cetinel S, Hancioğlu S, Sener E, Uner C, Kiliç M, Sener G, Yeğen BC (2010) Oxytocin treatment alleviates stress-aggravated colitis by a receptor-dependent mechanism. Regul Pept 25(160):146–152
17. Dusunceli F, Iseri SO, Ercan F, Gedik N, Yegen C, Yegen BC (2008) Oxytocin alleviates hepatic ischemia–reperfusion injury in rats. Peptides 29:1216–1222
18. Tugtepe H, Sener G, Biyikli NK, Yuksel M, Cetinel S, Gedik N, Yegen BC (2007) The protective effect of oxytocin on renal ischemia/reperfusion injury in rats. Regul Pept 140:101–108
19. Iseri SO, Gedik IE, Erzik C, Uslu B, Arbak S, Gedik N, Yegen BC (2008) Oxytocin ameliorates skin damage and oxidant gastric injury in rats with thermal trauma. Burns 34:361–369
20. Iseri SO, Sener G, Sağlam B, Gedik N, Ercan F, Yeğen BC (2005) Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. Peptides 26:483–491
21. Biyikli NK, Tugtepe H, Sener G, Velioglu-Ogünç A, Cetinel S, Midillioglu S, Gedik N, Yegen BC (2006) Oxytocin alleviates oxidative renal injury in pyelonephritic rats via a neutrophil-dependent mechanism. Peptides 27:2249–2257
22. Houshmand F, Faghihi M, Zahediasl S (2009) Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats. Peptides 30:2301–2308
23. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–1111
24. Erel O (2004) A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 37:112–119
25. Harma M, Harma M, Erel O (2003) Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly 133:563–566
26. Jankowski M, Hajjar F, Kawas SA, Mukaddam-Daher S, Hoffman G, McCann SM, Gutkowska J (1998) Rat heart: a site of oxytocin production and action. Proc Natl Acad Sci USA 24(95):14558–14563
27. Gutkowska J, Jankowski M, Mukaddam-Daher S, McCann SM (2000) Oxytocin is a cardiovascular hormone. Braz J Med Biol Res 33:625–633
28. Jankowski M, Wang D, Hajjar F, Mukaddam-Daher S, McCann SM, Gutkowska J (2000) Oxytocin and its receptors are synthesized in the rat vasculature. Proc Natl Acad Sci USA 97:6207–6211
29. Iseri SO, Sener G, Saglam B, Gedik N, Ercan F, Yegen BC (2005) Oxytocin protects against sepsis-induced multiple organ damage: role of neutrophils. J Surg Res 126:73–81
30. Houshmand F, Faghihi M, Saleh Z (2009) Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats. Peptides 30:2301–2308
31. Gutkowska J, Jankowski M, Lambert C, Mukaddam-Daher S, Zingg H, McCann S (1997) Oxytocin releases atrial natriuretic peptide by combining with oxytocin receptors in the heart. Proc Natl Acad Sci USA 94:11704–11709
32. Christian MS, Hoberman AM, Foss JA (1995) Perinatal and postnatal reproduction study of RWJ-22164–021 administered subcutaneously to Crl:CD®BR, VAF/Plus® rats (Segment III evaluation). Includes postnatal "behavioral/functional" evaluation. Argus Research Laboratories Inc., Horsham
33. Fuchs AR, Romero R, Keefe D, Parra M, Oyarzun E, Behnke E (1991) Oxytocin secretion and human parturition: pulse frequency and duration increase during spontaneous labor in women. Am J Obstet Gynecol 165(5 Pt 1):1515–1523
34. de Geest K, Thiery M, Piron-Possuyt G, Vanden Driessche R (1985) Plasma oxytocin in human pregnancy and parturition. J Perinat Med 13:3–13
35. Young LJ, Wang Z (2004) The neurobiology of pair bonding. Nat Neurosci 7:1048–1054
36. Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E (2005) Oxytocin increases trust in humans. Nature 435:673–676
37. Uvnas-Moberg K (1998) Antistress pattern induced by oxytocin. News Physiol Sci 13:22–25
38. Dawood MY, Wang CF, Gupta R, Fuchs F (1978) Fetal contribution to oxytocin in human labor. Obstet Gynecol 52:205–209
39. Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hübner CA, Represa A, Ben-Ari Y, Khazipov R (2006) Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. Science 314:1788–1792