

EXPERIMENTAL STUDY

Chorionic morphine, naltrexone and pentoxifylline effect on hypophyso-gonadal hormones of male rats

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Abstract: *Background:* Knowledge about harmful effects of morphine on hormone secretion seems to be necessary. The aim of the present study was to evaluate the effect of pentoxifylline on side effects derived by morphine on hypophyso-gonadal hormones of male rats.

Methods: 32 male rats were divided into the 4 groups of OSS: control (received 40 g Sucrose/l drinking water and intraperitoneal injection of 1 l/kg normal saline), OMS: morphine group (received 0.4 mg/l + 40 g Sucrose/l in drinking water and intraperitoneal injection of 1 l/kg normal saline), NMS: morphine+naltrexane group (received 0.4 mg/l + 40 g Sucrose/l in drinking water and IP injection dose of 10 mg/kg/ml/day Naltrexane) and PMS: morphine + pentoxifylline group (received 0.4 mg/dl + 40 g Sucrose/l in drinking water and IP injection dose of 12 mg/kg/ml/day Pentoxifylline) for 56 days, respectively.

Results: Serum levels of testosterone, LH, FSH hormones were measured. Pentoxifylline increased serum levels of testosterone, LH, FSH hormones compared to control, morphine and morphine-naltrexane groups.

Conclusion: Pentoxifylline has a significant efficacy for increasing serum levels of sexual hormones. Considering that Pentoxifylline is safe and cheap, with easy application, we suggest for the usage of this drug for improving semen parameter's quality before performing ART for the treatment of morphine addicts (Fig. 1, Ref. 31).
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Key words: morphine, naltrexane, pentoxifylline, rat.

Introduction

The side effects of opioids usage/misusage on endocrine system in both human and laboratory animals have been characterized. Although, hormones releasing from pituitary gland are affected by opioids, hypogonadism could be considered as a primary consequence of usage of these drugs. The potential consequences of hypogonadism include decreased libido and erectile dysfunction in men, oligomenorrhea or amenorrhea in women, and bone loss or infertility in both sexes (1–3).

Opioid receptor antagonists like Naltrexone are commonly used for the treatment of drug abused subjects by decreasing opioid intake and reversing the effects of opioid overdose (1). Studies showed that morphine decreased LH and also testosterone secretion while morphine antagonists like Naltrexone and Naloxone relieved this effects, but after a chronic treatment, a high level of testosterone along with the fact that morphine enhanced sensitivity of the hypothalamus to negative feedback by testosterone could induce a negative effect on male reproductive system in morphine abused subject treated with Naloxone (4, 5).

Pentoxifylline is a selective drug for the dilatation of the vessels in the limbs, brain and retina, it also increases diapedesis of red cells among the wall of capillaries, improves microcirculation of the vessels, blocks the action of neutrophils and increases or decreases production of some cytokines (6). Pentoxifylline (PTX) is effective for the treatment of diseases related to fibrotic tissue such as chorionic wound healing, necrotic wounds in the diabetic veins (Venous leg ulcer), pulmonary fibrotic inflammation, fibrosis of interstitial tissue of the kidney, phlegmona of the foot, and sarcoidosis (7–9).

The effects of pentoxifylline on male reproductive system have repeatedly been described and shown that pentoxifylline increased sperm motility and acrosome reaction and scavenges ROS (10–12). Here, the study was conducted to show the effect of PTX and Naltrexone on serum levels of FSH, LH and testosterone on morphine treated rats.

Materials and methods

Animals

Male Wistar rats (n = 32), weighing approximately 200–250 g, were purchased from Pasteur Institute of Iran (Tehran) and maintained in our animal resource center under controlled conditions of lighting (12-h light, 12-h dark cycle) and temperature (22±2 °C). The animals were given free access to standard chow and water (13).

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Experimental design

All animals received care as based on the Research Committee of our University. The animals were allocated to 4 groups. Each group consisted of 8 animals. All groups including OSS (control group) received 40 g sucrose/lit in their drinking water. The rats (In OMS, NMS, and PMS groups) were made dependent by chronic self-administration of morphine in drinking water. Each of the animals received morphine sulfate 0.1, 0.2 and 0.3 mg/ml for 48 hours and 0.4 mg/ml from 7th day to 56th day with 40 gr. sucrose/lit in their drinking water as previously described (13).

In NMS group, the rats were co-administrated with Naltrexone (Sigma) (10 mg/kg/day) intraperitoneally (i.p) and in PMS group with PTX (sigma) (12 mg/kg/day) (i.p).

On the 57th day, all rats (n = 32) were anesthetized with diethyl ether. Then, blood samples from all experimental rats were collected directly from the right atrium of the heart and separation of serum was achieved by centrifugation. The samples were stored in tubes with plastic caps at -70 until analyses.

Biochemical analysis

The levels of LH, FSH and Testosterone were measured by inductive coupled plasma-optical emission spectroscopy (Perkin Elmer, model 7300, USA) using specific ELISA kites for rat. The level of testosterone was examined by ALPCO (No; 55-TESTMS-E01), For LH by KUSABIO (No; CSB-E12654r), and for FSH by EIAab (No; E0830r) according manufactures' instructions. The data of testosterone was expressed as ng/ml and for FSH and LH as mIU/ml.

Statistical analysis

Data were presented as the mean \pm SD. Multiple comparisons were calculated by One-Way ANOVA. For comparing the two groups, statistical Student's t-test was used. The significance level chosen was $p < 0.05$.

Results

The data of serum levels of FSH, LH and testosterone are shown in the figure 1. The serum level of FSH (mIU/ml) in PMS Group (1.59 ± 0.4) (PTX treated with morphine) was increased in comparison to the control group (OSS) ($p = 0.000$). The serum level in OMS (Morphine treated) (0.54 ± 0.17) and NMS (Naltrexone treated with morphine) (0.56 ± 0.2) did not show significant differences compared to the control group (Fig. 1A). The serum level of FSH in PMS group also was increased in comparison with OMS ($p = 0.000$) and NMS ($p = 0.000$) groups (Fig. 1A). The serum level of LH was decreased in OMS (0.46 ± 0.03) ($p = 0.02$) and NMS (0.52 ± 0.04) ($p = 0.04$) groups, while it was increased in PMS group (1.28 ± 0.2) ($P = 0.000$) in comparison with the control (OSS) group (0.74 ± 0.07) (Fig. 1B). In PMS group, the serum level of LH was also significantly higher than OMS ($p = 0.000$) and NMS ($p = 0.000$) groups (Fig. 1B). The serum level of testosterone in PMS was (5.02 ± 0.9) that showed an increase in comparison with OSS (3.5 ± 0.6) ($p = 0.002$), NMS (2.1 ± 0.3) ($P = 0.000$) and OMS (1.73 ± 0.1) ($p = 0.000$) groups, while it was decreased in OMS ($p = 0.000$) and NMS ($p = 0.006$) in comparison with OSS group (Fig. 1C).

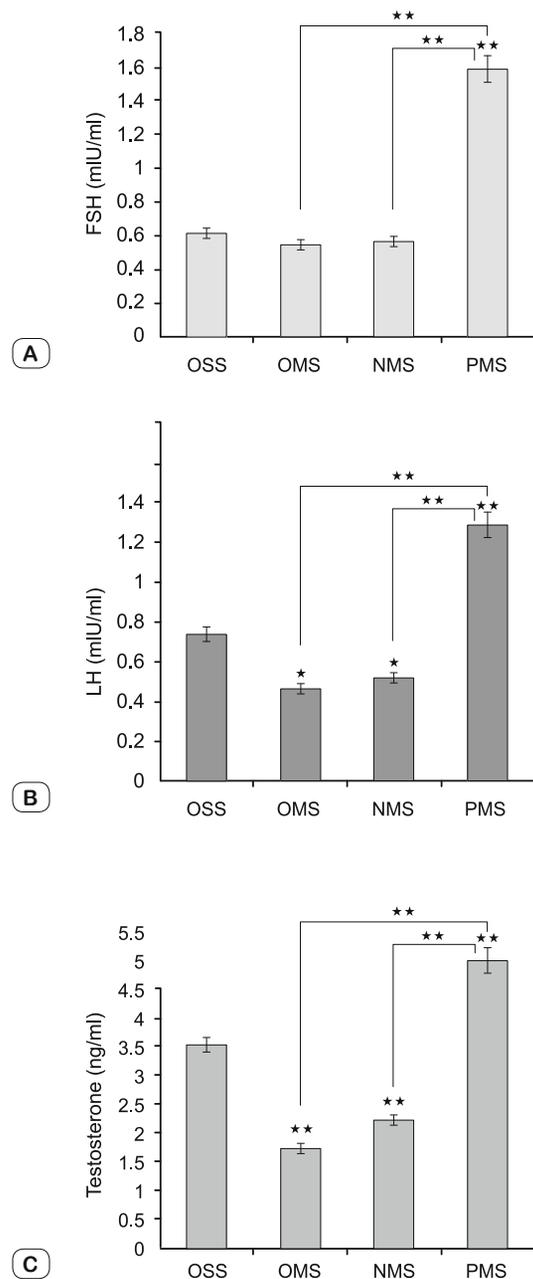


Fig. 1. Effect of morphine administration alone or in combination with naltrexone and pentoxifylline during 56 days of the study (n = 8) on serum levels of FSH(mIU/ml), LH(mIU/ml), and testosterone (ng/ml) of male rats. The rats received only oral sucrose (OSS), sucrose with morphine (OMS), Sucrose and morphine with naltrexone (NMS), Sucrose and morphine with pentoxifylline (PMS). The data showed that in pentoxifylline treated animals the serum level of FSH(A), LH (B) and testosterone (C) significantly increased (* $p < 0.05$, ** $p < 0.001$).

Discussion

In the present study, in Morphine and Morphine + Naltrexone treated animals, the serum level of FSH did not show significant change, but LH level was reduced in these two groups.

The influence of opioids and their antagonists on the hypothalamic-pituitary-gonadal (HPG) axis have been performed in laboratory animals (1). In female animals, exogenous opioids and endogenous opioid peptides caused a significant decrease in LH pulse frequency (14), whereas naloxone promoted an increase in the LH surge compared with saline controls (15). However, in very young (10 to 30 d-old) male rats, naloxone failed to increase serum LH (16) and did not induce pubertal changes. It seems that the concentration of testosterone in males has a positive preliminary effect on the action of opioid antagonist on the secretion of LH and as we showed in this study, in Morphine and Morphine + Naltrexone treated animals that the concentration of testosterone were lower than the control group, Naltrexone failed to increase the serum level of LH. Furthermore, Naltrexone alone in both sexes increased the secretion of LH and also following removing morphine, it could positively sensitize the brain region to negative feedback of testosterone that leads to more secretion of LH. But, in females, estrogen had an opposite effect as that for testosterone in males and when naloxone administered to immature female rats advanced the age of onset of puberty (17). This idea was repeated in ovariectomized rats when LH responses to opioids were antagonized by naloxone (18, 19). In contrast to their effects on LH, most opioids and their analogs appear not to affect FSH (20). Furthermore, treatment with opioid antagonists, naloxone and naltrexone, did not alter FSH concentration levels in either sex (21), suggesting that the endogenous opioids are not involved in regulation of FSH secretion. It is interesting to note that FSH levels are unchanged despite the ability of opioid treatments to stimulate hypothalamic GnRH.

Previous studies indicated that pentoxifylline improved all sperm parameters including sperm count, motility and morphology the serum level of LH, FSH and also testosterone in idiopathic infertile and also in asthenozoospermic men (22). The present study added that pentoxifylline increased serum level of LH, FSH and testosterone in morphine abused animals.

The stimulatory effect of pentoxifylline on sperm parameters can clearly be attributed to the increased intracellular levels of cAMP (23). Cyclic AMP, in turn, is believed to stimulate the cAMP-dependent kinase (24), which itself induces sperm tail protein phosphorylation (25) with subsequent increase in sperm motility (26). Improving acrosomal reaction is another beneficial effect of pentoxifylline drug, demonstrated that this induction is not due to a Ca^{2+} -influx in the sperm cell but is according to increasing the intracellular level of cAMP as the second messenger of acrosomal reaction (27, 28).

Scavenging ROS from spermatozoa is another action of pentoxifylline that is derived by reducing the superoxide release and increasing SOD-like and catalase-like activity (12, 29). This effect is possibly due to the reduction of the formation of endoperoxides as a consequence of the elevated cAMP levels that inhibit the cyclooxygenase within the arachidonic pathway (29). Furthermore, it has been shown that pentoxifylline both enhances morphine's analgesic effects by targeting microglial activation during morphine therapy/treatment in mice (30) and also decreases pro-inflammatory cytokines contributing to nociception in rats (31).

In conclusion, the present study adds that pentoxifylline also reverse side effects of morphine on reproductive system. The act that could be attributed to increased level of cAMP as the most prominent pharmacological effect of pentoxifylline in the response to decreased levels of cAMP derived by morphine administration.

References

- Vuong C, Van Uum SH, O'Dell LE, Lutfy K, Friedman TC.** The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 2010; 31 (1): 98–132.
- Hijazi RA, Cunningham GR.** Andropause: is androgen replacement therapy indicated for the aging male? *Annu Rev Med* 2005; 56: 117–137.
- Daniell HW.** Hypogonadism in men consuming sustained-action oral opioids. *J Pain* 2002; 3: 377–384.
- Bhanot R, Wilkinson M.** The inhibitory effect of opiates on gonadotrophin secretion is dependent upon gonadal steroids. *J Endocrinol* 1984; 102: 133–141.
- Gabriel SM, Simpkins JW, Kalra SP, Kalra PS.** Chronic morphine treatment induces hypersensitivity to testosterone-negative feedback in castrated male rats. *Neuroendocrinology* 1985; 40: 39–44.
- Yessenow RS, Maves MD.** The effect of pentoxifylline on random skin flap survival. *Arch Otolaryngol Head Neck Surg* 1989; 115 (2): 179–181.
- Marques LJ, Zheng L, Poulakis N, Guzman J, Costabel U.** Pentoxifylline inhibits TNF- α production from human alveolar macrophages. *AMJ Respir Crit Care Med* 1999; 159 (2): 508–511.
- Aviado DM, Porter JM.** Pentoxifylline: A new drug for the treatment of intermittent claudication. Mechanism of action, pharmacokinetics, clinical efficacy and adverse effects. *Pharmacotherapy* 1984; 4 (6): 297–307.
- Lin HY, Yeh CT.** Controlled release of pentoxifylline from porous chitosan-pectin scaffolds. *Drug Deliv* 2010; 17 (5): 313–231.
- Nassar A, Mahony M, Blackmore P, Morshedi M, Ozgur K, Oehninger S.** Increase of intracellular calcium is not a cause of pentoxifylline-induced hyperactivated motility or acrosome reaction in human sperm. *Fertil Steril* 1998; 69: 748–754.
- Nassar A, Morshedi M, Mahony M, Srisombut C, Lin MH, Oehninger S.** Pentoxifylline stimulates various sperm motion parameters and cervical mucus penetrability in patients with asthenozoospermia. *Andrologia* 1999; 31: 9–15.
- Gavella M, Lipovac V.** Pentoxifylline-mediated reduction of superoxide anion production by human spermatozoa. *Andrologia* 1992; 24: 37–39.
- Badawy AA, Evans CM, Evans M.** production of tolerance and physical dependence in the rat by simple administration of morphine in drinking water. *Br J Pharmacol* 1982; 75: 485–491.
- Ferin M, Vande Wiele R.** Endogenous opioid peptides and the control of the menstrual cycle. *Eur J Obstet Gynecol Reprod Biol* 1984; 18: 365–373.
- Orstead KM, Hess DL, Spies HG.** Opiatergic inhibition of pulsatile luteinizing hormone release during the menstrual cycle of rhesus macaques. *Proc Soc Exp Biol Med* 1987; 184: 312–319.
- Cicero TJ, Schmoeker PF, Meyer ER, Miller BT, Bell RD, Cytron SM, Brown CC.** Ontogeny of the opioid-mediated control of reproduc-

- tive endocrinology in the male and female rat. *J Pharmacol Exp Ther* 1986; 236: 627–633.
- 17. Sirinathsinghji DJ, Motta M, Martini L.** Induction of precocious puberty in the female rat after chronic naloxone administration during the neonatal period: the opiate ‘brake’ on prepubertal gonadotrophin secretion. *J Endocrinol* 1985; 104: 299–307.
- 18. Eason MG, Francis RS, Kuhn CM.** Opioid agonists stimulate growth hormone secretion in immature rats. *Neuroendocrinology* 1996; 63: 489–497.
- 19. del Valle-Soto ME, Iglesias L, Calzada B, Vega JA, Hernandez LC, Pérez-Casas A.** Effects of morphine on the pituitary-thyroid axis: morphological and analytical studies. *Funct Dev Morphol* 1991; 1: 3–6.
- 20. Yilmaz B, Konar V, Kutlu S, Sandal S, Canpolat S, Gezen MR, Kelestimur H.** Influence of chronic morphine exposure on serum LH, FSH, testosterone levels, and body and testicular weights in the developing male rat. *Arch Androl* 1999; 43: 189–196.
- 21. Abbott DH, Holman SD, Berman M, Neff DA, Goy RW.** Effects of opiate antagonists on hormones and behavior of male and female rhesus monkeys. *Arch Sex Behav* 1984; 13: 1–25.
- 22. Safarinejad MR.** Effect of pentoxifylline on semen parameters, reproductive hormones, and seminal plasma antioxidant capacity in men with idiopathic infertility: a randomized double-blind placebo controlled study. *Int Urol Nephrol* 2011; 43 (2): 315–328.
- 23. Calogero AE, Fishel S, Hall J, Ferrara E, Vicari E, Green S, Hunter A, Burrello N, Thornton S, D’Agata R.** Correlation between intracellular cAMP content, kinematic parameters and hyperactivation of human spermatozoa after incubation with pentoxifylline. *Hum Reprod* 1998; 13: 911–915.
- 24. Tash JS, Hidaka H, Means AR.** Axokinin phosphorylation by cAMP dependent protein kinase is sufficient for activation of sperm flagellar motility. *J Cell Biol* 1986; 103: 649–655.
- 25. Nassar A, Mahony M, Morshedi M, Lin MH, Srisombut C, Oehninger S.** Modulation of sperm tail protein tyrosine phosphorylation by pentoxifylline and its correlation with hyperactivated motility. *Fertil Steril* 1999; 71: 919–923.
- 26. Bracho GE, Fritch JJ, Tash JS.** Identification of flagellar proteins that initiate the activation of sperm motility in vivo. *Biochem Biophys Res Commun* 1998; 242: 231–237.
- 27. Blackmore PF, Neulen J, Lattanzio F, Beebe SJ.** Cell surface-binding sites for progesterone mediated calcium uptake in human sperm. *J Biol Chem* 1991; 266: 18655–18659.
- 28. De Jonge CJ, Han HL, Lawrie H, Mack SR, Zaneveld LJD.** Modulation of the human sperm acrosome reaction by effectors of theadenylate cyclase/cyclic AMP second-messenger pathway. *J Exp Zool* 1991; 258: 113–125.
- 29. Yovich JL.** Pentoxifylline: Actions and applications in assisted reproduction. *Hum Reprod* 1993; 8: 1786–1791.
- 30. Mika J, Wawrzczak-Bargiela A, Osikowicz M.** Attenuation of morphine tolerance by minocycline and pentoxifylline in naive and neuropathic mice. *Brain Behav Immun* 2009; 23: 75–84.
- 31. Wei T, Sabsovich I, Guo TZ.** Pentoxifylline attenuates nociceptive sensitization and cytokine expression in a tibia fracture rat model of complex regional pain syndrome. *Eur J Pain* 2009; 13: 253–262.

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