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Ovarian Polycystic Induction with Morphine in Wistar Rat

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ABSTRACT

Background and Objective: The effect of opioids on the reproductive system has been studied as an attractive research field. In this study, the effect of morphine as an analgesic opioid was evaluated on the ovarian polycystic induction in the rat.

Materials & Methods: In this study, 24 female virgin Wistar rats weighing 200-250 g were injected with morphine (1-10 mg/kg/day) for 9 days. Control group received normal saline (1 ml/kg, i.p.) throughout the experimental period. After completion of injection period, all animals underwent surgery to collect the ovaries. After biometrical measurements, the ovaries specimens were fixed in the formalin to use in future study.

Results: The ovaries from morphine-injected rats had thick-wall follicular cysts whilst the control samples showed the Graafian follicles. In addition, the higher doses of morphine (10 mg/kg) led to much thicker wall cysts than the lower doses (1 mg/kg). However, the experimental samples did not show a significant difference versus control in view of their size.

Conclusion: The enhanced irregularity in the opioid system activity has been reported in polycystic ovary syndrome (PCOS). Based on these findings, the induction of connective disturbance between the opioid and the reproductive systems due to chronic use of the morphine may crucially play a role in ovarian polycystic induction in the rat.`

Key Words:
Morphine
Polycystic
Ovary
Rat

1. Introduction

Opioid drugs are compounds of semi-opium characteristics which are extracted and produced from *Papaver somniferum* (L.). Opium accordingly, is a word derived from *opos*, the Greek word for" juice". Many opioid drugs are still derived from opium (1). In 1806, Frederich Serturner, a pharmacist's assistant, reported the isolation of morphine from opium by crystallization of pure alkaloids. He named the substance in opium as morphine, after *Morpheus*, the Greek GOD of dreams. Morphine is the most crucial one amongst the several alkaloids found in opium and morphine is the main member of opium family. Codeine is made synthetically from morphine and opioid word illustrates variety of synthetic and semi-synthetic drugs with opiate-like characteristics. The main action of opioid system is sedation of the pain. These materials act by attaching to opioid receptors. The opioid receptors are component of G protein-coupled receptors family (2). None of the opioid peptide family is exclusively associated with a specific opioid receptor type. The opioid system is composed of several major opioid receptor types, including mu (m, for morphine) (3,4), kappa (k, for ketocyclazocine) (5), delta (d, for vas deferens) (6), and ORL-1 (for opioid receptor-like) (7). Kappa-opioid-mediated neuro-transmission has been implicated in pain regulation in women, but not in men (7).

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Exploiting drugs out of doctor's prescription is misuse and according to the present finding, the opioid abuse may have unfavorable effects on the reproductive system. Infertility is a major concern in polycystic ovary syndrome (PCOS). Morphine disrupts the ovarian cycles and may reduce the fertility (8). Also, it has been shown in an experiment that prescribing morphine sulfate in influential dose cause teratogenic mice and fetal death. The PCOS is a reproductive system disorder that causes infertility and creates various physical and mental problems in the PCOS patient (9). Based on the documents, the reason of high estrogen, gonadotropin irregular secretion and chronic anovulation are assumed important (10). The μ-opioid receptors have special role in different aspect of female reproduction (11). Activation of μ-opioid receptors are associated with releasing of the endogenous opioid (12). In persons with PCOS, we observed that the level of the luteinizing hormone (LH) increases by opioid stimulation. It is observed that opioidergic receptors inhibition by using naloxone (μ-receptor selective antagonist) causes an increase in LH releasing in final follicular phase, a response that has negative feedback on the gonadotropin releasing hormone (GnRH). It has been shown that the endogenous opioids (like endorphin, enkephalin and dynorphin) have inhibition role on GnRH secretion. Therefore, the change in the action of the opioids leads to alteration of the neuroendocrine events. Another opioid role is blocking the action of hypothalamic-pituitary-ovary axis and stimulating the production of growth hormone (GH) and prolactin (13-15).

2. Materials and Methods

We used the morphine sulfate (purchased from TEMAD Company with certificate from the Ministry of Health Iran) and Hematoxylin-Eosin was from Merck Co., Germany.

2.1. Animals

The animals were Wistar female rats (body weight 200-250 g) purchased from Pasteur Institute of Iran. They were retained in the animal care center of School of Basic Sciences at Shahed University under standard conditions (21± 3°C and 12-h light / dark cycle). The animals were kept in the standard cages in groups of two. The rats had free access to the standard food (provided from Pars Co.) and tap water.

2.2. Experimental Method

In this study, 24 female rats were randomly chosen and divided into the experimental morphine receiving groups (1-10 mg/kg, i.p.) and the control (taking normal saline solution; 1 ml/kg, i.p.). The trial groups (n=6 per morphine dose) were drug injected intraperitoneally during a 9-day period. Control group was solely injected with saline throughout the experimental period. The weights of rats were determined before and after the completion of the injection period. By the end of the experiments, all animals underwent surgery; the ovaries were dissected out and then, after the biometrical measurements of the ovaries, the specimens were fixed in 10% formalin for tissue slicing. The tissue blocks were sectioned (3-4 μm) by using the microtome. They were stained with the aid of the Hematoxylin and Eosin (H&E) method.

2.3. Statistical analysis

All data were analyzed using the analysis of variance (ANOVA) by the SPSS software to compare the number of cysts between the experimental groups' ovaries and those obtained from the control group. For comparison between the groups, the Tukey Kramer's *post hoc* test was used. The statistical significance was considered at $p< 0.05$. All data are expressed as Means ± SEM. The Image Tool software was used for quality examination of the photos in an area of 100 μm^2 with the density calibration.

3. Results

Finding from this experimental study showed a significant effect of morphine (1-10 mg/kg, i.p.) on the rats' ovaries. These specimens had follicular cysts versus the control group receiving saline (Fig. 1).

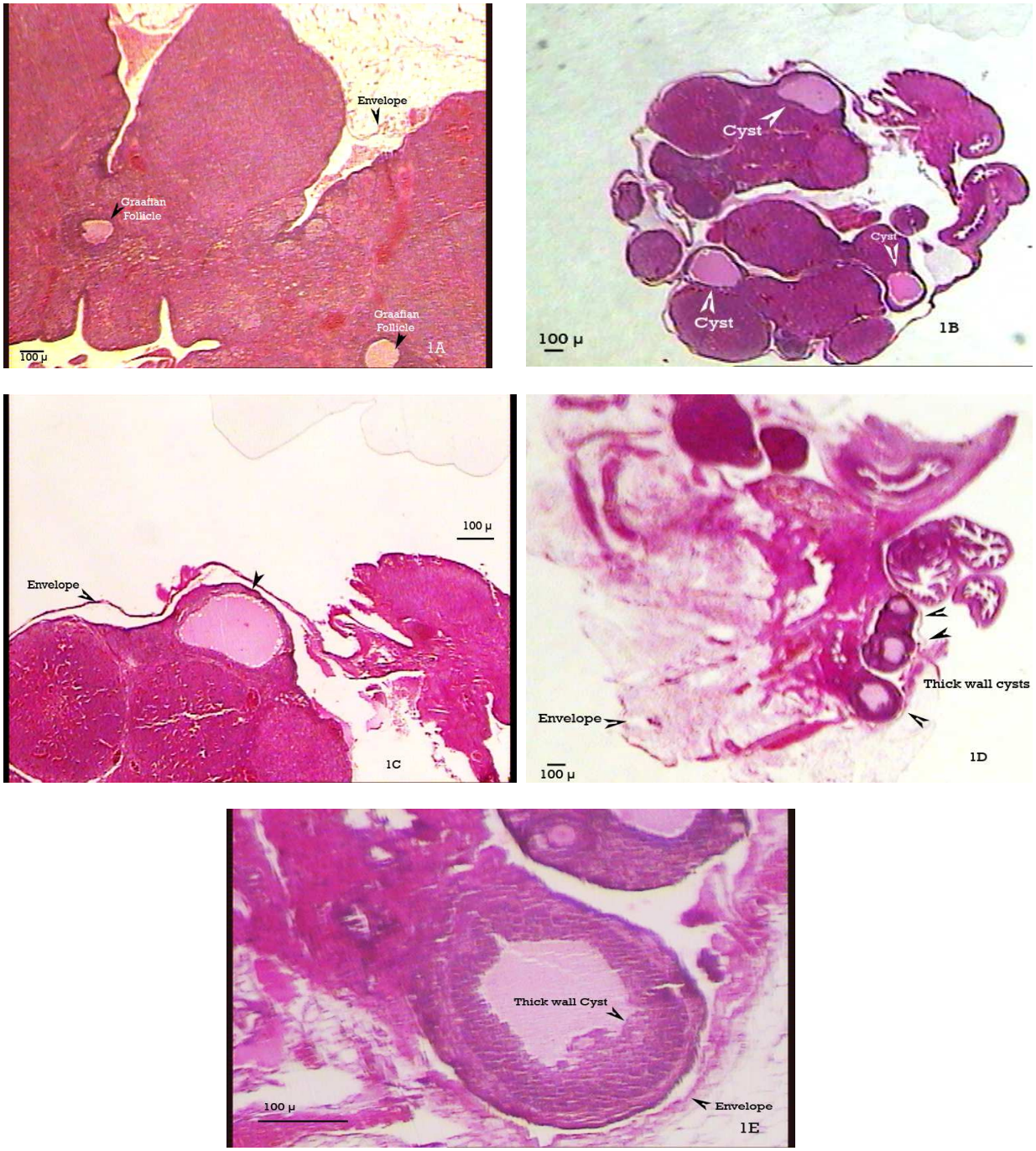

Fig. 1: Female virgin Wistar rats were injected with morphine (1-10 mg/kg, i.p.) throughout the experimental period (9 days/day). Control group was solely injected with saline (1 ml/kg, i.p.). The figure shows the control rats' ovaries with Graafian follicles (1A). The samples obtained from rats receiving morphine (1B-1E) consequently had cysts. The samples of the treatments with the higher doses (e.g. 10 mg/kg) (1D-1E) had much thicker wall cysts than the other groups (e.g. 1 mg/kg) (1B-1C). The figure shows more detail.

Interestingly the number of the cysts in the trial samples did not show the drug dose effect ($p< 0.05$), indicating that the chronic use of morphine could produce the cysts in the rat ovary with no significant effect of drug's density.

The present result reveals another considerable point that the drug has a dose-related thickness effect on the cysts walls which resemble to the PCOS thickened wall cysts.

According to our findings, the diameters of ovaries in the treated animals did not show significant differences when compared with the control group ($p> 0.05$) (Fig. 2).

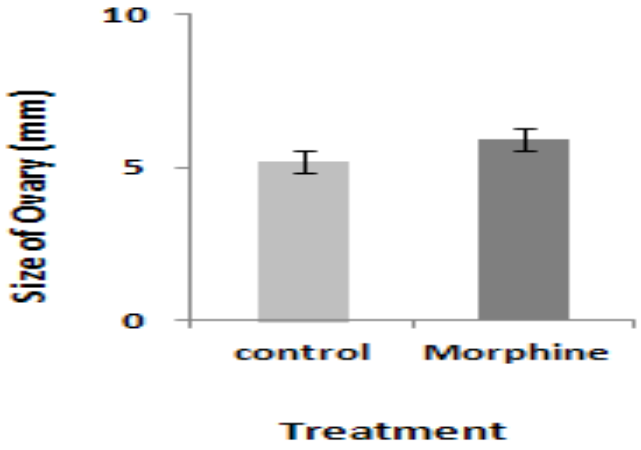

Fig. 2: This figure indicates the diameters of ovaries in rats treated by morphine (1-10 mg/kg, i.p., for 9 days and daily) in comparison with the controls. Control group was solely injected with normal saline (1 ml/kg, i.p., for 9 days and daily). X axis denotes the control and experimental groups.

4. Discussion

This research was conducted to show the negative effect of morphine on the rat ovary. Based on the present findings, a significant cystic induction due to chronic usage of morphine (1-10 mg/kg, i.p.) was observed in the rats' ovaries. The gathered samples showed the follicular cysts versus the control group (which received saline). But, the number of cysts between the samples indicated no significant differences. It seems that the chronic usage of this drug could result in cystic ovary induction in rats**,** without a significant effect by the drug's density. The considerable point was that the thickness of the cysts illustrated a drug dose effect on meaning that the cysts walls were much thickened at the higher concentrations of the drug. Based on these results, however, the diameters of ovaries in the treatment animals did not show the significant differences compared with the control saline group.

There is an obvious response to why ovaries were cystic due to morphine usage. By considering all findings that have been obtained by researchers up to now, some explanations are expressed. As we know, one of the important sign for the PCOS is known as the cystic ovaries. Studies show that women with the PCOS have an increased number of activated T cells in the follicular fluid (16, 17) compared with the controls. By considering these results there is possibly a more important interaction between the opioid morphine and the inflammatory factors in indication of this phenomenon (9, 16, 17). Present study may certificate that the opioid system plays a role in ovarian polycystic induction. Morphine, the sedative drug, has inflammatory effects on the rat's ovary (18). These results possibly suggest that the endocrine system may change as an irregular factor in reproductive system. Opioids increase the secretion of LH in response to GnRH. Also opioids can inhibit the clearance of insulin by the

liver and stimulate the production of insulin by the pancreas, the responses that are contributing to the hyperinsulinemia in the PCOS. Insulin and LH synergize to lead to excess androgen production by the ovaries (18). Insulin also inhibits the production of the sex hormone binding globulin, leading to increased free androgens. Hyperandrogenism predisposes toward central adiposity and insulin resistance, as free fatty acids inhibit the uptake of glucose into muscle cells. Thus, the opioid system, hyperinsulinemia, and hyperandrogenism appear to be all involved in the metabolic and reproductive disturbances seen in the PCOS (19, 20). Moreover, by using higher doses of morphine, the scenery of cysts becomes more similar to that seen in the PCOS. The mechanism is related to the nature of the opioid receptors pharmacokinetic. From the steroids synthesis by the gonadotropin factors to the activation of the inflammatory system are probably involved in the process. In order to clarify the hypothesis, more studies should be carried out in the future researches.

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