Original Article

Protective Effect of Baclofen on Ovarian Cystogenesis and Morphine-Induced Lipid Profile Change in Female Rats

Zahra Jamshidi, Manizheh Karami, Mohsen Khalili[1], Mehrdad Roghani[1]
Departments of Biology and [1]Physiology, Shahed University, Tehran, Iran

Abstract

Introduction: Morphine induces ovarian cysts that cause obesity and disrupt sex hormone secretion. Baclofen, a gamma-aminobutyric acid receptor agonist, can help regulate sex hormones and reduce harmful blood lipids by protecting against morphine-induced gamma-aminobutyric acid inhibition. We investigated the prophylactic effect of baclofen in rats receiving morphine by comparing with the untreated groups. **Materials and Methods:** Forty eight female Wistar rats were randomly divided into several groups, including control (saline 1 mL/kg, i.p.), morphine (5 mg/kg, i.p.), baclofen (10, 20, and 30 mg/kg, i.p.), and baclofen (10, 20, and 30 mg/kg) before morphine (5 mg/kg). Twenty four hours after the treatment, blood and serum samples were taken to check the levels of gonadotropins (LH & FSH) and lipid profile. The ovaries and uterus were also studied, and a proinflammatory nitric oxide (NO) diagnostic test was completed. The results were analyzed using analysis of variance ($\alpha = 0.05$). **Results:** In comparison with the control group, the levels of LH and not FSH decreased in the morphine group and the number of ovarian cysts was more in the morphine group. These problems were not observed in the group of baclofen alone and baclofen + morphine. However, the triglyceride level increased slightly in the baclofen 30 mg/kg + morphine group. But the LDL level somewhat decreased. The proinflammatory NO system did not show significant activation in the ovary and uterus, except for the baclofen 10 mg/kg + baclofen group. **Conclusion:** Morphine can cause ovarian cysts by lowering LH but baclofen prophylaxis can protect reproductive properties by adapting major metabolic changes.

Keywords: Baclofen, cyst, FSH, morphine, LH, lipid, ovary, NO

Introduction

Morphine, a pure alkaloid isolated from opium,[1] can relieve pain[2] and has a wide clinical application. Studies have shown that morphine can induce ovarian cysts[3] and alter lipid metabolism.[4] Gamma-aminobutyric acid (GABA), which is known as the main inhibitory neurotransmitter, is involved in preventing the development of ovarian cysts.[5,6] All parts of the hypothalamus, including GnRHergic neurons, communicate with GABA neurons.[7] The present study investigated the protective effect of baclofen, GABA receptor agonist, on ovarian cyst production, and changes in lipid profile and gonadotropin levels in female rats at reproductive age treated with morphine.

Materials and Methods

Animal subject

In this experimental study, 48 female Wistar rats, with an average weight of 235 g, were housed in the animal room under standard conditions with free access to water and food, 12 hours of darkness and light, humidity 60%, and they were kept at a temperature of 21°C-23°C.

Code of ethics

The code of ethical approval of this study is IR.SHAHED.REC.1399.114.

Animal groups

Animals (48 rats) were randomly divided into eight groups: the first group only received placebo (saline 1 mL/kg). The second group was administered morphine (5 mg/kg). The three

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Address for correspondence: Dr. Manizheh Karami, Associate Professor, Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran.
E-mail: karami@shahed.ac.ir

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other groups received baclofen (10, 20, and 30 mg/kg). The last three groups were given baclofen (10, 20, and 30 mg/kg), 20 minutes before morphine (5 mg/kg). All injections were performed only once and intraperitoneally (i.p.).

Drugs and materials used

Baclofen and morphine were provided from Temad Co., Tehran, Iran. Ketamine 10% and xylazine 2% were bought from Veterinary Organization, Tehran, Iran. NADPH-d and nitro blue tetrazolium were purchased from Merck, Germany. Paadco ELISA kits were used to measure blood lipid and gonadotropins profiles.

Time of blood sampling and preparation of serum samples

Twenty four hours after treatment, the rats were anesthetized with ketamine-xylazine and blood samples were taken from the rats' hearts. Blood samples were 30 minutes after collection centrifuged at 3,000 rpm for 10 minutes. Serums were isolated and tested for LH and FSH and lipid profiles. By making a longitudinal incision in the abdominal surface, the ovaries and uteri were removed from the abdominal cavity and examined histologically and biochemically.

Analytical studies

FSH and LH levels

Radioimmunoassay was used to measure the levels of LH and FSH. At first the blood serum without labeling was poured into a container and then the labeled hormone was added to it. Both antigens compete with each other for binding to standard and marked antibodies that were added to the solution. In these steps, the antibody first binds to the unlabeled antigen and the excess binds to the labeled antigen. After discarding the supernatant, which contains the free-labeled antigen and the unlabeled antigen bound to the antibody, its precipitate, which contains the labeled antigen and binds to the antibody, remains at the bottom of the container. The resulting precipitate was placed in the gamma counter and the resulting numbers which indicate the amount of antigen bound to the radioactive materials were read. The higher the numbers, the lower the amount of hormone in the serum because it causes more of the labeled antigens to bind to standard and labeled antibodies.

Evaluation of triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol

ELISA kit was used to measure blood lipid profile. To diagnose the serum triglyceride and total cholesterol, the following steps were performed separately. First, 10 µL of distilled water, serum, and standard solution were introduced into each of the control, sample, and standard tubes, respectively, and then 1,000 µL of reagent solution was added to all tubes. After mixing, they were incubated for 20 minutes at ambient temperature (20 ºC-25ºC) and for a maximum of 60 minutes and the standard light absorption of samples were read against the blank at 546 nm.

HDL cholesterol measurement: 200 µL of the calibrator, 200 µL of the control, and 200 µL of the sample were mixed with 500 µL of precipitate and placed at 20ºC to 25°C for 10 minutes, and then centrifuged at 4,000 rpm for 10 minutes. After centrifugation, 100 µL of distilled water, supernatant, and standard solution were added to each of the control, sample, and standard tubes, respectively, and then 1,000 µL of reagent solution was added to all tubes. After mixing, it was incubated for 20 minutes at ambient temperature and then for 10 minutes at 37°C and for a maximum of 60 minutes, the standard light absorption of samples were read against the blank at 546 nm.

Measurement of LDL cholesterol: Low-density serum lipoproteins were precipitated by a precipitating reagent. Then LDL absorption at 294 nm was inspected by spectrophotometer.

Histological examination

After fixing the ovarian and uterine tissue in 10% formalin with microtome device, we prepared slices with a thickness of 3-4µm. Ovaries and uterus were examined histologically after staining with hematoxylin-eosin.

Survey on the involvement of proinflammatory NO with the help of NADPH-diaphorase

To determine the role of proinflammatory factor NO in ovaries and uterus, specialized staining with NO biochemical marker (NADPH-diaphorase) was used. To show the activation of NOS in ovarian and uterine tissues, after preparing the 4-micron slices, the incisions were pasted on a poly L-lysine (Sigma) slide. Then clarified in two stages with xylene and after dilution in alcohol 100 to 50 exposed to equal proportions of NADPH-d (2 mg per 1 mL of phosphate buffer) and nitro blue tetrazolium (1 mg per 1 mL of phosphate buffer). It was placed in an incubator at 37°C for 16 hours. After rinsing with tap water, washing steps were performed in 50 to 100 alcohol and then clarification was completed in xylol.

Statistical tests used

The obtained data were evaluated by one-way analysis of variance (ANOVA) and Tukey's *post hoc* test was followed to show the between groups differences, and $P < .05$ was considered as a significant level.

Ethical aspects

All the ethical principles of working with animals are considered based on the Declaration of Helsinki (DoH) and the code of ethics has been assigned to this research by the local ethics committee of Shahid University as follows: (IR.SHAHED.REC.1399.114.).

Results

Histological results

Results of involvement of proinflammatory NO by NADPH-d Marker

Involvement of proinflammatory agent NO using specific staining with NO biochemical marker (NADPH-d) on the surface of ovary and uterus shows that in morphine-receiving

group, this proinflammatory system has not been activated in the area of germinal tissue [Figure 1]. Examination of ovarian and uterine tissue in the groups receiving single baclofen and preinjection of baclofen to morphine showed that this proinflammatory system was somewhat activated in the morphine + baclofen treatment at the lowest dose.

Lipid measurement results

Levels of triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol

Examination of blood lipid profile showed that in the morphine receiving group compared to the control group, the level of total cholesterol, HDL cholesterol, and triglyceride somewhat increased but the level of LDL cholesterol slightly decreased. The triglyceride level in the group receiving preinjection of baclofen 30 mg/kg was higher than the other groups. LDL cholesterol was lower in this baclofen (30 mg/kg) group [Figure 2].

Results of measurements of FSH and LH levels

Another biochemical study in this study was to evaluate the levels of LH and FSH in tall groups. In this study, it was shown that the level of FSH in the morphine group did not show a significant difference compared to the control group. The level of LH decreased relatively. However, in the groups receiving single baclofen and those receiving preinjection of baclofen, a relative increase in the level of LH was observed, which albeit was not statistically significant [Figure 3].

Discussion

In addiction treatment centers, baclofen is given to patients to detoxify from morphine abuse. Baclofen therefore appears to counteract the effects of morphine.[8] This study was performed to evaluate the protective effect of baclofen against cyst production and change in lipid and gonadotropins levels in female rats treated with morphine.

In the present study, staining with biochemical NO marker (NADPH-d) showed the involvement of the proinflammatory NO in the morphine + lower dose baclofen at the marginal level of the ovary. However, the combination of baclofen at higher doses and morphine did not show stimulation of the NO system. This indicates a dose-dependent response. Studies have shown accordingly that baclofen modulates the proinflammatory effects of materials.[9]

Studies have proposed that about half of all women suffering from ovarian cysts are obese. These people have excess adipose tissue in their body that produces free radicals during metabolism, which have destructive effects on the body and cause more cysts.[10] Therefore, in this study, by measuring the blood lipid profile, we examined whether morphine is effective in increasing the lipid levels and whether baclofen can prevent ovarian cystogenesis by preventing the destructive effects of morphine. The results of this study showed that in the morphine receiving group compared to the control group, the concentration of total cholesterol, HDL cholesterol, and triglyceride somewhat increased. But, except for high doses of baclofen and morphine, we did not see a significant change in LDL level.

Studies have previously shown that baclofen is effective in reducing obesity and body fat mass.[11] Other studies have indicated that substances such as metformin, which decrease body fat, reduce the number of cysts in the ovaries, and improve fertility. Authors, therefore, have indicated that by reducing body fat level, the destructive effects of materials on fertility can be prevented.[12] Accordingly, it seems that baclofen can be effective in increasing fertility by reducing adipose tissue.

Studies have shown that morphine inhibits LH release by reducing the release of norepinephrine in the preoptic area.[13] In the present study, the level of FSH in the morphine group did not significantly change. However, the level of LH in the morphine group showed a relative decrease compared to the control group. Studies have previously demonstrated that the central GABAergic system controls the function of the

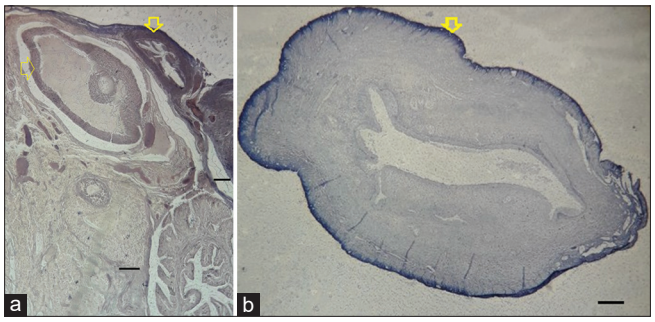

Figure 1: Evaluation of involvement of proinflammatory NO using specific staining with NADPH-d in ovarian (a) and uterine (b) tissue in morphine + baclofen and control groups respectively: Arrow indicates activation of proinflammatory NO system in the peripheral area of the ovary of morphine + baclofen (10 mg/kg) group (a). (b) indicates the activation of the NO system in uterine connective tissue of control animal. Line is 50 μm

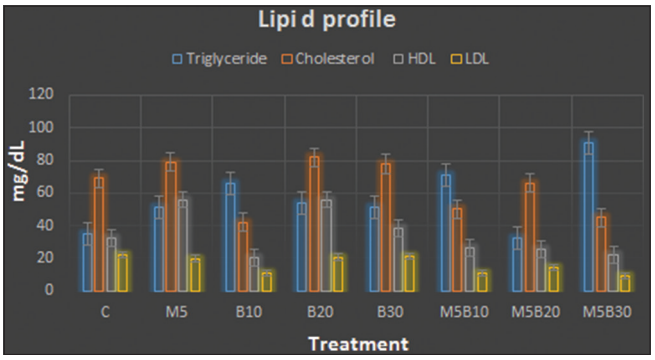

Figure 2: Evaluation of triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol in different groups. Examination of blood lipid profile showed that in the morphine receiving group compared to the control group, the quantity of total cholesterol, HDL cholesterol, and triglyceride somewhat increased. In the baclofen 30 mg/kg + morphine group, the triglyceride level relatively increased but LDL cholesterol somewhat decreased compared to the control group

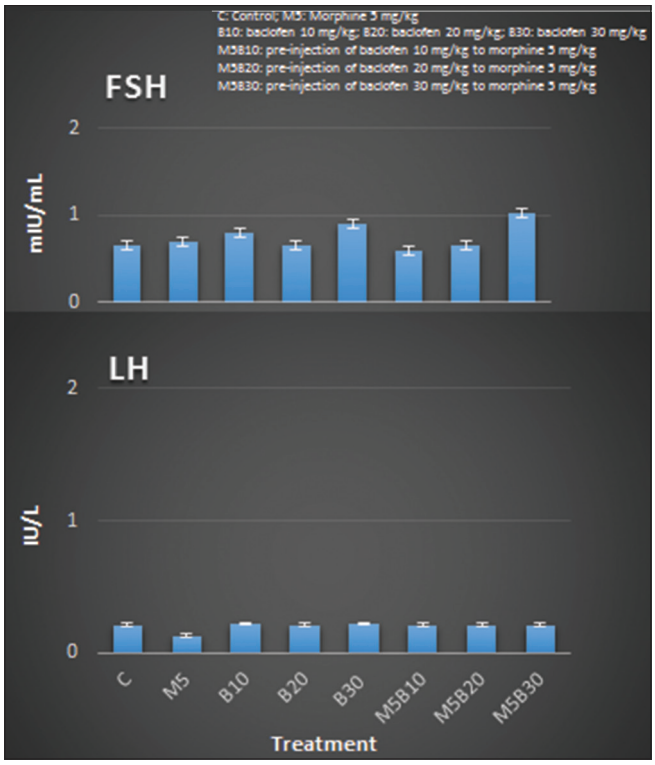

Figure 3: Evaluation of LH and FSH levels in different groups. In the morphine group, LH levels decreased relatively compared to the control. However, in the groups receiving single baclofen and those receiving preinjection of baclofen to morphine, the level of LH showed relatively increase. There was no significant difference in FSH levels in the morphine group compared to the control

hypothalamic GnRH neurons, the HPG axis, and the function of peripheral steroid-producing organs by neuroendocrine mechanism.[14] Another study has indicated that GABAB receptors regulate the excitability of GnRH neurons. Baclofen, as a GABAB receptor agonist, modulates pulsed LH secretion.[15] It has been shown that selective stimulation of GABAA fibers in the arcuate nucleus of the hypothalamus neurons leads to a significant and long-term increase in LH secretion in male and female mice.[5] In the present study, baclofen caused a relative increase in LH levels in the baclofen group and baclofen + morphine group compared to morphine group. This finding is consistent with the aforementioned other data and this indicates reliable experimental design by this laboratory.

Conclusion

Morphine induces ovarian cystogenesis, which can cause obesity and hormone secretion disorders. Baclofen probably protects against the inflammatory effects of morphine and helps to regulate sex hormones and reduce harmful change of blood lipids.

Author contribution statement

M.K. designed the experiments; Z.J. performed experiments and collected data; M.K. supervised, directed, and managed the study; Z.J. wrote initial version of paper and M.K. edited the paper. All authors approved final version of the paper.

Data availability

All data are provided in this article.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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