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A Mouse Model of Primary Dysmenorrhea-Associated Mood Disorder – A Preliminary Study

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Abstract

Primary dysmenorrhea, or painful menstruation, is known to affect many reproductively active females. Besides the chronic pain that affects daily well-being, the inflicted patients are exposed to a higher risk of developing mood disorders. Various clinical studies exhibited that patients with prolonged primary dysmenorrhea showed an elevated level of depression. To date, there's no report on a representative model of primary dysmenorrhea-associated mood disorders. It is crucial for a new model to be present, as it will be a convenient tool for drug discovery. In the present study, employing an established mouse model of induced primary dysmenorrhea, we further examined the associated mood disorders via various behavioural tests, e.g., the open field test (anxiety-like behaviour), the tail suspension test (depression-like behaviour), and the social interaction test (anhedia). The findings showed that depressive and anxiety-like behaviours were present in the primary dysmenorrhea-induced mice. Thus, this model has the potential to be further employed as a drug screening model for novel treatments for primary dysmenorrhea-associated mood disorders.

Keywords: Menstrual cycle, depression, anxiety, psychological wellbeing, preclinical model

Introduction

Dysmenorrhea, also known as menstrual pain, is characterised by cramping of the uterus during menstrual cycles. In the female population, dysmenorrhea is pervasive, neglected, underdiagnosed, and undertreated. According to pathophysiologicalclassification, dysmenorrhea can be divided into two categories: secondary, which entails underlying pelvic pathology, and primary, which occurs in normal pelvic anatomy and is the subject of this study[1]. The precise cause of primary dysmenorrhea is unknown, but it is typically associated with an increase in uterine contractions due to an excess of prostaglandin secretion in the endometrium[2].

The potential consequences of dysmenorrhea can be investigated from two perspectives: psychological and physiological. If they are in great discomfort, women with dysmenorrhea may withdraw from social and physical activities. Multiple psychological disorders, such as anxiety and depression, can result from prolonged exposure to perceived stress and pain sensations[3,4]. In addition, women with dysmenorrhea have a lower quality of life than those without the condition, which may be the root cause of their mood disorders[5]. Due to the fact that dysmenorrhea often neglected as severe illness and there is a dread of experiencing negative side effects from therapy, the issue does not receive adequate attention, and its tendency increases over time. According to studies, dysmenorrhea impairs women's ability to perform daily activities, resulting in diminished physical, psychological, and social quality of life, particularly emotional, mental, and social functioning[6]. This can lead to psychological disorders such as anxiety and depression.

Despite the high prevalence of primary dysmenorrhea-associated mood disorders in clinical settings, there is no animal model that mimics these psychological symptoms in a preclinical laboratory setting. A preclinical model is critical for the development of novel pharmacotherapies or complementary treatments for primary dysmenorrhea-associated mood disorders. The present study expanded the previously reported mouse model of primary dysmenorrhea[7] and examined whether depressive- and/or anxiety-like responses are present.

Material and Methods

Chemicals and Reagents

Estradiol and oxytocin were purchased from Merck KGaA (Darmstadt, Germany).

Animals

Eighteen nonpregnant female Institute of Cancer Research (ICR) mice weighing 24–37 g (8-week-old) were housed in a temperature (25°C), humidity (72%) and light (12-hour light/dark cycle) controlled cages with food and water. The mice were allowed to acclimatize to the laboratory environment for at least 1 week before the commencement of the study.

Experimental protocol

The primary dysmenorrhea model was adapted from previous literature with some modification. Each mouse was given intraperitoneal (i.p.) injection of estradiol (1mg/kg/day) for 9 days in the morning. On the 4th day onwards, the mice in negative control (CTRL, n=9) group received vehicle twice per day, with minimum of 6 hrs apart. The dysmenorrhea group (DYS, n=9) received oxytocin injections (0.4U, i.p.) instead of vehicle.Behavioural experiments were conducted on the 10th day.

Open Field Test

The open field test was done referring to previous reports, with minor adaptations[8,9]. The test arena was constructed using black corrugated plastic boxes (100 x 100 x 76 cm). The box was divided into four quadrants, each measuring 50 cm × 50 cm. Before each test, a 75% ethanol wipe was used to clean and disinfect each quadrant. ICR mice's movements are being captured on camera. The mice were allowed to move for 30 minutes in each area. The distance traveled by the mice in the arena was analysed.

Social Interaction Test

The study was done based on previous reports with minor modifications[9,10]. The open field test area was constructed using black corrugated plastic boxes (100 x 100 x 76 cm). The box was divided into four chambers measuring 50 cm × 50 cm. Before each test, a 75% ethanol wipe was used to clean and disinfect each quadrant. Four identical blue wire cup-like plastic containers and four orange wire cup-like plastic containers were used as apparatus. A naïve mouse of the same sex was present in each blue plastic container while the orange plastic container does not hold any naïve mice. Blue and orange plastic containers were placed on each side of the chamber. Adhesive tape was used to stick the container tightly with each chamber wall. ICR mice's movements are being captured on camera. The mice were allowed to move for a total of 30 minutes in each area. The time spent by pairs of female mice in the arena was analysed.

Tail Suspension Test

A black background was set up with black corrugated plastic cardboard. Video camera was set up to capture the movement of ICR mice and recorded by Logitech Capture. Mice were suspended for 6 minutes above the ground by their tails each round. The immobility duration of each mouse was analysed. The test protocol was done as per previous study, with minor modifications[11].

Statistical analysis

All data were presented as mean ± standard error of mean (SEM). Student’s t-test were employed to analayse the statistical

difference between groups. Statistical differences were considered significant if $P < 0.05$.

Results and Discussion

In the present study, primary dysmenorrhea was first established in mice by using the oxytocin-induced model, which was previously shown to mimic painful responses in primary dysmenorrhea in the form of abdominal writhing response. It was previously shown that this mouse model presented similar pathophysiology and clinical features of human primary dysmenorrhea such as elevated prostaglandin levels particularly $PGF_{2\alpha}$ or PGE_2, fall in the velocity of the arterial blood flow, thicker and smaller area of myometrium as well as uterine ischemia and unusual uterine contraction[7].

The open field test is a conventional behavioural test for detecting anxiety-like responses in rodents[12]. Mice are compelled to investigate a perceived threat despite their natural aversion to brightly illuminated open spaces. Increased anxiety is characterised by decreased locomotion and an increased propensity to remain near the arena's boundaries. Treatment with anxiolytics will reduce the apprehensive behaviour of animals in open fields, thereby encouraging locomotion. In the present study, the total distance travelled (in metres) by the mice during the 30-minute test was measued and analysed. Figure 1 compares the total distance travelled by mice injected with oxytocin (DYS) to that of mice injected with vehicle (CTRL). In this instance, the mice with primary dysmenorrhea exhibited a higher level of anxiogenic-like behaviour than their counterparts administered with the vehicle, instead of oxytocin. This is consistent with clinical studies that found a substantially higher level of anxiety in patients with dysmenorrhea.

Next, we examined whether mice with primary dysmenorrhea exhibited social withdrawal. The objective of the social interaction test is to examine the sociability of mice[13] by placing them in an open area with two cages situated against the wall, one of which contains an unfamiliar mouse of the same sex and the other of which is empty. In rodents, sociality is the tendency to interact with other mice and spend time with other mice. Several neuropsychiatric disorders, such as depression and anxiety, are distinguished by abnormalities in social behaviour and cognitive recognition[14]. Figure 2 demonstrates that the primary dysmenorrhea model group (DYS) spends less time in the enclosure with unfamiliar mice compared to the control group (CTRL). These results are consistent with previous research indicating that women with dysmenorrhea experience deleterious effects on their social lives[15,16].

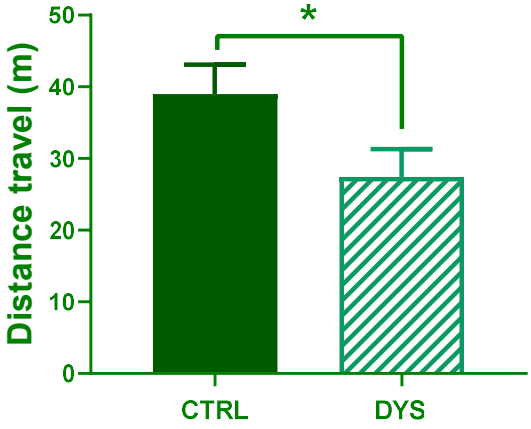

Figure 1: Open fieldtest

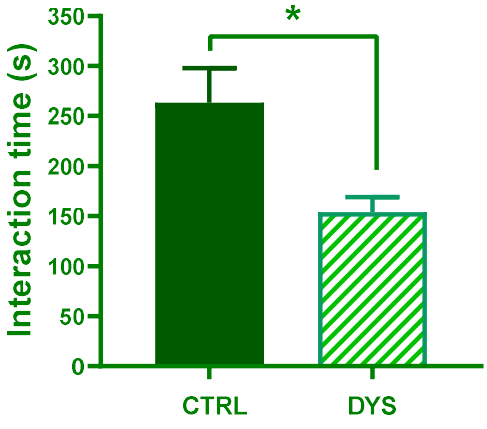

Figure 2: Social interaction test

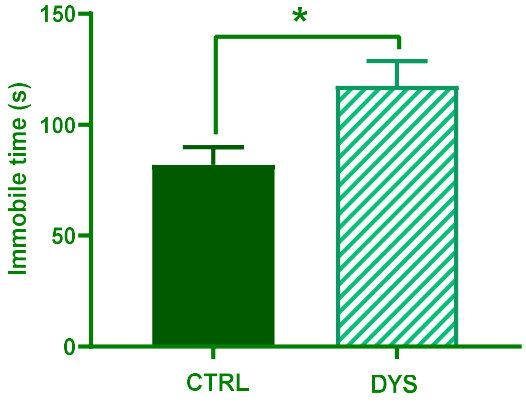

Figure 3: Tail suspension test

The tail suspension test is an animal model for the study of depressive-like behaviours. Short-term stress was imposed by hanging the mouse by its tail, allowing the body to be suspended upside down in the air. The assumption behind this test is that the mouse will try to escape stressful situations up until a certain time, when it stops struggling and becomes immobile. Immobileness is a sign of depressive behaviour, as is a failure to persist in attempting escape. Hence, the longer the immobile time, the higher the level of depressive behaviour[17]. Clinically used antidepressant treatments were reported to cause a significant reduction in this depressive behaviour. The immobile phase of mice was significantly longer after administration of oxytocin (DYS) compared to that with vehicle (CTRL). This showed that oxytocin-induced primary dysmenorrhea caused depressive-like symptoms in this model (Figure 3). This observation is in line with previous literature that reported an association between depression and dysmenorrhea in clinical settings[6].

Conclusion

In the present study, we have demonstrated a mouse model mimicking depressive- and anxiety-like behaviours as reported in patients with primary dysmenorrhea. This experimental model has the potential to be applied in future studies to discover novel pharmacotherapies for primary dysmenorrhea-associated mood disorders.

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Conflict of Interest

The authors declare no conflict of interest.

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