



The Effect of Duration of Use of Depomedroxyprogesterone Acetate on the Thickness of the Vaginal Epithelium of Mice

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Abstract

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BACKGROUND: Depomedroxyprogesterone acetate (DMPA) is one of the most effective contraceptive methods currently widely used. Injectable contraceptives, including DMPA, are growing in popularity because of their ease of use, effectiveness and affordability. The use of DMPA also has advantages such as not interfering with sexual relations, does not need male participation, does not require storage space, and is easy to obtain and helps increase body weight.

AIM: The aim of the study was to analyze the effect of the duration of use of DMPA on the thickness of the vaginal epithelium of mice.

METHODS: The research design used in this study was true experimental with a post-test only control group design approach. This research was carried out treatment at a dose of 0.39 mg/mouse/day DMPA exposure in mice. The phenomenon observed in this study was the thickness of the vaginal epithelium of mice. This study used mice with the completely randomized design method and was carried out at the Pharmacology Laboratory and the Anatomical Pathology Laboratory, FKH Unsyiah. The collection of data technique was conducted by observation with a light microscope to calculate the average thickness of the vaginal epithelium of mice injected with DMPA in the four groups. The data analysis technique was carried out in five stages, namely, the normality test of the sample data with the Shapiro-Wilk test, the homogeneity test of variance with the Levene test, and comparing the thickness of the vaginal epithelium in each of the two treatment groups using the independent t-test.

RESULTS: The finding revised that there was a significant difference in the thickness of the vaginal epithelium in the two treatment groups 10 days and 15 days of DMPA exposure which was indicated by a $p = 0.003$ ($p < 0.05$). DMPA exposure levels cause thinning of the vaginal epithelial thickness. The average thickness of vaginal epithelium exposure to DMPA for 10 days in the control group was 182536.33 ± 5773.465 . In the treatment group, exposure to DMPA for 10 days, the thickness of the vaginal epithelium was 168322.33 ± 7611.300 . The mean vaginal epithelial thickness in the 15-day DMPA exposure group in the control group was 177242.83 ± 9689.558 . In the 15-day DMPA exposure treatment group, the thickness of the vaginal epithelium was 138674.83 ± 17176.124 .

CONCLUSION: There was a difference in the mean vaginal epithelial thickness between the control group and 10 days of DMPA exposure. There was a difference in the mean vaginal epithelial thickness between the control group and 15 days of DMPA exposure. The longer the exposure to DMPA, the thinner the thickness of the vaginal epithelium.

Introduction

Sustainable development (SDGs), namely, ensuring good health and well-being, furthermore achieving gender equality and empowering all women and girls include universal sexual and reproductive health. Both developed and developing countries around the world have shown increasing use of contraception to control populations [1]. Indonesia is a developing country with the most populous population in Southeast Asia. This situation encourages the government to establish policies regarding population control, namely, by establishing a family planning program (KB). Family planning can also help achieve other SDGs by enabling women to remain in employment or complete education, reducing poverty, and gender inequality [2], [3]. Although contraception has been used globally, contraceptive use remains

lower in developing countries [4]. In Indonesia, the prevalence of contraceptive use is still low and varies between provinces, economic status, education level, and location of residence [5]. Injectable contraceptives, including DMPA, are gaining popularity because of their ease of use, effectiveness, and affordability [6]. Birth control in family planning is used to control the time between pregnancies. Although the number of family planning users is increasing, the determinant of contraceptive use in married women in Indonesia is still insufficient [7]. There are several types of contraceptive methods in family planning. The use of contraceptive methods in 2012 showed that most family planning participants chose to use injection contraception [8]. The highest percentage of active contraceptive participants according to contraceptive methods was injection (46.84%), followed by pills (25.13%), intra-uterine device (IUD) (11.53%), implants (9.17%), female operative (MOW) (3.49%), condoms (3.13%), and male

operative method (0.70%) [9] Injectable contraceptives include depomedroxyprogesterone acetate (DMPA) that is gaining popularity because of its ease of use, effectiveness, and affordability [1]. DMPA is one of the most effective contraceptive methods because of the various benefits that can be obtained including not affecting sexual relations, does not affect breast milk, and has few side effects [10]. The main side effects of DMPA contraception at 6 months are menstrual disorders/bleeding patterns (93.60%), weight gain (48%), pain in joints (24%), and vaginal dryness (10.4%) [10]. The use of DMPA has the disadvantage of increasing the Human Papilloma Virus and the Human Immunodeficiency Virus (HIV) infection. HIV infection in women is mostly found in women of productive age. Eleven injectable contraceptives are responsible for 27,000–130,000 HIV-1 infections per year globally [11]. It remains controversial because several studies have failed to observe the overall effect of hormonal contraception on the incidence of HIV-1 infection or disease progression [12]. Biologically, women are more vulnerable than men. It is because sexual intercourse usually causes a woman's vagina to become blistered, and HIV enters through the wound. The concentration of HIV in the seminal fluid is much higher than the concentration of HIV in the vaginal fluid, which causes HIV and STD transmission to be more effective from men to women than vice versa. Women also face a greater risk associated with violence, such as rape, incestuous intercourse, and forced sexual intercourse, not through the vagina, such as through the rectum, where bleeding occurs more easily [13]. Women have a greater chance of being infected with HIV through sexual contact than men. The medical vulnerability of women is probably because the inner lining of the female genitalia is more prone to blisters during sexual intercourse than the inner lining of men. It is through this blistered part that the HIV penetrates and enters the bloodstream. HIV infection in women is mostly found in women of productive age [8]. Based on the background, the purpose of this study was to analyze the effect of DMPA use on the thickness of the vaginal epithelium of mice.

Methods

The research design used in this study was true experimental with a post-test only control group design approach. The post-test only control group design approach is that observations are made after the intervention is given. In this study, the treatment was carried out at a dose of 0.39 mg/mouse/day DMPA exposure in mice. The phenomenon (response) that occurs due to the treatment is observed after the treatment is complete. The phenomenon observed in this study was the thickness of the vaginal epithelium

of mice. This study used mice with a completely randomized design method. The project was conducted at the Pharmacology Laboratory and the Anatomical Pathology Laboratory, FKH Unsyiah. The role of the researcher starts from selecting experimental animals, assisting in surgery, and analyzing using Image J software. Mice were obtained from the Pharmacology Laboratory of FK Unsyiah and were selected as research subjects because they were easy to maintain and were relatively healthy animals and suitable for use in various types of research.

Balb/c mice were divided into four categories: Control Group 1: Injection of normal saline for 10 days. Control Group 2: Injection of normal saline for 15 days. Treatment Group 1: DMPA dose 0.39 mg/mouse/day for 10 days. Treatment Group 2: DMPA dose 0.39 mg/mouse/day for 15 days. Inclusion criteria for study subjects: Strain Balb/c, female gender, age 2 months, weight 18–35 g, healthy condition, and no visible anatomical disability. Exclusion criteria: Mice died during the research process. The number of experimental animal subjects for each group was six mice. To anticipate if there are experimental animals that die during the adaptation and treatment period. Hence, the group is added 10% of the number of experimental animals, namely, one as a reserve so that the number of experimental animals in each experimental group is 28. The data collection technique in this study was observed with a light microscope to calculate the average thickness of the vaginal epithelium of mice injected with DMPA in the four groups. In this study, the data analysis technique was carried out in five counting stages. There are five consecutive stages, namely, (1) normality test of sample data with the Shapiro–Wilk test, (2) homogeneity test of variance with the Levene test, (3) to compare the thickness of the vaginal epithelium in each of the two treatment groups an independent t-test was performed. All calculations were carried out with the aid of the SPSS for Windows 19.0 software.

Results

Vaginal epithelial thickness

The results of observing the number of spiral arteries with a light microscope and analyzed using Olivia software (Image 1). The vaginal epithelium, as illustrated below:

Parametric prerequisite test results

They are testing the comparison of the average thickness of vaginal epithelium in the control group and treatment group for 10 days, and the

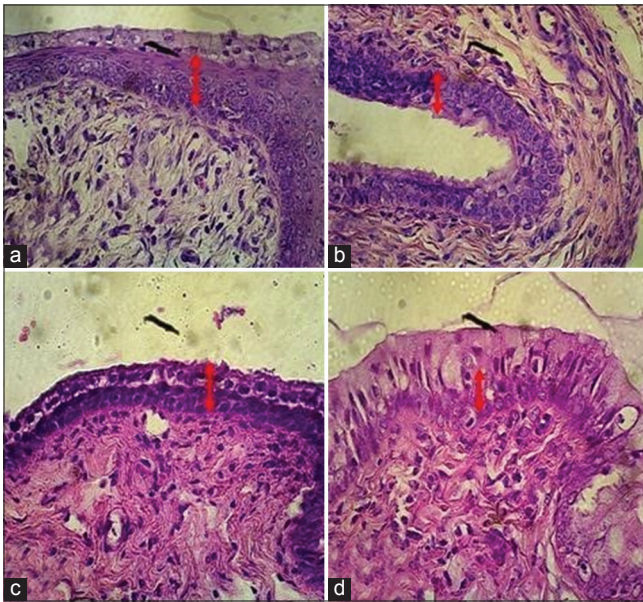


Image 1: Observation of vaginal thickness using a light microscope and *Olivia* software. The red color indicates the thickness of the vagina (indicated by an arrow). The differences in the mean total vaginal thickness are shown in pictures A, B, C, and D. Captions: (a) Control group 10 days (without treatment), (b) 10 days DMPA exposure group, (c) 15 days control group, (d) the DMPA exposure group was 15 days

control group and treatment group 15 days using the independent t-test. However, before the independent t-test is carried out, the assumption testing based on the parametric test is carried out. Namely, the normality assumption is carried out using the Shapiro–Wilk test. The assumption of normality is said to be fulfilled if the p-value of the calculation results is more significant than $\alpha = 0.05$. Using SPSS software, the following normality assumption test results are obtained:

Based on Table 1, the variable vaginal epithelial thickness obtained a p-value of more than $\alpha = 0.05$ ($p > 0.05$). This value indicates that the assumption of normality in the 10-day vaginal epithelial thickness variable has been fulfilled. Likewise, the variable thickness of the vaginal epithelium thickness for 15 days, the p-value was more than $\alpha = 0.05$ ($p > 0.05$). The normality assumption test shows that both variables have been fulfilled.

Table 1: Normality assumption test

| Group | Duration of DMPA exposure | Coefficient | p-value | Information |
|--------------------------|---------------------------|-------------|---------|-------------|
| 10 days of DMPA exposure | Control | 0.868 | 0.218 | Normal |
| | Treatment | 0.901 | 0.377 | Normal |
| 15 days of DMPA exposure | Control | 0.953 | 0.763 | Normal |
| | Treatment | 0.899 | 0.367 | Normal |

The assumption of homogeneity of variety is tested using the Levene test

The assumption of homogeneity of variance is said to be fulfilled if the p-value of the calculation result is more significant than $\alpha = 0.05$. The following are the results of testing the assumption of homogeneity of variety using the help of SPSS software:

Based on Table 2, in testing the assumption of homogeneity of the variability of the number of endothelial cells, the p-value is more significant than $\alpha = 0.05$ ($p > 0.05$) which indicates that the assumption of homogeneity of the variety of the number of endothelial cells has been fulfilled.

Table 2: Variety homogeneity test

| Variable | Coefficient | p-value | Information |
|---|-------------|---------|-------------|
| The thickness of the vaginal epithelium in the 10-day group | 0.242 | 0.633 | Homogeneous |
| The thickness of the vaginal epithelium in the 15-day group | 1.054 | 0.329 | Homogeneous |

Testing the effect of 10 days and 15 days of DMPA exposure on vaginal epithelial thickness

The following are the results of testing the effect of DMPA on the thickness of the vaginal epithelium using the independent t-test.

Based on Table 3, it is shown that the average thickness of vaginal epithelium exposure to DMPA for 10 days in the control group is 182536.33 ± 5773.465 . In the treatment group exposure to DMPA for 10 days, the thickness of the vaginal epithelium was 168322.33 ± 7611.300 . Descriptively, it was shown that the mean vaginal epithelial thickness of the control group with 10 days of DMPA exposure was higher than the 10-day DMPA exposure treatment group or in other words, it was descriptively proven that there was thinning of the vaginal epithelium due to exposure to DMPA. Using the independent t-test, the p-value was 0.005 ($p < 0.05$). From this test, it is shown that there is a significant difference in the mean vaginal epithelium between the control group and the DMPA exposure treatment group for 10 days. Table 3 also shows that the mean vaginal epithelial thickness in the 15-day DMPA exposure group in the control group was 177242.83 ± 9689.558 . In the 15-day DMPA exposure treatment group, the thickness of the vaginal epithelium was 138674.83 ± 17176.124 . Descriptively, it was shown that the mean vaginal epithelial thickness in the control group was higher than the treatment group with 15 days of DMPA exposure or in other words, it was descriptively proven that there was thinning of the vaginal epithelium due to 15 days of exposure to DMPA. Using the independent t-test, the p-value was 0.001 ($p > 0.05$). From this test, it is shown that there is a significant difference in the mean vaginal epithelium between the control group and the 15-day treatment group.

Table 3: Differences in average vaginal epithelial thickness due to DMPA exposure with independent t-test

| DMPA exposure | Group | Mean \pm SD (%) (μm) | p-value |
|---------------|-----------|-------------------------------------|---------|
| DMPA days 10 | Control | 182536.33 \pm 5773.465 | 0.005 |
| | Treatment | 168322.33 \pm 7611.300 | |
| DMPA days 15 | Control | 177242.83 \pm 9689.558 | 0.001 |
| | Treatment | 138674.83 \pm 17176.124 | |

Table 4 shows the difference in the mean value of vaginal epithelial thickness between 10

day and 15-day DMPA exposure. The difference in vaginal epithelial thickness in the two treatment groups with 10 days and 15 days of DMPA exposure was significantly different, which was indicated by a p-value of 0.003 ($p < 0.05$). It means that the longer the exposure to DMPA and the thinner the thickness of the vaginal epithelium.

Table 4: Difference in average vaginal epithelial thickness due to duration of DMPA exposure with independent t-test in the treatment group

| Duration of DMPA exposure | Mean \pm SD (%) | p-value |
|---------------------------|---------------------------|---------|
| 10-day exposure | 168322.33 \pm 7611.300 | 0.003 |
| 15-day exposure | 138674.83 \pm 17176.124 | |

Discussion

The finding indicated that exposure to DMPA will dilute the thickness of the vaginal epithelium due to exposure to DMPA for 10 days and 15 days. The difference in the thickness of the vaginal epithelium in the two groups treated with DMPA exposure for 10 days and 15 days was significantly different, which was indicated by a p-value of 0.003 ($p < 0.05$). The prolonged exposure to DMPA results in the thinning of the vaginal epithelial thickness. In some studies, DMPA can attenuate vaginal epithelium to the level of the luteal phase and increase the number of HIV target cells in vaginal tissue although other studies have found no effect of DMPA on cells [14]. The results also found that the average vaginal epithelial thickness of the DMPA exposure control group at 10 days was higher than the 10-day DMPA exposure treatment group. The independent t-test results obtained a $p = 0.005$ ($p < 0.05$), which means that there is a significant difference in the average vaginal epithelium between the control group and the DMPA exposure treatment group for 10 days. There is thinning of the vaginal epithelium due to exposure to DMPA. Vaginal epithelial thinning or Langerhans cell count did not differ between long-term DMPA users and copper-bearing IUD users who had never used DMPA [15]. Studies using non-human primate models indicate that DMPA increases the risk of acquisition of simian immunodeficiency virus through vaginal exposure [16], [17]. DMPA suppresses ovulation, where the active component of DMPA, medroxyprogesterone binds to glucocorticoid receptors and has a more substantial immunosuppressive effect than progesterone [18]. The use of DMPA increases the incidence of endothelial dysfunction, which is characterized by reduced flow-mediated dilation in the brachial artery [19]. The contraceptive component of DMPA has been shown to suppress systemic immune regulators, reduce cytokine production by plasmacytoid dendritic cells, and increase HIV-1 replication in blood mononuclear cells [14]. The decrease in estrogen levels frequently occurs with the length of time using DMPA.

These levels are the same as estrogen levels in the follicular phase of the menstrual cycle. Long-term use of progesterone contraceptives can change the vascular structure of the vagina and the morphology of vaginal tissue, along with the occurrence of oxidative stress and apoptosis due to a deficiency in the endogenous hormone estrogen [20]. The decrease in estradiol occurs with prolonged use of DMPA contraceptives and results in vaginal dryness and dyspareunia and can increase body weight and organ weight. DMPA can also make the endometrium secretory, and if used long-term, the endometrial lining becomes very thin [21]. DMPA reduces the risk of iron deficiency anemia, pelvic inflammatory disease, ectopic pregnancy, and disorders associated with uterine fibroids, pain symptoms associated with endometriosis, and the risk of endometrial cancer [22].

Conclusion

There was a difference in the mean vaginal epithelial thickness between the control group and 10 days of DMPA exposure. There was a difference in the mean vaginal epithelial thickness between the control group and 15 days of DMPA exposure. The longer the exposure to DMPA, the thinner the thickness of the vaginal epithelium is.

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