Влияние женских половых гормонов на функционирование белка-транспортера гликопротеина-Р

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Гликопротеин-Р (Pgp, ABCB1-белок) — белок-транспортер, локализующийся в мембране гепатоцитов, энтероцитов тонкого и толстого кишечника, эпителиоцитов почечных канальцев, эндотелиальных клеток гистогематических барьеров, опухолевых клеток.

Цель исследования — изучить в эксперименте влияние физиологических концентраций эстрадиола и прогестерона на функциональную активность Pgp на уровне целостного организма.

Материал и методы. Работа выполнена на 17 половозрелых кроликах-самках породы шиншилла. 1-й группе кроликов была проведена «ложная операция» (*n*=5); 2-й группе (*n*=6) выполняли овариоэктомию; 3-й группе (*n*=6) проводили овариоэктомию и в течение 14 дней, начиная с 14-х послеоперационных суток, вводили эстрадиол (по 0,5 мг), а затем комбинацию эстрадиола (0,5 мг) и прогестерона (5 мг) в течение 14 сут. За 7 дней до начала исследования, на 14, 28 и 42-е сутки после операции у кроликов всех групп методом ВЭЖХ определяли функциональную активность Pgp по фармакокинетике его маркерного субстрата фексофенадина. Накопление фексофенадина в организме свидетельствует об ингибировании Pgp, а снижение его содержания — об индукции Pgp.

Результаты. Овариоэктомия приводила к снижению функциональной активности Pgp. Введение эстрадиола в течение 14 сут после овариоэктомии существенно не влияло на функциональную активность белка-транспортера. Введение комбинации эстрадиола и прогестерона в течение 14 дней привело к повышению активности Pgp относительно показателя у овариоэктомированных животных и исходного уровня.

Заключение. Влияние физиологических концентраций эстрадиола и прогестерона на функционирование белка-транспортера свидетельствует о том, что эффективность фармакотерапии субстратами Pgp может зависеть от фазы менструального цикла, а для повышения эффективности соответствующей фармакотерапии возможна корректировка доз лекарственных средств.

Ключевые слова: гликопротеин-Р, ABCB1-белок, функциональная активность, фексофенадин, ВЭЖХ, прогестерон, эстрадиол.

Influence of female hormones on the P-glycoprotein functioning

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The P-glycoprotein (Pgp, ABCB1-protein) is a transport protein localized in the membrane of hepatocytes, small and large intestine enterocytes, renal tubular epitheliocytes, endothelial cells of histohematic barriers, and tumor cells.

Aim — to study the effect of estradiol and progesterone physiological concentrations on the Pgp functional activity on the whole body.

Material and methods. The work was performed on 17 adult female chinchilla rabbits. The first group of rabbits underwent a "false operation" (n=5); the second group (n=6) underwent ovariectomy. The third group (n=6) was ovariectomized and received estradiol (0.5 mg/rabbit) for 14 days (starting on the 14th postoperative day), followed by combined administration of estradiol (0.5 mg/rabbit) and progesterone (5 mg/rabbit) for 14 days. The Pgp functional activity was determined in rabbits of all groups using HPLC analysis of fexofenadine pharmacokinetics on day 7 before the study onset and on day 14, 28, and 42 after the operation. Accumulation of fexofenadine in rabbits indicates Pgp inhibition, and a decrease in its content means Pgp induction.

Results. Ovariectomy led to a decrease in the Pgp functional activity. The estradiol introduction for 14 days after ovariectomy did not significantly affect the transport protein functional activity. Combined administration of estradiol and progesterone for 14 days resulted in an increase in the Pgp activity, compared to that in the ovariectomy series and baseline values.

Conclusions. The dependence of the transport protein activity on the dynamics of physiological estradiol and progesterone concentrations suggests that the effectiveness of pharmacotherapy with Pgp substrates may depend on the menstrual cycle phase, and drug dose correction may be used to increase the pharmacotherapy effectiveness.

Keywords: P-glycoprotein, ABCB1-protein, functional activity, fexofenadine, HPLC, progesterone, estradiol.

Background

Glycoprotein-P (Pgp, ABCB1-protein) is an ATP-dependent protein transporter located in the cytoplasmic membrane of hepatocytes, small and large intestine enterocytes, renal tubular epitheliocytes, endothelial cells of histogematic barriers, and tumor cells. It performs the same function in all these cells: it removes xeno- and endobiotics to the extracellular space, biological fluids (blood, bile or urine) and the lumen of the intestine. Therefore, Pgp plays an important role in pharmacokinetics (absorption, distribution and excretion) of its substrate drugs, transport of endogenous substances (e.g., steroid and thyroid hormones), and development of resistance of tumor cells to chemotherapy [1].

The functional activity of Pgp is affected by a number of factors. Some substances act as inductors (rifampicin, glucocorticosteroids, thyroxine) and increase the activity

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of this transporter protein, which can reduce the effectiveness of therapy with Pgp substrates (digoxin, dabigatran etexilate, statins, etc.), whereas others act as Pgp inhibitors (verapamil, amiodarone, ketoconazole), which can lead to a relative overdose by its substrates [2]. The ability of drugs to inhibit the functional activity of Pgp can be used in cancer therapy to overcome the multiple drug resistance caused by increased activity of this transporter protein.

Conflicting results regarding the effect of female sex hormones on functional activity and Pgp expression have been obtained in in vitro studies of different cell lines. For example, it has been demonstrated that estrogens and progesterone at a concentration of 50 μM increase the expression of mRNA of *MDR1* gene which encodes the transporter protein in cells of LS180 line (human colon cancer cells) [4]. At the same time, estradiol at concentrations of 10 pM - 10 nM reduced the level of Pgp in the breast cancer cells transduced with the *MDR1* gene [5]. In the L-MDR1 and P388/dx cell lines, progesterone inhibits the activity of the transporter protein at a concentration of 13.3 ± 3.2 μM and 30.2 ± 9.8 μM, respectively [6]. Progesterone at a concentration of 50 μM also reduced the functional activity of Pgp in the vinblastine-resistant cell line, as evidenced by 4—5-fold increase in accumulation of vinblastine (substrate Pgp) in the cells [7]. It should be noted that in vitro studies used hormones concentrations significantly higher than their serum levels in humans and animals (3×10^{-9} M). In addition, the combined effect of estradiol and progesterone has not been studied.

Throughout the menstrual cycle, concentrations of sex hormones in a female body constantly changes. It suggests a change in the functioning of Pgp in different phases of the cycle, which may affect the pharmacokinetics of drugs that act as substrates of this transporter protein. This assumption is indirectly supported by inconsistency of data on gender differences in the pharmacokinetics of Pgp substrates (verapamil, cyclosporine, etc.), which may be associated with different phases of the cycle in women enrolled in the study [8].

Aim

To conduct an experiment on the effect of physiological concentrations of estradiol and progesterone on Pgp functional activity at the level of the whole organism.

Methods

Study Design

We have conducted an experimental prospective controlled study on laboratory animals.

Inclusion Criteria

The study was performed on 17 mature female chinchilla rabbits with a mass of 3000—3500 g. At the time of enrollment all animals reached puberty and were in a state of estrus (polyestros).

Conditions of the study

The animals were obtained from the Kasimov-Miakro kennel, they had the necessary veterinary certificates and were kept in the standard conditions of the vivarium of the Ryazan State Medical University of the Ministry of Health of the Russian Federation. The work with animals was conducted in accordance with the rules of laboratory practice (the order of the Ministry of Health of the Russian Federation No. 1999 of April 1, 2016).

Duration of the study

The study was conducted in 2017, the duration of the study for each animal was 47 days.

Subgroup analysis and description of medical intervention

The animals were divided into 3 groups.

The first group of rabbits underwent a "false surgery" (n = 5), which consisted of the opening of the skin and subcutaneous adipose tissue of the anterior abdominal wall, followed by layered wound suturing. The second group of animals (n = 6) underwent ovariectomy. The third group of rabbits (n = 6) had ovariectomy and was administered estradiol 0.5 mg/rabbit starting from 14th postoperative day for 14 days, followed by a combination of estradiol (0.5 mg) and progesterone (5 mg) for another 14 days .

Estradiol (Proginova, Bayer, Germany) and progesterone (Utrozestan, Besins Healthcare, Belgium) were administered to rabbits per os once a day. This scheme of the experiment mimics the menstrual cycle, during which the levels of estrogens and progesterone in the blood decreases during menstruation, the level of estrogens increases in the ovulatory phase, and the level of both estrogens and progesterone in the blood increases in the luteal phase.

The ovariectomy and the "false surgery" were performed in the operating room of the Ryazan State Medical University vivarium under anesthesia, which was administered by intramuscular injection of xylazine hydrochloride (Rometar, SPOFA, Czech Republic) in a dose of 4.0—6.0 mg/kg body weight and zoletil-50 (Virbac, France) in a dose of 5—10 mg/kg body weight.

The primary endpoint of the study

The functional activity of Pgp (based on the pharmacokinetics of the marker substrate) was determined 7 days before the start of the study, as well as on the 14th, 28th and 42nd days after the surgery.

Additional endpoints of the study

In addition, the serum concentrations of sex hormones (testosterone, estradiol, progesterone) were determined in the animals at the above-mentioned timepoints.

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Methods of recording endpoints

The functional activity of Pgp was evaluated by pharmacokinetics of fexofenadine (Allegra "Sanofy Aventis", France) after its single oral administration (67.5 mg/kg body weight, in a volume of 5 ml) as an aqueous suspension [9,10]. Fexofenadine does not undergo biotransformation and its pharmacokinetics depends primarily on the activity of this transporter protein. The accumulation of fexofenadine in rabbits indicates Pgp inhibition, whereas the decrease in its content indicate the induction of Pgp activity.

To determine the concentration of fexofenadine, blood in a volume of 5 ml was taken from the marginal vein of the rabbit ear into the heparinized tubes at 1, 2, 3, 4, 5, 6, 12 and 24 hours after the administration of the drug. Blood samples were centrifuged (1000 g, 10 min), the plasma obtained was stored until analysis at 29 °C for a month The concentration of fexofenadine in plasma was determined using a high performance liquid chromatograph "Stayer" equipped with a UV spectrophotometric detector UVV 104 at a wavelength of 220 nm using a reversed-phase chromatographic column Ultrasphere 4.6 × 250 mm (5 μm mesh) by Beckman Coulter. Fexofenadine was extracted from the plasma with dichloromethane ("ACROS ORGANICS"), ethyl acetate ("ACROS ORGANICS") and diethyl ether ("KHIMMED"). The following mobile phase was used for the elution (200 ml): 64 ml of acetonitrile, 133.7 ml of twice distilled water containing 2.33 ml of glacial acetic acid ("KHIMMED") and 0.936 ml of triethylamine ("ACROS ORGANICS") [9, 10]. The pH of the mobile phase was adjusted to 5.0 using triethylamine. The retention time of fexofenadine peak was 12.6 minutes.

The following pharmacokinetic parameters of fexofenadine were calculated using a model-independent method [11]: C_{max} is the maximum concentration (ng/ml), AUC_{0-t} is the area under the pharmacokinetic concentration-time curve from zero to the time of the last blood sampling (ng × h/ml); Cl is the total clearance (l/h). The concentration of sex hormones was determined in the Central Scientific Research Laboratory of Ryazan State Medical University by radioimmunoassay using the standard test system manufactured by IMMUNOTECH (Czech Republic), with subsequent processing of the results obtained on the Immunotest analyzer (Russia).

Ethical expertise

The study protocol No. 12 of April 8, 2016 was reviewed and approved at a meeting of the local ethical committee of the Ryazan Medical State University of the Ministry of Health of the Russian Federation.

Statistical analysis

Principles of calculating the sample size: the number of animals used in the study was defined by the minimum requirement for their numbers in an experimental pharmacokinetic study [12].

*Methods of statistical data analysis***.** The obtained results were processed with the help of the "StatSoft Statistica 7.0" software (USA). The significance of the differences between the indicators of the animals hormonal status was assessed using the Friedman criterion. In case of statistical significance, paired comparisons were performed using Wilcoxon test. The results obtained are presented in the form of a median, a lower and an upper quartile. The statistical significance of differences between the pharmacokinetic parameters of fexofenadine was assessed based on the assumption of log-normal distribution of data. The comparison of the studied pharmacokinetic parameters was carried out by means of ANOVA after their log-transformation. Differences compared to the baseline values within groups and intergroup comparisons were performed using the Fisher multiple comparison test. The results obtained are presented in tables as geometric mean and its 95% confidence interval. Differences were considered statistically significant at $p < 0.05$.

The relationship between the hormonal value of the animals in the second and third groups and the pharmacokinetic parameters of fexofenadine was evaluated using the Pearson correlation coefficient (Rs).

In addition, two-sided 90% confidence interval (CI) was calculated for the ratio of the geometric means of the pharmacokinetic parameters of fexofenadine to compare the parameters after the exposure to those of intact animals (within groups), as well as 90% CI of the ratio of the geometric means of the pharmacokinetic parameters of fexofenadine after the exposure (rabbits of the third group) to the parameters of animals after ovariectomy (rabbits of the second group). According to U.S. Food and Drug Administration, Center for Drug Evaluation and Research the differences between pharmacokinetic parameters are considered to be significant if the two-sided 90% CI of their ratio is outside the range of 0.8—1.25 (80—125%), since only a change of more than 25% in pharmacokinetic parameters can lead to a change in the pharmacodynamics of the drugs.

Results

Subjects (participants) of the study

All animals completed the study and were included in the final analysis.

Main outcomes of the study

The concentrations of sex hormones (testosterone, progesterone and estradiol) in the serum of animals from the different groups did not differ before the beginning of the experiment. After the "false surgery", the hormonal status of the rabbits did not change significantly throughout the entire experiment.

In animals in the second group (ovariectomy only), there was a significant decrease (29.7%, $p = 0.028$) of es-

tradiol level on Day 42 after surgery (Figure 1), as well as a decrease in the level of progesterone by 30.9% (p = 0.028) on Day 14 of the experiment, 54.9% (p = 0.028) on Day 28 and 48.7% (p = 0.028) at Day 42 (Figure 2) compared to the baseline.

In rabbits of the third group (administration of estradiol and progesterone after the ovariectomy), the concentration of estradiol throughout the entire experiment was not significantly different from the baseline (before ovariectomy) and exceeded the values for the rabbits in the second group (ovariectomy only) on Day 42 of the experiment by 62.3 % (p = 0.004) (Figure 2). The level of progesterone decreased by 46.9% (p = 0.028) on Day 14 of the study , by 88.1% (p = 0.028) on Day 28, and it increased by 168.5% (p = 0.046) on Day 42. During this period, the concentration of progesterone in animals of the third group exceeded the values for animals in the second group by 500.0% (p = 0.002).

The changes in concentration of testosterone were not significant in animals of all study groups throughout the experiment.

The functional activity of Pgp was evaluated based on the pharmacokinetics of its marker substrate, fexofenadine. Fexofenadine does not undergo biotransformation and its pharmacokinetics depends on this transporter protein. The accumulation of fexofenadine in rabbits (increase in C_{max} and AUC_{0-t}), and a decrease in its excretion (decrease in Cl), indicate a decrease in the functional activity of Pgp in the body, and the opposite changes in values indicate the increase in Pgp activity.

The pharmacokinetic parameters of fexofenadine in animals of different groups did not differ prior to the start of the experiment (Table 1).

In the group of animals which underwent false surgery, the pharmacokinetic parameters of fexofenadine did not differ significantly compared to the baseline values throughout the experiment, which indicates that there was no change in Pgp activity in the body.

In animals from the second group (ovariectomy only) on Day 14 after the surgery the following changes compared to the baseline were recorded: 1.61-fold increase in fexofenadine C_{max} (90% CI 1.09, 2.38, p = 0.039), 1.56-fold increase in AUC_{0-t} (90 % CI 1.08, 2.24, p = 0.05), and 0.325-fold decrease in Cl (90% CI 0.12, 0.95, p = 0.036). On Day 28 there was a 0.52-fold decrease in Cl (90% CI 0.31, 0.87, p = 0.07) compared to the baseline. On Day 42 there was 2.43-fold increase in C_{max} of fexofenadine (90% CI 1.87, 3.15, p = 0.0008), 2.24-fold increase in AUC_{0-t} (90% CI 1.53; 3.28, p = 0.0017), and 0.37-fold decrease in Cl (90% CI 0.17, 0.82, p = 0.06) (Table 1) compared to the baseline values. The data obtained indicate the accumulation of fexofenadine in rabbits and slowing of its excretion, which reflects a decrease in the functional activity of Pgp. The maximum and clinically significant differences (90% CI of the ratio of the geometric means of pharmacokinetic parameters of fexofenadine in animals subjected to ovariectomy compared to the parameters of the intact animals, which fall outside the 0.8—1.25 range) were recorded on the Day 42 after the operation.

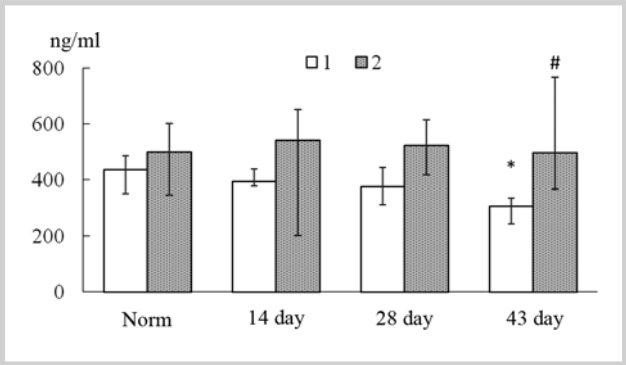

Figure 1. **The concentration of estradiol in the sera of rabbits after ovariectomy and the subsequent administration of a combination of estradiol and progesterone. The data are presented as a median, upper and lower quartiles.**

Note: 1 - ovariectomy group, 2 - group of animals that received a combination of estradiol and progesterone after ovariectomy. * - p <0.05 in comparison with the value for intact animals (norm), # - the same when compared with the values for animals of the ovariectomy group.

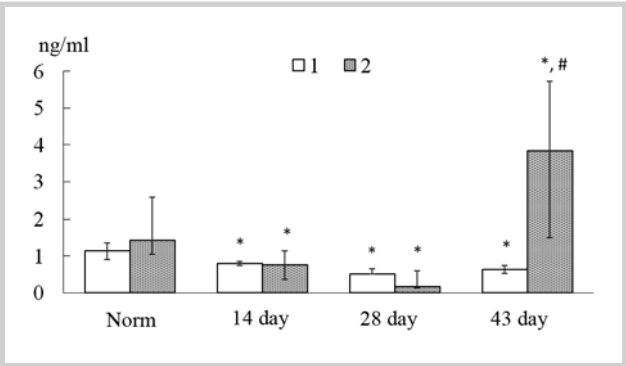

Figure 2. **The concentration of progesterone in the sera of rabbits after ovariectomy and the subsequent administration of a combination of estradiol and progesterone. The data are presented as a median, upper and lower quartiles.**

Note: Assignments as on Figure 1.

In animals of the third group (administration of estradiol and progesterone after the ovariectomy) Cl of fexofenadine decreased 0.62 fold (90% CI 0.45, 0.86, p = 0.024) on Day14 after ovariectomy, compared to the baseline values. On Day 28 (Day 14 after the initiation of estradiol administration), AUC_{0-t} of fexofenadine increased 1.4-fold (90% CI 1.16, 1.7, p = 0.038), and Cl decreased 0.5-fold (90% CI 0.4, 0.64, p = 0.042). On Day 42 of the experiment (Day 14 after the initiation of estradiol administration and Day 14 after the initiation of the administration of the combination of estradiol and progesterone), C_{max} decreased 0.73-fold (90% CI 0.56, 0.96, p = 0.041) and AUC_{0-t} decreased 0.618-fold (90% CI 0.46, 0.83, p = 0.044).

The observed changes in the pharmacokinetics of fexofenadine point to its accumulation and slowing of its

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Pharmacokinetic parameters of fexofenadine in ovariectomy and administration of a combination of estradiol and progesterone

		Dates of the experiment			
		Baseline	Day 14	Day 28	Day 42
C_{max}, ng/ml	Ovariectomy	276.46 (175.03; 436.67)	445.97 (322.8; 616.07)*	409.23 (222.85; 751.47)	671.55 (344.9; 1307.5)*
	Estradiol + progesterone	352.06 (247.49; 500.81)	366.75 (252.83; 532.00)	467.82 (414.49; 528.00)	258.33 (196.08; 340.34)*#
AUC_{0-t}, ng*h/ml	Ovariectomy	3198.09 (1883.65; 5429.77)	4977.98 (3338.49; 7422.59)*	3852.71 (2451.45; 6054.95)	7169.97 (3840.12; 13387.2)*
	Estradiol + progesterone	3491.2 (2680.56; 4547.08)	3757.87 (2551.21; 5535.26)	4904.0 (4282.64; 5615.61)*	2157.73 (1659.80; 2805.03)*#
Cl, l/h	Ovariectomy	16.17 (9.25; 28.27)	5.26 (1.59; 17.41)*	8.42 (5.75; 12.33)*	6.04 (2.49; 14.62)*
	Estradiol + progesterone	14.72 (10.51; 20.62)	9.13 (5.81; 14.34)*	7.44 (5.15; 10.77)*	19,25 (10,45; 35,49)#

Notes: *, P <0.05 when compared with the baseline, # - the same when compared with the мфдгуы in the ovariectomy group. The parameters are represented as the geometric mean and its 90% confidence interval.

excretion during 28 days of the study, i.e., to inhibition of Pgp. The decrease in fexofenadine levels in rabbits after the administration of the combination of estradiol and progesterone over 14 days compared with the baseline values reflects the induction of the transporter protein.

When comparing the pharmacokinetic parameters of fexofenadine in animals of the second and third groups over 28 days, no significant differences were revealed. On Day 42, the C_{max} of fexofenadine was 0.385-fold lower in animals of the third group than in the rabbits of the second group (90% CI 0.23, 0.64, p = 0.031), AUC_{0-t} was 0.30-fold lower 90% CI 0.19, 0.49, p = 0.016) and Cl was 3.19-fold higher (90% CI 1.49, 6.81, p = 0.009).

Additional endpoints of the study

Correlation analysis in the second group revealed a direct relationship between the concentration of progesterone and the total clearance of fexofenadine (Rs = 0.536, p = 0.007), whereas the concentration of estradiol showed no association with any of the pharmacokinetic parameters studied.

In the third group, the inverse relationship was observed between the concentration of progesterone and C_{max} (Rs = -0.435, p = 0.034 and AUC_{0-1} (Rs = -0.497, p = 0.014) of fexofenadine, and between the concentration of estradiol and C_{max} of fexofenadine (Rs = -0.388, p = 0.061). The dependence between the concentration of progesterone and Cl of fexofenadine (Rs = 0.733, p = 0.00024) was found to be direct.

Adverse events

No deaths were recorded during the course of the study.

Discussion

Summary of the main outcome of the study

Starting with Day 14 after ovariectomy, the functional activity of Pgp at the level of the whole organism decreases and reaches its minimum on Day 42 after the operation. Given the absence of significant changes in the concentration of estradiol in the first 28 days, the decrease in the activity of the transporter protein during this period is most likely due to the decrease in the level of progesterone, which is confirmed by the correlation between the concentration of progesterone and the total clearance of fexofenadine. The greatest decrease in Pgp activity was observed on Day 42 day of the study, when the levels of both estradiol and progesterone decreased.

Administration of 0.5 mg of estradiol for 14 days after ovariectomy did not significantly affect the functional activity of Pgp; it remained below the baseline and did not differ significantly from that of the animals of the second group. The administration of a combination of estradiol and progesterone for 14 days increased Pgp activity compared not only with the parameters of the second group, but also with the initial values. We believe that the administration of a combination of hormones results in normalisation of the level of estradiol (it does not differ significantly from the norm), and an increase in the concentration of progesterone.

Discussion of the main outcomes of the study

The revealed changes in Pgp activity are consistent with the data obtained earlier on its lower activity in male rabbits compared to females [13]. Apparently, one of the reasons for gender differences in the functional activity of the transporter protein is the stimulating effect of estradiol and progesterone on its functional activity.

The effect of estradiol and progesterone on Pgp activity can cause variability in the pharmacokinetics of substrates of the transporter protein in women [8]. However, we have not found any studies in which the relationship between safety and efficacy of pharmacotherapy with Pgp substrates and the menstrual phase have been studied.

The functioning of Pgp can change as a result of direct influence substances on its activity, or as a result changes in the expression of ***MDR1***, the gene that encodes the protein-transporter [1]. The effect of sex hor-

mones in physiological (relatively low) concentrations on the transporter protein is most likely due to a change in its expression.

The putative estrogen response element is found above the transcription initiation site [14] in the TRANSFAC database, but not in the promoter of the *MDR1* gene. However, binding sites for AP-1 transcription factor have been identified, which indicates the possibility of the mediated effect of estradiol on Pgp expression. Estrogens reduce the expression of the c-Jun protein, which is the main component of AP-1. Increased c-Jun expression is associated with the suppression of *MDR1* gene expression in various human cell lines [16].

An experiment with T47D cells transfected with the promoter of *mdr1b* gene showed that at a physiological concentration of 5×10^{-7} M the agonist of the progesterone receptors R5020, acting through the progesterone receptor PR_A, stimulates the expression of the gene encoding Pgp in the uterus of mice [17].

Estrogens and gestagens at high concentrations (10 μM) are also able to act as typical xenobiotics through other types of nuclear receptors, such as the X-receptor (PXR) [18,19], which increases the expression of the *MDR1* gene [20].

Limitations of the study

Pgp expression in organs and tissues has not been studied as part of this project, which does not allow one to unequivocally answer the questions: what causes the changes in the functioning of the transporter protein (modulation of expression or actual activity), and also in which specific organs the modulation of Pgp activity (intestine, kidneys , liver) leads to a change in the pharmacokinetics of its marker substrate, fexofenadine.

Further research in this area will allow to establish organ-specific molecular mechanisms of the change in the functioning of this transporter protein in different phases of the menstrual cycle.

Conclusion

In rabbits, ovariectomy leads to a decrease in the functional activity of Pgp; administration of estradiol (0.5 mg) starting from Day 14 after the surgery does not affect the activity of the transporter protein, and the subsequent use of a combination of estradiol (0.5 mg) and progesterone (5 mg) for 14 days increases Pgp activity.

The dependence of the function of the transporter protein on the physiological fluctuations in the concentrations of estradiol and progesterone suggests that the efficacy and safety of pharmacotherapy with Pgp substrates may differ in different phases of the menstrual cycle, which requires correction of dosage depending on the phase of the cycle.

Supplementary information

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Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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