HISTOMORPHOLOGICAL CHANGES IN THE OVARY EXPOSED TO CALCIUM ANTAGONISTS

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Goal. To study the comparative influence of representatives of different chemical groups of CA on histomorphological changes in the ovaries of female white rats. **Material and methods.** The control group was administered 0.2 ml of 0.9% NaCl solution daily, the experimental animals were administered verapamil at a dose of 5 mg/kg and 25 mg/kg, nifedipine at a dose of 5 mg/kg and 10 mg/kg, and diltiazem at a dose of 5 mg/kg and 20 mg/kg. After 21 days, the animals were decapitated, ovaries were removed and were fixed in 10% formalin solution, were clarified in xylene, paraffinized and formed into blocks. Sections of 5 microns thickness were prepared from the blocks using a microtome (Leica RM 2125 RTS, Germany). Sections were stained with hematoxylin and eosin (Merck, Germany). The preparations were examined under a light microscope (Leica DM 750, Germany). Changes were recorded using a camera attached to the microscope (Leica ICC 50, Germany). **Results.** Verapamil at a dose of 5 mg/kg, the number of preantral follicles was (2.3±0.4), which is 16.7% ($p>0.05$) less compared to the control group. The number of antral follicles was (7.4 ± 0.6), which is 42% less ($p < 0.05$). In the group of nifedipine (5 mg / kg), the quantitative indices of antral and preantral follicles decreased by 28.5% and 21%, respectively. In the group receiving nifedipine (10 mg/kg), this reduction was 71% compared to the control group and was 16.7% higher than in the group receiving verapamil ($p>0.05$). In females receiving diltiazem (20 mg/kg), the number of preantral follicles decreased by 41.7% compared to the control group ($p>0.05$). **Conclusion.** Has been founded potential negative impact of verapamil, as well as diltiazem, on the ovaries, and when high doses are required, the advantage of the nifedipine in this aspect has been determined.
Keywords: calcium channel blockers, antireproductive effect, ovary, follicle

Calcium antagonists (CA) are among the drugs used in the treatment and prevention of cardiovascular diseases (CVD) [1, 2]. The mechanism of action of these drugs is based on the blockade of potential-dependent calcium channels (PVCC) of the cell membrane and the reduction of the intracellular concentration of the corresponding ions by preventing the transmembrane Ca2+ ion influx. It is known that Ca2+ ions are universal secondary mediators and participate in practically all intracellular processes – cell aggregation, hormone secretion, mitosis, as well as the maturation of sex cells, and, as a result, in reproductive function [3].

The increase in the number of people suffering from UDS diseases in the reproductive age from year to year, and the fact that this increase is observed not only among men but also among women, respectively, necessitates the intake of CA at a younger age [4]. The above-mentioned creates the basis for studying the effect of the relevant substances on reproductive function. Most authors emphasize the possibility of the effect of drugs on reproductive function, and in the literature there is information about the effect of CA on reproductive function [5, 6], but there is no information about its effect on the ovaries in females. The discovery of the antiplatelet, antioxidant, etc. properties of CA, which are widely used in cardiology, neurology, gynecology, etc. fields of medicine [7] further expands their areas of application, and the above-mentioned creates the need for a comprehensive study of the effect of the relevant drugs on reproductive function. Verapamil, nifedipine and diltiazem, which have the same mechanism of action at the cellular level, are classic representatives of different chemical groups of CA, and differ sharply from each other due to their various properties. Our **goal** is to study the comparative effect of representatives of different chemical groups of CA on reproductive function, including the ovaries of female white rats.

Material and methods. The animals of the 1st control group were injected daily with 0.2 ml of 0.9% NaCl solution, the experimental animals of the 2nd and 3rd groups were injected with verapamil (isoptin, Abbott Italy) at a dose of 5 mg/kg and 25 mg/kg, respectively, the experimental animals of the 4th and 5th groups were injected with nifedipine (Pharmodipin, "Farmak" Ukraine) at a dose of 5 mg/kg and 10 mg/kg, respectively, and the experimental animals of the 6th and 7th groups were injected with diltiazem (Diltizem-L, MNIS-Istanbul) at a dose of 5 mg/kg and 20 mg/kg, respectively. The drugs were injected into the abdominal cavity of the animals and the doses were determined based on the available literature data [8]. The treatment course for the animals was continued for 21 days, provided that they were fed under the same feeding and

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housing conditions. On the last day of treatment, the animals were decapitated under ether anesthesia and the abdominal cavity was opened and both ovaries were removed. The biopsy samples were fixed in 10% formalin solution. Alcohols of various concentrations were used for dehydration. In the next stage, the slices were clarified in xylene and paraffinized and made into blocks. 5-micron sections were prepared from the blocks using a microtome (Leica RM 2125 RTS, Germany). The sections were stained with hematoxylin and eosin dyes (Merck, Germany). The prepared preparations were examined with a light microscope (Leica DM 750, Germany). Changes were recorded with a camera attached to the microscope (Leica ICC 50, Germany).

During the statistical analysis of the quantitative indicators obtained from the results of the study, the variational statistical method (Student's t-test) and the non-parametric method U (Wilcoxon-Mann-Whitney) test were applied. Statistical studies were carried out using MS EXCEL and S-PLUS programs.

Results and discussion. Histological analysis of ovarian tissue in female animals receiving a long-term treatment course against the background of a dose of 5 mg/kg verapamil showed that there was no significant change in the number of preantral follicles. Thus, in females receiving a course of treatment with verapamil at a dose of 5 mg/kg, the number of preantral follicles was (2.3±0.4), which was a 16.7% ($p>0.05$) decrease compared to the control group. In the same group, the number of antral follicles was (7.4±0.6) and a 42% decrease ($p<0.05$) was observed. In the nifedipine (5 mg/kg) group, the quantitative indicators of antral and preantral follicles decreased by 28.5% and 21%, respectively, compared to the control group, which is statistically insignificant. Slightly different results were obtained in females in the diltiazem (5 mg/kg) group. Thus, the number of antral follicles decreased by 37% ($p<0.05$) compared to the control group, and the decrease in the number of preantral follicles was 8% ($p>0.05$) (Tab. 1). The rate of follicle degeneration against the background of low doses of KA did not differ significantly from that of the control group, and the increase in the number of degenerative follicles in the nifedipine (5 mg/kg) and diltiazem (5 mg/kg) groups was statistically insignificant compared to the control group ($p>0.05$). The number of corpus luteum in all study groups changed in accordance with the listed indicators and no statistically significant results were obtained. The results obtained show that, against the background of low doses of verapamil (5 mg/kg), nifedipine (5 mg/kg) and diltiazem (5 mg/kg), there was a change in the quantitative composition of structural and functional elements of ovarian tissue compared to the indicators of the control group females, but the corresponding changes were within the norm, that is, this proves that the drugs do not affect reproductive function.

Table 1

Changes in the quantitative indicators of structural and functional elements in the ovaries of white rats against the background of low doses

Groups	Preantral follikul	Antral follikul	Degeneraiv follikul	Corpus luteum
Kontrol (n-10)	2.0±0.4 (0-3)	10.5±1.0 (6-15)	0.6±0.3 (0-2)	7.3±1.3 (0-13)
Verapamil 5 mg/kg	2.3±0.4 (0-5)	7.4±0.6 (3-11)	1.3±0.2 (0-2)	5.6±1.0 (0-12)
p_1	>0.05	<0.05	<0.05	>0.05
Nifedipin 5 mg/kg	2.4±0.3 (0-4)	8.2±5.6 (5-11)	1.1±0.2 (0-2)	8.4±0.9 (4-13)
$p_1; p_2$	$p_1>0.05$; $p_2>0.05$	$p_1>0.05$; $p_2>0.05$;	$p_1>0.05$; $p_2>0.05$	$p_1>0.05$; $p_2<0.05$
Diltiazem 5 mg/kg	2.2±0.4 (0-5)	7.7±0.6 (4-12)	1.0±0.2 (0-2)	6.2±0.3 (5-8)
$p_1; p_2$	$p_1>0.05$; $p_2>0.05$	$p_1<0.05$; $p_2>0.05$	$p_1>0.05$; $p_2>0.05$	$p_1>0.05$; $p_2>0.05$

Note: p1 – comparison with the control group; p2 – comparison with verapamil; $p<0.05$ statistical significance of the difference between groups.

As a result of the conducted studies, it is known that against the background of high doses of KA, the tendency to decrease in preantral and antral follicles increased. In female rats receiving a long-term course of treatment with verapamil at a dose of 25 mg/kg, the number of antral follicles was (1.2±0.2)

and was 8 times lower than the indicators of the control group animals ($p<0.001$). In females receiving a course of treatment with nifedipine at a dose of 10 mg/kg, this indicator was (6.2±0.3), which was 70% lower than the indicators of the control group ($p<0.005$) and was 4 times higher than the indicators of the verapamil (25 mg/kg) group animals. Long-term treatment with diltiazem at a dose of 20 mg/kg caused a 1.7-fold decrease in the number of antral follicles in the same group of females, which was 2.2 times higher than in the verapamil (25 mg/kg) group ($p<0.001$). As for the number of preantral follicles in the verapamil group, this indicator was (1.0±0.2) and was 2 times lower than in the control group ($p<0.05$). In the nifedipine (10 mg/kg) group, this decrease was 71% compared to the control group ($p<0.05$) and was 16.7% higher than in the verapamil group ($p>0.05$). In females treated with diltiazem (20 mg/kg), the number of preantral follicles decreased by 41.7% compared to the control group ($p>0.05$) and was 1.8 times higher than the verapamil (25 mg/kg) group ($p<0.005$). On the other hand, it is known that the rate of increase in the number of degenerated follicles in females treated with verapamil at doses of 25 mg/kg slightly increased. Thus, the number of corresponding follicles in the verapamil (25 mg/kg) group was 2 times higher than in the control group. In the nifedipine (10 mg/kg) group, this indicator was 1.4 times higher than in the control group and 35.1% lower than in the verapamil

Table 2

Changes in the quantitative indicators of structural-functional elements in the ovaries of white rats against the background of high doses

Groups № (n=12)	Preantral follikuls	Antral follikuls	Degeneraiv follikuls	Corpus luteum
Kontrol (n=10)	2.0±0.4 (0-3)	10.5±1.0 (6-15)	0.6±0.3 (0-2)	7.3±1.3 (0-13)
Verapamil 25 mg/kg	1.0±0.2 (0-2)	1.2±0.2 (0-2)	1.9±0.5 (0-4)	2.8±1.0 (0-9)
p_1	<0.05	<0.001	>0.05	p<0.05
Nifedipin 5 mg/kg	1.2±0.2 (0-2)	6.2±0.3 (5-8)	1.4±0.4 (0-3)	5.4±0.9 (0-12)
$p_1; p_2$	$p_1<0.05$; $p_2>0.05$	$p_1<0.005$; $p_2<0.001$	$p_1>0.05$; $p_2>0.05$	$p_1>0.05$; $p_2<0.05$
Diltiazem 5 mg/kg	2.8±0.5 (1-5)	3.8±0.3 (3-6)	0.3±0.1 (0-1)	5.0±0.8 (0-9)
$p_1; p_2$	$p_1>0.05$; $p_2<0.005$	$p_1<0.001$; $p_2<0.001$	$p_1>0.05$; $p_2<0.05$	$p_1<0.05$; $p_2<0.05$

Note: p1 – comparison with the control group; p2 – comparison with verapamil.

(25 mg/kg) group ($p>0.05$). The number of degenerated follicles in the diltiazem group was 1.4 times higher than in the control group ($p>0.05$) and 6 times lower than in the verapamil group ($p<0.05$) (Tab. 2).

As for the corpus luteum indicators, it is clear that the most significant decrease occurred in the verapamil (25 mg/kg) group: the number of corpus luteum was (2.8±1.0) and was 1.6 times lower than in the control group ($p<0.05$). This is most likely due to the change in follicle maturation. In the nifedipine (10 mg/kg) group, the decrease in he number of corpus luteum was 45% compared to the control group and was statistically insignificant ($p>0.05$). However, the number of corpus luteum in the corresponding group was approximately 2 times higher than in the verapamil (25 mg/kg) group. In females in the diltiazem (20 mg/kg) group, the number of corpus luteum was found to be 4 times lower than in the control group, which was 2 times higher than in the verapamil (25 mg/kg) group. The results show that increasing the dose of the respective drugs significantly changes the quantitative composition of the structural and functional elements of the ovarian (Figure).

As can be seen in the figure, in the control group, antral follicles of various sizes are found in a wide stroma consisting of a large number of interstitial cells, as well as follicles preparing for ovulation (preovulatory). Antral follicles are not found in the cross-section of the preparation prepared from the ovaries taken from animals in the verapamil (25 mg/kg) group. A large number of degenerated follicles are found in the narrow stroma. In the ovaries of females administered nifedipine (10 mg/kg) and dil-

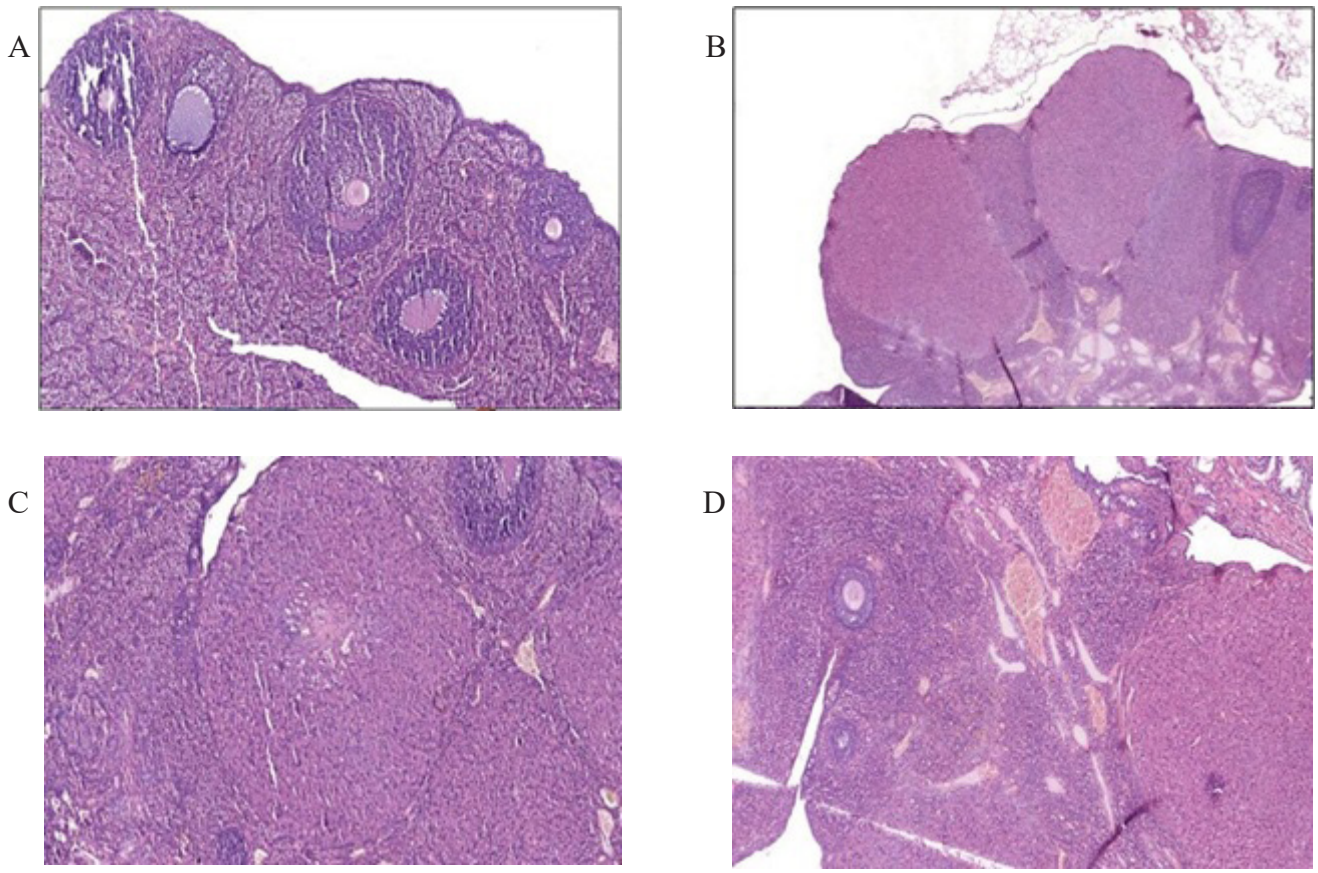

Figure. Histomorphological structure of the ovary against the background of high doses of CA; A – control group; B – verapamil; C – nifedipine; D – diltiazem.

tiazem (20 mg/kg), a corpus luteum surrounded by a moderate amount of interstitial cells and a small number of antral follicles are found.

The results of the study show that a significant decrease in the number of follicles and corpus luteum in females receiving long-term treatment with verapamil at a dose of 25 mg/kg (compared to the control group) indicates that high doses of the drug have an undesirable effect on the histo-morphological structure of the ovary and, consequently, on reproductive function. Against the background of a high dose of diltiazem (20 mg/kg), similar changes were found, although not as much as verapamil. In animals receiving long-term treatment with high doses of nifedipine (10 mg/kg), the quantitative composition of the structural and functional elements of the ovarian tissue changed less than in the other study groups.

Zhang C. et al. in their study found that the proliferation of ovarian cells was slowed down by the effect of CA [9]. The results of another study conducted on male rats showed that nifedipine, verapamil, and diltiazem significantly reduced testicular mass and also reduced the number of male germ cells [10]. Recent research results also show that Calcium channels are pivotal to male fertility and contraceptives, offering diagnostic and therapeutic potential [11]. The research results found in the literature sources are consistent with our results. From this we can conclude that Ca ions play an important role in follicle maturation, and its deficiency leads to disruption of the relevant processes.

Thus, the results of the study, taking into account the above, revealed to us the possibility of using representatives of various chemical groups of CA in low doses in the pharmacotherapy of a number of diseases of women of reproductive age. Also, in clinical practice, in cases where treatment is required against the background of high doses or when there is a need to increase the dose, it is important to take into account the potential negative effect of verapamil, as well as diltiazem on the ovaries, and when it is necessary to use high doses, the advantage of the dihydropyridine derivative nifedipine from this aspect was determined.

Conflict of interests. The authors declare no conflict of interest.

Transparency of financial activity. The authors have no financial interest in the presented materials or methods.

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XÜLASƏ

KALSİUM ANTAQONİSTLƏRİNİN TƏSİRİNƏ MƏRUZ QALAN YUMURTALIQDA HİSTOMORFOLOJİ DƏYİŞİKLİKLƏR

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Məqsəd. Müxtəlif kimyəvi qrupların nümayəndələrinin dişi ağ siçovulların yumurtalıqları da daxil olmaqla reproduktiv funksiyaya müqayisəli təsirini öyrənmək. **Material və metodlar**. 1-ci nəzarət qrupuna gündəlik 0,2 ml 0,9% NaCl məhlulu vuruldu, 2-ci və 3-cü qrupların eksperimental heyvanlarına müvafiq olaraq 5 mq/kq və 25 mq/kq dozada verapamil vuruldu, 4-cü və 5-ci qrupların eksperimental heyvanlarına müvafiq olaraq 5 mq/kq və 10 mq/kq dozada nifedipin vuruldu, 6-cı və 7-ci qrupların eksperimental heyvanlarına isə 5 mq/kq və 20 mq/kq dozada diltiazem vuruldu. 21 gündən sonra heyvanların başları efir anesteziyası altında kəsildi və qarın boşluğu açıldı və hər iki yumurtalıq çıxarıldı. Biopsiya nümunələri 10% formalin məhlulunda fiksasiya edildi. Növbəti mərhələdə dilimlər ksilolda təmizləndi və parafinləşdirilərək bloklara çevrildi. Bloklardan mikrotom (Leica RM 2125 RTS, Almaniya) istifadə edilərək 5 mikronlu kəsiklər hazırlanmışdır. Kəsiklər hematoksilin və eozin boyaları ilə boyanmışdır (Merck, Almaniya). Hazırlanmış preparatlar işıq mikroskopu (Leica DM 750, Almaniya) ilə müayinə edilmişdir. Dəyişikliklər mikroskopa qoşulmuş kamera ilə qeydə alınmışdır (Leica ICC 50, Almaniya). **Nəticələr.** Verapamillə 5 mq/kq dozada müalicə zamanı preantral follikulların sayı (2.3±0.4) olmuşdur ki, bu da nəzarət qrupu ilə müqayisədə 16.7% ($p>0.05$) azalma deməkdir. Antral follikullar (7.4±0.6) olmuşdur və 42% azalma ($p<0.05$) müşahidə edilmişdir. Nifedipin (5 mq/kq) qrupunda antral və preantral follikulların kəmiyyət göstəriciləri müvafiq olaraq 28.5% və 21% azalmışdır. Nifedipin (10 mq/kq) qrupunda bu azalma nəzarət qrupu ilə müqayisədə 71% ($p<0.05$) və verapamil qrupundakından ($p>0.05$) 16.7% yüksək olmuşdur. Diltiazem (20 mq/kq) ilə müalicə olunan qadınlarda preantral follikulların sayı nəzarət qrupu ilə müqayisədə 41.7% azalmış ($p>0.05$) və verapamil (25 mq/kq) qrupundan 1.8 dəfə çox olmuşdur ($p>0.05$). **Yekun.** Klinik praktikada, yüksək dozalar fonunda müalicə tələb olunduqda və ya dozanın artırılmasına ehtiyac olduqda, verapamilin, eləcə də diltiazemin yumurtalıqlara potensial mənfi təsirini nəzərə almaq vacibdir və yüksək dozalardan istifadə etmək lazım gəldikdə, dihidropiridin törəməsi olan nifedipinin bu baxımdan üstünlüyü müəyyən edilmişdir.

Açar sözlər: kalsium kanal blokerləri, reproduktiv təsir, yumurtalıq, follikul

РЕЗЮМЕ

ГИСТОМОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В ЯИЧНИКАХ ПОД ВОЗДЕЙСТВИЕМ АНТАГОНИСТОВ КАЛЬЦИЯ

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Цель. Изучить сравнительное влияние представителей различных химических групп карбоангидразы на гистоморфологические изменения в яичниках самок белых крыс. **Материал и методы**. Контрольной группе ежедневно вводили 0,2 мл 0,9% раствора NaCl, экспериментальным животным — верапамил в дозах 5 мг/кг и 25 мг/кг, нифедипин в дозах 5 мг/кг и 10 мг/кг, а также дилтиазем в дозах 5 мг/кг и 20 мг/кг. Через 21 день животных обезглавливали, яичники извлекали и фиксировали в 10% растворе формалина, осветляли в ксилоле, парафинировали и формировали блоки. Из блоков готовили срезы толщиной 5 мкм с помощью микротома (Leica RM 2125 RTS, Германия). Срезы окрашивали гематоксилином и эозином (Merck, Германия). Препараты исследовали под световым микроскопом (Leica DM 750, Германия). Изменения регистрировали с помощью камеры, прикрепленной к микроскопу (Leica ICC 50, Германия). **Результаты.** При дозе верапамила 5 мг/кг количество преантральных фолликулов составило (2,3±0,4), что на 16,7% ($p>0,05$) меньше по сравнению с контрольной группой. Количество антральных фолликулов составило (7,4±0,6), что на 42% меньше ($p<0,05$). В группе нифедипина (5 мг/кг) количественные показатели антральных и преантральных фолликулов снизились на 28,5% и 21% соответственно. В группе, получавшей нифедипин (10 мг/кг), это снижение составило 71% по сравнению с контрольной группой и было на 16,7% выше, чем в группе, получавшей верапамил ($p>0,05$). У самок, получавших дилтиазем (20 мг/кг), количество преантральных фолликулов уменьшилось на 41,7% по сравнению с контрольной группой ($p>0,05$). **Вывод.** Установлено потенциальное негативное воздействие верапамила, а также дилтиазема, на яичники, и при необходимости применения высоких доз в этом аспекте определено преимущество нифедипина.

Ключевые слова: блокаторы кальциевых каналов, антирепродуктивный эффект, яичник, фолликул

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