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PATHOMORPOLOGICAL RESEARCH OF VAGINAL SUPPOSITORIES "KLIMEDEKS" IN RATS WITH THE IRRYTATIVE VAGINITIS

Vaginitis is one of the most common reasons of women during calls to the gynecologist. The best method of their treatment is vaginal suppositories that contain antibacterial and anti-inflammatory components. We have studied vaginal suppositories "Klimedeks" in female-rats with experimental vaginitis caused by irritants mixture of a gum turpentine and dimethylsulfoxide. Treatment of vaginitis was 8 days. At 5th and 8th days animals were taken out of the experiment by method of euthanasia and researched vaginal mucosa by histologically. Vaginal suppositories "Klimedeks" at the fifth day of the experiment positively impact on the mucosa of the vagina in 87.5 % female rats of the animals had no signs of damage and inflammation of the lining of the vagina and superior comparator vaginal tablets "Mikozhynaks" for therapeutic effect. On the eighth day of the experiment, 100 % structural and functional state of animals mucous membrane of the vagina fully recovered. Placebo did no positive impact on the vagina of rats.

Key words: experimental vaginitis; postmortem study; vaginal suppositories; rats

INTRODUCTION

Vaginitis is one of the most common reasons women during calls to the gynecologist. The literature suggest a wide range of prevalence of the disease [4, 14], is due to the fact that a lot of patients don't consult their doctor, are treated yourself, cases of asymptomatic disease happen. Infection, inflammation or change the normal vaginal microflora can be the causes of vaginitis. Symptoms that accompany vaginitis are common and often lead to self-diagnosis and self-treatment [9, 11].

The conditions lead to vaginitis often include: contraception, pregnancy, level of estrogen, intake antibiotics, sexually transmitted infection, and so on [12]. More than 90% of vaginitis are diagnosed during bacterial vaginosis, vulvovaginal candidiasis, trichomoniasis [2, 11].

But there vaginitis without infectious origin [7]. There are atrophic vaginitis, allergic vaginitis, traumatic vaginitis, desquamative inflammatory vaginitis, and so on. Prolonged uncontrolled treatment by antiseptics or antibiotics, a menopause, neuroendocrine disorders, using of oral contraceptives can be the causes of these types of vaginitis, which leads to chronic process [8].

Thinning of the vaginal epithelium makes it more susceptible to injury and leads to exposure of connective tissue, which makes it vulnerable to inflammation and infection. Low glycogen content in the vaginal epithelium leads to reduces the synthesis of lactic acid by lactobacilli and to increase the vaginal pH. This promotes the growth of excessive intestinal flora, disappearance of lactobacilli and increases inflammation of the vagina and surrounding skin.

Vaginal suppositories that contain antibacterial and anti-inflammatory components are one of the best treatments for vaginal atrophy [13].

Thus, interest presented study the impact of new vaginal suppositories "Klimedeks" [5] include clindamycin, metronidazole, dexamethasone, hippophaes oleum, fluconazole, developed led by prof. Yarnih T. G. (the department TL NUPh), on morphological status vaginal mucosa against the background of the pilot irritative vaginitis.

MATERIALS AND METHODS

In the research female rats nonlinear were used. Pet care (including euthanasia) in the experiment was carried out according to the principles of "European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1985) [3], approved by the I National Congress on Bioethics (Kyiv, 2000) consistent with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Before the experiment, the females estrous cycle was investigated and selected animals in oestrus-prooestrus phases to minimize differences in the assessment of the functional state of the mucous membrane of the vagina when comparing the results. Irytatyvnyy vaginitis modeled introduction gum turpentine mixture of dimethylsulfoxide in a ratio of 1: 1 vaginally [1]. Animals were divi-

ded into groups as follows: Group 1 – intact animals, Group 2 – pathological control (positive control), Group 3 – animals were treated with vaginal suppositories "Klimedeks", Group 4 – animals treated with the drug compared vaginal tablets "Mikozhynaks" (Mekofar, Vietnam), Group 5 – animals which used suppository base (placebo) in the equivalent amount (negative control). Suppository doses of study drugs and administered in terms of comparison by commonly used in experimental pharmacology rate of species stability of Rybolovlev Yu. R. [10].

Vaginitis treatment began on the first day of disease and continued for 8 days. Investigated suppositories and preparations comparison was administered 1 time per day. At 5 and 8 day experiment on animals were taken out by euthanasia. After opening the macroscopic study of vaginal mucosa of all groups animals was investigated and vaginal samples were taken for histological investigation.

All samples were fixed in 10 % formalin solution, dehydrated in alcohols of increasing strength and embedded in paraffin [6]. The evaluation of the micropreparations was performed by microscope Granum, microphotography carried digital video camera Granum DSM 310. Photos processed on a computer Pentium 2,4 GHz with using Toup View.

Morphological study did with the consulting of Senior Researcher Yu. B. Lar'yanovskaya.

RESULTS AND DISCUSSION

The wall of the vagina of intact control animal consists of mucous, muscular and adventive layers in the microphotographs. Structural organization of epithelial mucosa in different parts of the vaginal wall is somewhat varied. In the vestibule of vagina is a typical skin type. At the distal (tail vagina) is multilayer flat epithelium with keratinized symptoms (called transitional type). Basal and intermediate layers were well differentiated and traced moderate dipping of epithelial tongues deep into mucosa. In the proximal part (close to the odd part of the uterus) epithelial structure depended on the phase of the estrous cycle. In the microphotographs in phase prooestrus epithelium was a multilayered-prismatic with mucinous cells on the surface, in the oestrus phase it was multilayered flat keratinized epithelium. The stratum corneum is expressed very moderate (less expressive than in the distal). It should be noted that often in different microphotographs observed different stages of development phases prooestrus-oestrus, which differ among themselves by severity of features. Vaginal mucosa lamina propria (stroma) had features of dense connective tissue. Closer to the muscle layer is changed in the submucosal layer, which looked like loose connective tissue. Vascularization was normal, the lumen of blood vessels was free and moderately wide. Submucosal layer directly changed in the muscle layer. Adventive layer is located outside of the muscle layer, consisted of loose connective tissue containing blood vessels (Fig. 1).

On the fifth day after administration of a mixture irritants in 87.5 % of females observed widespread destructive inflammatory changes all layers of the vaginal wall. The mucous layer is in a state of complete destruction, heavily infiltrated by mononuclear cells. Submucosal layer is very edematic, enlarged, collagen fibers altered degenerative, inflammatory cell response is located closer to the muscular layer. The blood vessels of the stroma mucous layer and submucosal layer paralytic expanded, in some of their observed blood cells located on the edge (Fig. 2a).

The vagina epithelial layer of other animals was saved, but without differentiation layers, superficial epithelium layer was in the state of the morphologic alterations, sometimes whole layer started to exfoliate off completely and stroma at this place was without outer layer. Collagen component of the lamina propria of mucous layer was very edematic. The stroma was penetrated by red blood cells, expressive inflammatory reaction was in the submucosal layer (Fig. 2b).

In the intact parts of the vaginal wall epithelium retains the ability to functional physiological changes in-

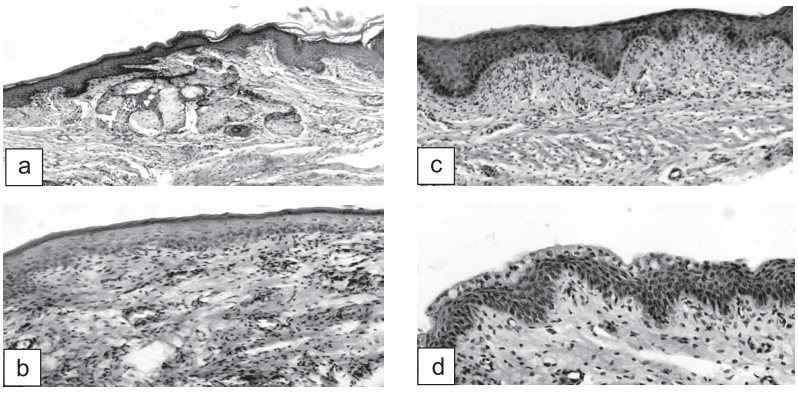

Fig. 1. *The epithelium of various parts of mucous membrane of wall of the vagina intact female rats: a – vestibule of vagina (typical skin); b – distal part (multilayered keratinized); c-d – proximal part (phase oestrus, prooestrus). Hematoxylin-eosin. a-c – x100, d – x200*

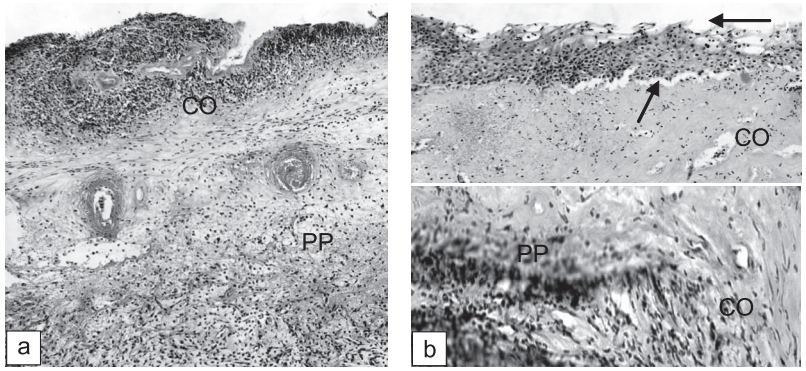

Fig. 2. *The wall of the vagina of female rats on the 5th day after administration of a mixture irritants: a – necrosis with mononuclear infiltration of mucous layer (CO), edema, degeneration of collagen fibers of submucosal layer (PP), paralysis of the vascular wall, inflammatory reaction; b – necrobiosis of the surface layers of the epithelium, absence of layers differentiation, exfoliation of layer, stroma of mucous is without outer layer and with edema (CO), erythrocyte penetration. Inflammation of submucosal layer (PP). Hematoxylin-eosin. x100*

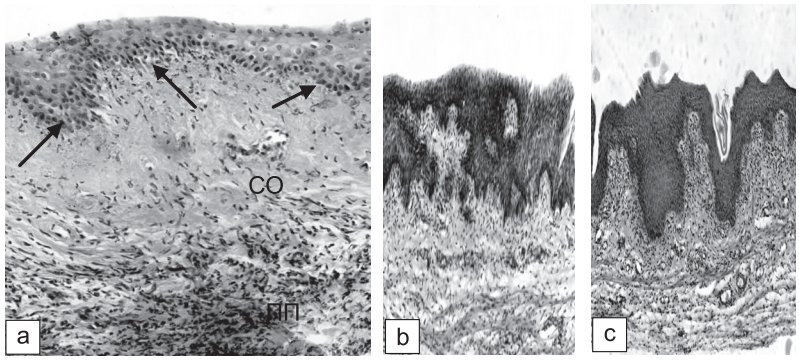

Fig. 3. *The wall of the vagina of female rats on the 5th day after administration of a mixture irritants. The area beyond the zone of destruction: a – proliferation of basal cells (arrows), degeneration of cells intermediate layer of the epithelium, edema of stroma mucous membrane (CO), inflammatiory cell reaction in the submucosal layer (PP); b – expressive proliferation of epithelial layer. Hematoxylin-eosin. x200; c – distal part (thickened epithelial layer, hyperkeratosis, inflammation in the stroma. Hematoxylin-eosin. x100*

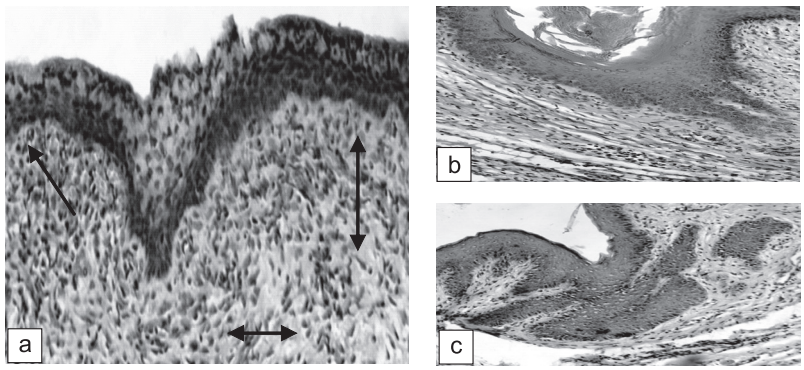

Fig. 4. *The wall of the vagina of female rats on the 8th day after administration of a mixture irritants. Proximal part: a – normal state epithelium, remains of inflammation in the stroma, small subepithelial hemorrhage (arrow), expansion and plethoric of capillary network (x250). Phase prooestrus. Distal part: b – thickened epithelial layer, hyperkeratosis (x200). Phase oestrus. Hematoxylin-eosin*

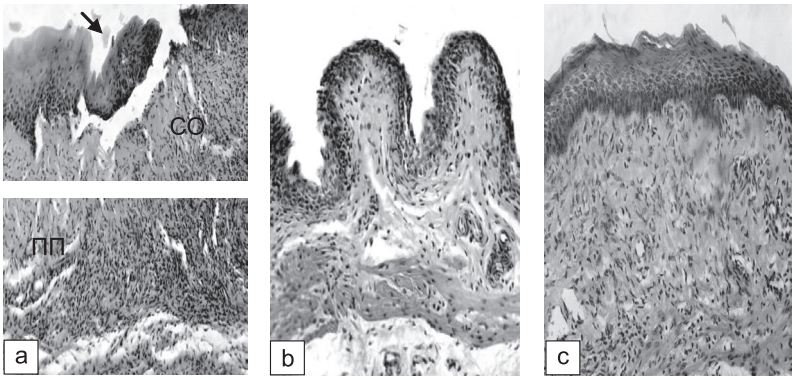

Fig. 5. *The wall of the vagina of female rats after vaginal suppositories "Klimedeks" treatment during the 5th day after administration of a irritants mixture: a – micro-defects of epithelial layer, its separation from lamina propria mucosa, moderate edema of the stroma (CO) of the submucosal layer (PP) inflammatory response; b – the normal state of the epithelial layer of the proximal and c – distal vaginal wall (phase oestrus). Hematoxylin-eosin. x200*

herent phases of the estrous cycle, although sometimes thickening of the epithelial layer traced.

In areas beyond the destructive changes observed stromal mucosal edema, inflammation in the submucosal layer. The epithelium, on the whole, kept the ability to physiological changes (phase rrooestrus-oestrus). At the same time, in the epithelial layer you can see the proliferation of basal cells, degeneration cell of the intermediate layer, sometimes layer lost the typical cells location (Fig. 3a). In some places you can see proliferation of epithelial (Fig. 3b).

On the 8-th day of the experiment pathologycal changes in mucous membrane of the vagina in control group were insignificant. Epithelial structure and physiological cycle were without changes. Plethoric capillary network noted in proximal subepithelial mucous layer some females, small focal hemorrhages, inflammation remains. In places of the distal part epithelial layer thickening, hyperkeratosis were saved (Fig. 4a, b, c). In the vaginal mucosa only remains vascular and inflammatory reactions and focal hypertrophic proliferation of epithelial layer in the distal area of the vaginal wall traced.

Administration of vaginal suppositories "Klimedeks" causes positive impact on the state of the mucous membrane of the rats' vagina, injured by mixture of irritants. On the fifth day of the experiment only 12.5 % female rats (1 of 8) in the distal mucosa small zone of destruction and separation from lamina propria mucosa of the epithelial layer revealed. Unlike reference pathology were less expressive edema, inflammatory cell and vascular stroma reaction as in mucosal and submucosal layer in it (Fig. 5a). 87.5 % of the animals this group structure epithelial layer is preserved, was typical for a particular phase of the estrous cycle, stroma mucosa and submucosal layer were not changed (Fig. 5b-c) already on the fifth day of the experiment.

On the 8-th day after administration of irritants structural organization of epithelial cells vaginal mucosa of rats treated with vaginal suppositories "Klimedeks" was physiological normal (Fig. 6).

Vaginal tablets do "Mikozhynaks" cause a similar impact on the vaginal wall. On the fifth day after administration irritants mixture in 2 of 8 females have been very extensive damaged areas of vaginal wall that microsco-

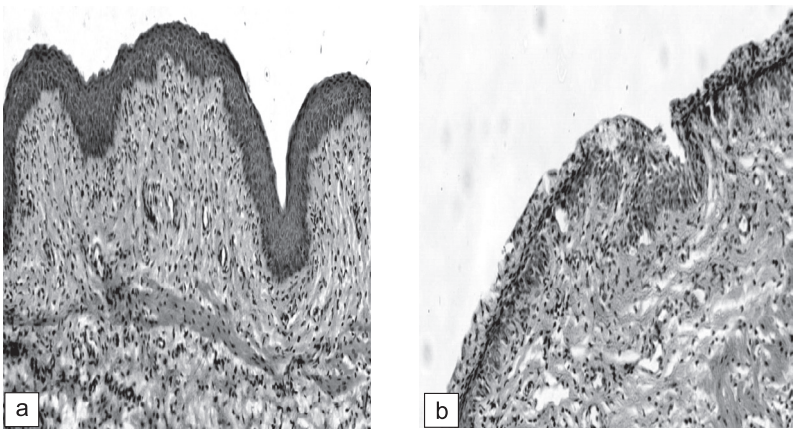

Fig. 6*. The wall of the vagina of female rats treated with vaginal suppositories "Klimedeks" on the 8-th day after administration of a mixture irritants. Structural organization of the mucous membrane was normal (a – distal, b – proximal). Phase oestrus. Hematoxylin-eosin. x200*

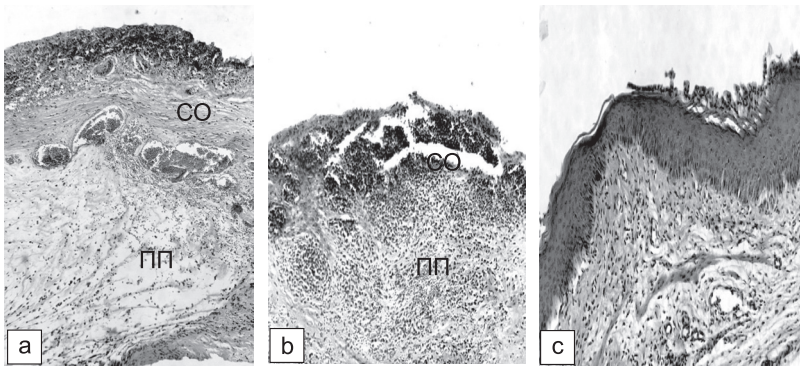

Fig. 7. *The wall of the vagina of female rats treated with vaginal suppositories «Mikozhynaks» on the fifth day after administration of a mixture irritants: a – the destruction of layers; b – the destruction of the mucosa (CO) inflammation in submucosal layer (PP); c – epithelium is thickened, but functionally normal in intact segment (phase oestrus). Hematoxylin-eosin. x100*

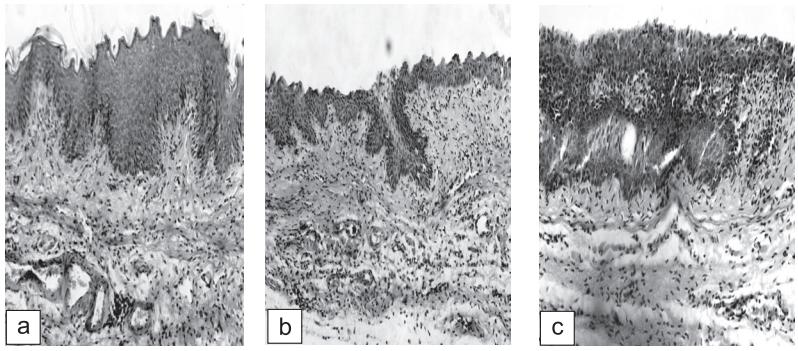

Fig. 8. *The wall of the vagina of female rats treated with vaginal suppositories «Mikozhynaks» on the fifth day after administration of a mixture irritants: a – a thickening of the epithelium of the distal (x200); b – normal epithelium in the proximal (phase oestrus, x100); c – the proliferation of the epithelium, loss of structural layers, deep penetration into the surrounding stroma (x200). Hematoxylin-eosin*

pically was not different from control disease (Fig. 7a). Even 2 rats had smaller destructive changes of mucosa (Fig. 7b). In the intact parts of the epithelial layer was thickened slightly, but structurally and functionally mucosa and submucosal stroma layer were normal (Fig. 7c). The vaginal wall of remaining animals was without destruction. Epithelial layer of the distal vaginal wall was often thickened, epithelial layer of the proximal vaginal wall ranged in thickness from normal to thin. In one case traced focal proliferation in violation of structural characteristics of epithelial layer and significant penetration into subordinate stroma were registered (Fig. 8).

The reference-drug of vaginal tablets "Mikozhynaks" during 5 days of the experiment were worse than vaginal suppositories "Klimedeks" in expressive positive impact on the vaginal wall of female rats.

On the background of placebo administration during 5 days of the experiment vaginal wall of rats practically do not have positive changes compared to the control pathology. On the 8th day, as in the control pathology group was a natural regression of pathological process.

CONCLUSIONS

1. Vaginal suppositories "Klimedeks" reduced edema, inflammatory cellular and vascular reactions in the stroma of the mucous membrane, and in the submucosal layer and helped restore the vaginal mucosa, which indicates apparent anti-inflammatory effect on the experimental models of irritative vaginitis caused by a mixture of turpentine oil and dimethylsulfoxide.
2. Vaginal suppositories "Klimedeks" during the experimental study exceeded reference-drug of vaginal tablets "Mikozhynaks" with the ability to restore the mucous membrane in the background irritative vaginitis.
3. Vaginal suppositories "Klimedeks" are perspective for using as a drug for the treatment of vaginitis.

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ПАТОМОРФОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ВАГИНАЛЬНЫХ СУППОЗИТОРИЕВ «МЕЛАНИЗОЛ» НА ФОНЕ ИРРИТАТИВНОГО ВАГИНИТА

Представлены патоморфологические исследования влияния вагинальных суппозиториев «Меланизол» на ход ирритативного вагинита, вызванного смесью живичного скипидара и диметилсульфоксида у крыс. Вагинальные суппозитории «Меланизол» на фоне вагинита проявили лечебный эффект, который проявился в предотвращении возникновения некротически-воспалительного повреждения и способствовании заживлению дефектов слизистой оболочки и подслизистого слоя стенки влагалища. На модели экспериментального вагинита было доказано, что суппозитории «Гравагин» уступали по выразительности положительного влияния на состояние влагалищной стенки крыс-самок, что проявлялось в наличии достаточно крупных очагов деструкции влагалищной стенки, очагов воспаления, отека коллагеновых волокон. Вагинальные суппозитории «Меланизол» являются перспективными для применения в качестве препарата для лечения неспецифических вагинитов, сопровождающихся деструктивно-воспалительными реакциями.

Ключевые слова: патоморфологические исследования; суппозитории вагинальные; масло чайного дерева; метронидазол

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ПАТОМОРФОЛОГІЧНЕ ДОСЛІДЖЕННЯ ВАГІНАЛЬНИХ СУПОЗИТОРІЇВ «МЕЛАНІЗОЛ» НА ТЛІ ІРИТАТИВНОГО ВАГІНІТУ

Представлені патоморфологічні дослідження впливу вагінальних супозиторіїв «Меланізол» на перебіг іритативного вагініту, викликаного сумішшю живичного скипидару і диметилсульфоксиду у щурів. Вагінальні супозиторії «Меланізол» на тлі вагініту виявили лікувальний ефект, який проявився у запобіганні виникненню некротично-запального пошкодження та сприянні загоєнню дефектів слизової оболонки та підслизового прошарку стінки піхви. На моделі експериментального вагініту було доведено, що супозиторії «Гравагін» поступалися за виразністю позитивного впливу на стан піхвової стінки щурів-самиць, що проявлялося у наявності достатньо великих осередків деструкцій піхвової стінки, вогнищ запалення, набряку колагенових волокон. Вагінальні супозиторії «Меланізол» є перспективними для застосування в якості препарату для лікування неспецифічних вагінітів, що супроводжуються деструктивно-запальними реакціями.

Ключові слова: патоморфологічні дослідження; супозиторії вагінальні; олія чайного дерева; метронідазол

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