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Efficacy study of vaginal suppositories with anti-inflammatory and antimicrobial effect on a model of experimental vaginitis

Исследование эффективности вагинальных суппозиториев с противовоспалительной и противомикробной активностью на модели экспериментального вагинита

Abstract

The study has been carried out in 35 female Vistar rats weighing 200–220 g. The animals were divided into 5 groups: 1 – intact animals, control; 2 – animals with modeled experimental vaginitis (EV); 3 – EV + base (B); 4 – EV + new combined investigated medicinal product with anti-inflammatory and antimicrobial effect in the form of vaginal suppositories (ID); 5 – EV + reference medicinal product neo-penotran in the form of vaginal suppositories (RP). The investigated medicinal product contained ibuprofen, clotrimazol and metronidazol in the form of suppositories. neo-penotran contained miconazol and metronidazol. Both medicinal products included the identical base: witepsol. 5 days after modeling EB a histological examination of rats' vagina was carried out.

While modeling EB in 2 group of animals an evident atrophy of vaginal mucosa epithelium, desquamation of the areas of dead mucosa and an evident inflammatory reaction (macrophage and neutrophil infiltration). In groups 4 and 5 the drugs' effect was manifested as rejection of a dead layer into the organ lumen. A statistically significant reduction of structural changes in the vaginal wall (total index, epithelial thickness) was detected in group 4 and there was no true difference in group 5.

The investigated medicinal product's effect consisted in a faster rejection and elimination of the damaged epithelium and a formation of favorable conditions for the reconstructive process. A conclusion about the absence of the drug influence in group 5 was made, because an increase of the average epithelial thickness resulted from a mucosal edema, but not from an activation of reconstructive processes.

Keywords: experimental vaginitis, suppositories, ibuprofen, metronidazol, clotrimazol, morphometry.

Резюме

Исследование проведено на 35 крысах-самках линии Vistar весом 200–220 г. Животные были разделены на 5 групп: 1 – интактные животные, контроль; 2 – животные с моделируемым экспериментальным вагинитом (ЭВ); 3 – ЭВ + основа; 4 – ЭВ + новый комбинированный исследуемый препарат с противовоспалительной и противомикробной активностью в виде вагинальных суппозиториев; 5 – ЭВ + референтный препарат нео-пенотран в виде вагинальных

суппозиториев. Исследуемый препарат содержал ибупрофен, клотримазол и метронидазол в виде суппозиториев. Нео-пенотран в своем составе имеет миконазол и метронидазол. Оба лекарственных средства включают идентичную основу – витепсол. Через 5 дней с момента моделирования ЭВ было проведено гистологическое исследование влагалища крыс.
При моделировании ЭВ во 2-й группе животных наблюдали выраженную атрофию эпителия слизистой оболочки влагалища, десквамацию участков поврежденной слизистой оболочки и выраженную воспалительную реакцию (инфильтрация макрофагов и нейтрофилов).
В 4-й и 5-й группах влияние препаратов отразилось в виде отторжения погибшего слоя эпителия в просвет органа. Статистически значимое уменьшение структурных изменений в стенке влагалища (total index, epithelial thickness) установлено в группе 4 и не имело достоверной разницы в группе 5.
Действие исследуемого препарата заключалось в более быстром отторжении и элиминации поврежденного эпителия и создании благоприятных условий для восстановительного процесса. Сделан вывод об отсутствии влияния препарата в группе 5, поскольку увеличение средней толщины эпителия было обусловлено отеком слизистой оболочки, а не активацией восстановительных процессов.
Ключевые слова: экспериментальный вагинит, суппозитории, ибупрофен, метронидазол, клотримазол, морфометрия.

■ INTRODUCTION

Atrophic vaginitis is common in postmenopausal women. This pathological condition is observed in approximately 50–70% female patients [1], what has negative influence on their quality of life [2]. It has been proved that the reduction of estrogen level inhibits physiological processes in vaginal epithelium, which manifest as blood flow reduction, glandulae cervicales uteri secretion reduction [3] and lack of physiological vaginal moisture. The loss of glycogen and the lack of lactobacteria which transform glycogen into lactic acid to support normal vaginal pH in the range 3,5–4,5 leads to pH elevation up to 5,0–7,5 [1]. Atrophic alterations of mucosa, its thickness reduction give rise to vaginal infections, because an alterated mircomedium enables a reproduction of pathogenic microflora, such as Gardnerella, Prevotella, Streptococcus, Atopobium, Candida, Anaerococcus, Sneathia, Aerococcus, Peptoniphilus, Dialister and Finegoldia, while physiological microflora (Lactobacillus) is lost [4,5]. An inflammatory process progresses being affected vaginal mucosa [6]. As the above-mentioned mucosa does not contain its own organized lymphoid tissue, the development of infectious process may be dynamic [7]. Therefore, atrophic vaginitis is the result of structural changes of vaginal mucosa with bacterial inflammation.

Nowadays the agents of local protection of vaginal mucosa affected with different vaginal infections are actively studied [8,9]. Different forms with clotrimazol, imidazol, miconazol, triazol, nitromidazole, metronidazol, tinidazol, clindamyzin are effective in case of mycotic and bacterial etiology [10, 11, 12]. Antimicrobial drugs have direct antimycotic, antibacterial and antiprotozoal effect due to deterioration of cellular wall components' synthesis in microorganisms. In order to stop a local inflammatory process it is proposed to use ibuprofen [13]. The effect of ibuprofen is directed to the inhibition of cyclooxigenase (COX-1 i COX-2), which reduces

the formation of prostaglandins and tromboxan A2, the inflammatory mediators which support the inflammation locally. It is also known than ibuprofen has antimicrobial effect first of all directed to gram-negative stains, Gardnerellavaginalis, as well as Candida albicans [13]. It is indicated that ibuprofen has therapeutic advantages comparing to other nonsteroidal antiinflammatory drugs (NSAD) and is used for the development of complex drugs, particularly for the treatment of vaginitis [14]. These characteristics have made it possible to offer a potential medicinal product which has anti-inflammatory and antimicrobial effect due to the presence of ibuprofen, metronidazol and clotrimazol, for the treatment of vaginites of different etiology, in order to reduce the risk of adverse effects caused by polypragmasy.

■ PURPOSES OF RESEARCH

To study the effecacy of a new potential combined medicinal product in the form of vaginal suppositories on a model of experimental vaginitis and to compare its efficacy with the selected reference medicinal product neo-penotran.

■ MATERIALS AND METHODS

The study has been carried out in 35 female Vistar rats weighing 200–220 g. A model of experimental vaginitis (EB) reconstructed in the way of introduction of a tampon moistened with 10% silver nitrate solution into the vagina and its exposition for 5 minutes [15]. As the study results demonstrated, the vaginal introduction of 10% silver nitrate solution causes burns and necrosis of mucosal surface layer. Beginning from the next day after the model reconstruction, the investigated medicinal products were administered in the investigated groups of rats: 1) the base – witepsol; 2) the investigated potential medicinal product; 3) the reference medicinal product. The investigated medicinal product contained ibuprofen, clotrimazol and metronidazol in the form of suppositories. Neo-penotran contained miconazol and metronidazol. Both medicinal products included the identical base: witepsol. The period of administration was 5 days. When the experiment had finished, the animals were removed from the trail in accordance with the ethical principles of experiments in animals approved by I National Congress on Bioethics (Kyiv, 2000), which are consistent with the terms and conditions of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes.

The vagina was isolated for a histological examination. The investigated samples were fixed in 10% formalin solution on a cold phosphate buffer. The period of fixation was 24 hours. The fixed samples were rinsed with running water, desiccated with ascending concentrations of ethanol and placed into paraplast (Leica Surgipath Paraplast Regular). Dehydration protocol: ethanol (from 70% to 100% ethanol solution), dioxane, xylol, xylol/papaplast (1:1), paraplast. The blocks were aligned in a way which made it possible to study the whole organ surface with the cavity in a projection of a histological section. Paraffin sections 5 μm thick were obtained on the microtome ThermoMicrom HM 360 RotaryMicrotome. The sections were deparaffinized, rehydrated and dyed with hematoxylin-eosin. The microslides were examined with the use of a microscope Olympus BX 51. Microphotos were

Table 1
Measurement scale of vaginal structural changes (Index)

Group	Index				Points Me [Q1–Q2]
	Mucosal edema	Hyperemia of mucosa vessels	Hemorrhage	Infiltrates	
1	–	–	–	–	0
2	n	N	n	n	Σn
3	n	N	n	n	Σn
n	n	N	n	n	Σn

taken from randomly selected areas of mucosa. A morphometric analysis was carried out with the use of the software CarlZeiss (AxioVision SE64 Rel.4.9.1) with an increase × 400. A morphometric assessment consisted in mucosal thickness measurement based on two points: first – an epithelial contour wrapped around the organ lumen, second- an external epithelial contour which touches the submucous layer. 10 values were measured from each section and they were averaged in the group. The thickness was measured in μm.

To assess general organ morphological changes an analysis of organ changes was carried out according to the criteria: mucosal edema, hyperemia of mucosal vessels, hemorrhages, infiltrates. Each criterion was assessed by points: 1 point – changes were detected in <25% of total areas, 2 points – changes were detected in 25–50% of sample area, 3 points – changes were detected in 50–75% of sample area. According to the results of the assessment the total points were taken in accordance with the criteria as indicated in table 1.

The statistic assessment was carried out with the use of non-parametric methods. Kolmogorov – Smirnov's test was used to determine the normality of division of the data sample. The intergroup difference was determined according to a non-parametric Kruskal – Wallis test. The results of morphometric examination were given in the form of median and quartile intervals [Q1–Q3]. The data samples were analyzed with the use of the software OriginLab version 8.0.

■ RESULTS

Of histological examination confirmed structural changes in vaginal mucosa and submucous layer. Model EB was characterized with a significant grade of destructural changes of the epithelial layer, desquamation of the dead mucosa areas, submucous layer "exposure" and an evident inflammatory reaction. The inflammation had such structural manifestations as focal or diffuse-focal infiltration of macrophages and neutrophils, what is a manifestation of an immune aggression towards the bacterial infection. The main structural changes of mucosa in atrophic vaginitis are shown on figure 1. In certain cases infiltrative events were detected in organ lumen.

In the group with EV+B the rejection of epithelium damages with silver was revealed, what was manifested as high level of conservation of the mucosa. The structural changes of the epithelium were polymorphous: loss and cornification of surface layers, leucocyte infiltration. Vascular hyperemia,

Table 2
Results of histological and morphometric evaluation of vaginal structural changes on the model of atrophic vaginitis

№	Group	Model of experimental vaginitis (EV)			
		Points		Mucosal thickness, μm	
		Me [Q1–Q2]	p-values	Me [Q1–Q2]	p-values
1	Control	0	–	161,5 [118,0–209,7]	–
2	EV	9 [7–10]	P1–2<0,05	100,6 [74,0–124,9]	P1–2<0,05
3	EV+B	7 [6–7,5]	P1–3<0,05 P2–3>0,05	149,9 [127,5–176,1]	P1–3>0,05 P2–3<0,05
4	EV+ID	4 [4–6]	P1–4<0,05 P2–4<0,05 P3–5<0,05	174,8 [126,7–203,4]	P1–4>0,05 P2–4<0,05
5	EV+RP	5 [4,5–8]	P1–5<0,05 P2–5>0,05	149,2 [109,1–256,0]	P1–5>0,05 P2–5<0,05

focal increase of phybroblast thickness and leucocyte infiltration were detected in submucous layer. The morphometric examination revealed a reduction of mucosa lesion, what was represented as an increase of epithelial thickness concerning group 2 (149,9 [127,5–176,1] μm vs 100,6 [74,0–124,9] μm, p<0,05 (tabl. 2).

In the group with EV+ID rejection of the dead layer of epitheliocytes into the organ lumen was also detected. The epithelium in a significant extension of the lumen is characterized with multilayer structure, and in certain zones some foci of proliferation were detected, what confirms an activation of reconstructive processes. In the submucous layer the rests of oxygenated silver, some foci of hemosiderin, a moderate blood filling of the vessels and single leucocyte infiltration were detected. A statistically significant reduction of number of points which confirms the grade of structural changes in the organ wall was determined concerning groups 3 and 4. The epithelial thickness in group 4 was 174,8 [126,7–203,4] μm, what is statistically more significant than in group 2 (p<0,05). At the same time, the obtained values had no difference vs the control group (p>0,05). The obtained results were assessed as a manifestation of the protective effect of the investigated medicinal product, which manifested on structural level as fast rejection and elimination of the damaged epithelium and creation of favourable conditions for the reconstructive process.

In the group with EV+RP an evident local infiltration and partial rejection of the damaged epithelium were detected. According to the results of morphometric analysis a conclusion about the absence of pharmacological impact was made, due to the fact that an increase of the average epithelial thickness was conditioned with mucosal edema, but not with an activation of reconstructive processes. It is also confirmed with the loss of surface epithelial layers. The focal immune reaction was assessed as 1 point, because the lesion area was less than 1/3 of the investigated organ lumen, but these changes had a significant impact on the development of structural changes of mucosal epithelium.

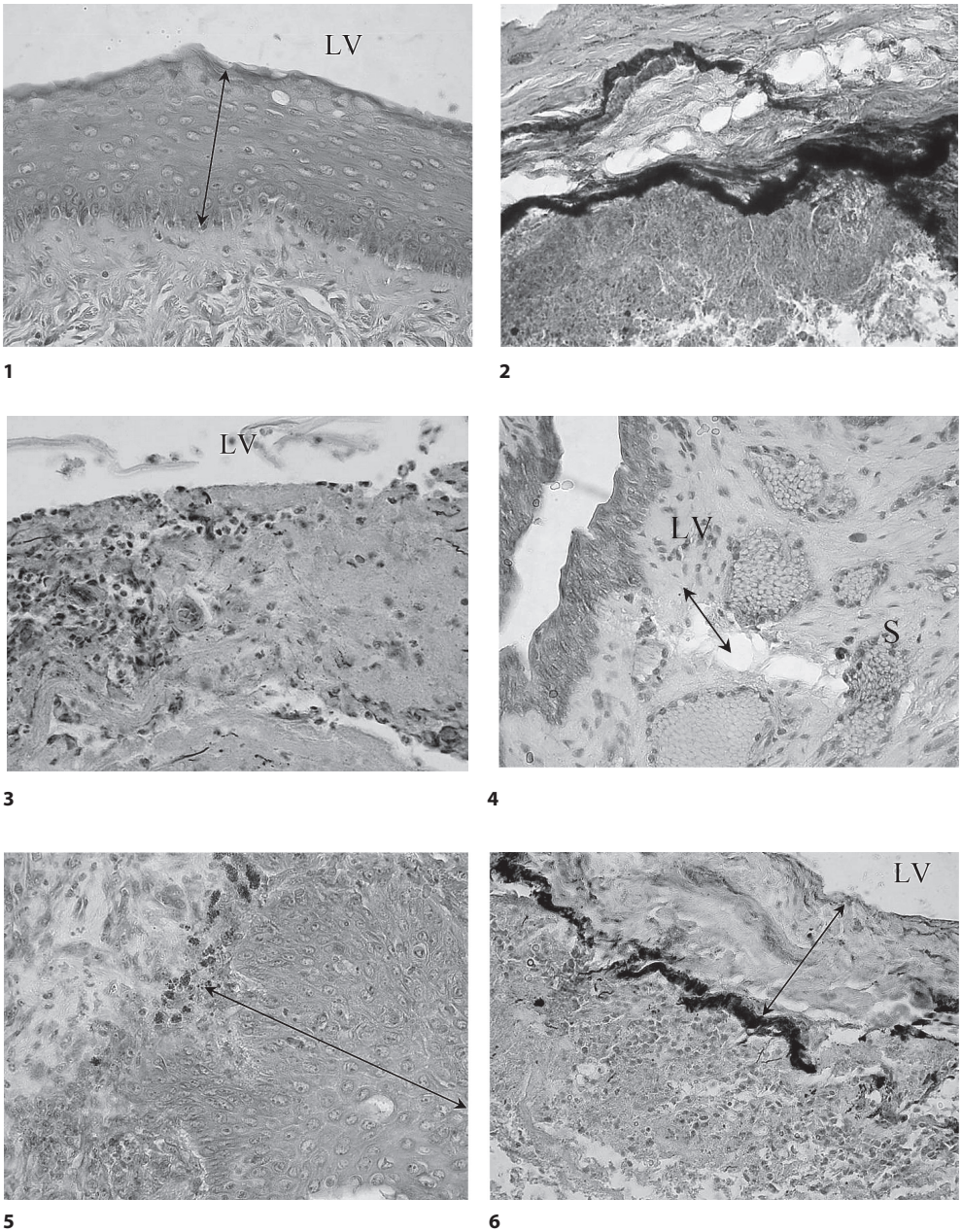

Fig. 1. Histological alteration of vaginal mucosa in control and experimental groups on the model of atrophic vaginitis (EV): 1 – control: unaffected epithelium; 2 – EV: dystrophic alteration of mucosal epithelium (dark inclusions – oxygenated silver); 3 – EV: epithelium desquamation; 4 – EV+base: partially conserved epithelium, accumulation of hemosiderin in the submucous layer; 5 – EV+ID: significant conservation of epithelium, accumulation of hemosiderin; 6 – EV+RP: dystrophic mucosal alterations

Notes: VL – vaginal lumen; ↔ mucosal thickness; S – stasis, vascular hyperemia. Hematoxylin with eosin, × 400.

■ DISCUSSION

Based on the exposed histological and morphometric examinations which have been carried out, some conclusions concerning the particularities of an inflammatory process development were made. In case of an experimental pathology a significant atrophic mucosal lesion and an evident inflammatory reaction are observed, what is not limited only with the site of silver nitrate administration and it covers the submucous layer and the muscular membrane. The topical administration of the investigated medicinal products had an impact on partial conservation of mucosa and favoured the reconstructive processes. The pharmacological effect of the medicinal products favoured an elevation of the damaged tissue rejection and an activation of epithelial regeneration. A comparative analysis confirmed the presence of pharmacological effect of the investigated medicinal product, and the use of the base was assessed as prevention from progressive epithelial dystrophy and a tendency to a reconstructive process.

A therapeutic effect of ibuprofen for treatment of vaginites is indicated in other studies [16, 17]. Moreover, the relief of vaginitis symptoms (vaginal pruritus, pain, burning, erythema and redness) was effective comparing to bendizamin, clotrimazol. The medicinal products are well-tolerated, safe, but high doses of metronidazol may cause adverse reactions [18]. It is expected, that suppositories with antimicrobial agents in a complex with ibuprofen may be characterized with high efficacy and low probability of adverse consequences in case of vaginal administration [19]. The authors made a conclusion that the use of topical medicinal products makes unnecessary a systemic therapy.

There have been some reports on stable strains of certain pathogenic agents of vaginitis to the medicinal products. In such cases high doses of medicinal products are usually used, what may cause an adverse effect [20]. In other reports it is exposed that high-dose intravaginal combinations with metronidazol are well-tolerated and demonstrate an evident therapeutic efficacy during the treatment [21]. Upon such conditions an additional inclusion of ibuprofen suppositories may have positive effect concerning the reduction of adverse effects and the inhibition of inflammation. In the own studies the base itself did not have any protective impact on vaginal epithelial atrophy, although it was supposed that an additional moisture with the suppository base may facilitate the elimination of dead epithelium and prevent from submucous layer lesion in the area of vaginal wall.

■ CONCLUSIONS

1. The intravaginal administration of 10% silver nitrate solution caused a necrotic lesion of vaginal mucosa in rats on the 6th day of the experiment, this lesion covered the submucous and the muscular layer as well.
2. The administration of witepsol as a base in the form of suppositories prevented from progressive epithelial dystrophy and had a tendency to reconstructive process. A morphometric investigation detected the reduction of the mucosal lesion, what manifested as an increase of epithelium thickness 149,9 [127,5–176,1] μm, vs a control pathology 100,6 [74,0–124,9] μm.

3. The administration of a potential investigated medicinal product favoured the activation of reconstructive processes due to the presence of the foci of proliferation, the reduction of the grade of pathological and structural alterations in the vaginal wall. In the above-mentioned experimental conditions the potential combined medicinal product was more effective as a therapy comparing to the reference agent, according to the results of morphometric analysis. It has been established that a statistically significant increase of epithelium thickness in the group of investigated medicinal product was 174,8 [126,7–203,4] μm vs 149,2 [109,1–256,0] μm in reference medicinal product. The obtained results evidence the expediency of further deep study of the investigated medicinal product in the form of vaginal suppositories as a prospective combined medicinal product for the treatment of inflammatory diseases of female reproductive organs.

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