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Experimental Study of the Effect of Serum Hydrogen Sulfide on the Course of the Inflammatory Process in the Vaginal Wall

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Various pathological conditions are characterized by the influence of hydrogen sulfide level on the course of the pathological process. This study examines the effect of serum hydrogen sulfide levels on the inflammatory process in the vaginal wall of rats.
Aims: To evaluate the effect of excess and deficiency of serum hydrogen sulfide on the course of the inflammatory process in the vaginal wall of rats.
Methodology: The study was performed on 125 female Wistar rats under 1 year of age and weighing 160.0 to 200.0 grams. All animals were divided into 7 groups: control (intact rats) and 6 experimental groups with different H_2S levels and different treatment approaches of inflammation in the vaginal wall. The level of serum hydrogen sulfide was studied and the levels of TNF-α and IL-1β in the tissue homogenate of the vaginal wall were determined. In all experimental groups, the study was performed in dynamics - 10 min, 4, 8 and 24h after simulation of inflammation.
Results: The dynamics of local levels of TNF-α and IL-1β in all groups had a similar trend and was characterized by the rapid development of the inflammatory process from its simulation to 4 hours of study, followed by gradual attenuation of inflammation and almost complete normalization of the studied indicators for 24 hours. Preliminary serial introduction of sodium hydrosulfide, as a donor of hydrogen sulfide, allowed to reduce the degree of manifestation of the inflammatory process and to achieve faster normalization of the studied parameters. At the same time, the artificially created deficiency of serum hydrogen sulfide (previous serial administration of propargylglycine) prolonged the duration and increased the studied indicators of inflammation in the vaginal wall.
Conclusions: The course and intensity of the inflammatory process in the vaginal wall of rats are directly dependent on the background level of serum hydrogen sulfide.

Keywords: Hydrogen sulfide; vaginal wall; inflammation; TNF-α; IL-1β; clindamycin phosphate; rats.

1. INTRODUCTION

Despite the constant progress in clinical microbiology and pharmacology of antibacterial drugs, inflammatory diseases of the lower genital tract continue to occupy a leading place in the structure of obstetric and gynecological pathology. The most significant of these is bacterial vaginosis [1,2].

Inflammatory diseases of the vagina are a serious health problem for women of childbearing age, their children and partners, as they are associated with an increased risk of pelvic inflammatory disease, increased miscarriages and premature births, and an increased risk of human immunodeficiency virus infection [3,4].

The social and practical significance of this pathology encourages the search for new mechanisms of control and modulation of the inflammatory process in the lower parts of the genital tract.

One of the little-studied modulators of the inflammatory process is hydrogen sulfide. In 1777, the Swedish chemist Carl Wilhelm Scheele first synthesized hydrogen sulfide (H_2S). Since then, our ideas about this compound have undergone a significant transformation. From the beginning, this compound was considered only as a highly toxic exogenous protein breakdown product [5]. The knowledge accumulated over 2.5 centuries allows to take away hydrogen sulfide an important role among endogenous factors in maintenance of homeostasis. Today, hydrogen sulfide together with nitrogen monoxide (NO) and carbon monoxide (CO) belong to the family of gas transmitters. Hydrogen sulfide is produced by cystathionine-β-synthase, cystathionine-γ-lyase and 3-mercapto-pyruvate-sulfur-transferase in mammalian cells [6].

Hydrogen sulfide plays an important role in the regulation of vascular tone, angiogenesis, neuromodulation, cytoprotection, inflammation, apoptosis, oxidative stress, allergic reactions [6,7,8].

For many years, various studies have indicated the role of hydrogen sulfide in the inflammatory process. Reactive oxygen species from activated neutrophils can oxidize H_2S to form sulfite, which further enhances leukocyte adhesion regulation and neutrophil function by activating beta-integrin Mac-1 (CD11b / CD18) and Ca^{2+}/calmodulin dependent protein kinase, respectively [9]. In addition, hydrogen sulfide has been shown to provoke short-term granulocyte survival by inhibiting the cleavage of caspase-3 and

mitogen-activated protein kinase p38 (MAPK) and, therefore, to promote bactericidal activity of neutrophils [10].

It is proved that various pathological conditions can be characterized by a decrease or increase in the basal content of H_2S in blood plasma. However, the level of hydrogen sulfide itself can modulate the course of pathological processes [11].

At present, in the scientific literature there is almost no information on the peculiarities of gynecological inflammatory diseases, in particular inflammatory diseases of the vagina, in conditions of excess and deficiency of serum hydrogen sulfide.

The aim of the study – to evaluate the effect of excess and deficiency of serum hydrogen sulfide on the course of the inflammatory process in the vaginal wall of rats.

2. MATERIALS AND METHODS

The experimental study was performed on the basis of a research laboratory of preclinical study of pharmacological substances of National Pirogov Memorial Medical University, Vinnytsya.

All experiments were performed in accordance with the "Regulations on the use of animals in biomedical experiments" with the permission of the Bioethics Committee and in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes". The study protocol was approved by the Committee on Bioethics, National Pirogov Memorial Medical University, Vinnytsya, Vinnytsia, Ukraine (Protocol Nr. 9, October 25th 2021).

The study included 125 female Wistar rats under 1 year of age and weighing 160.0 to 200.0 grams (181.5 ± 12.4 grams).

All rats were randomly divided into 6 groups:

1. Control group (CG, n = 5) - intact animals.
2. Experimental group 1 (EG 1, n = 20) - inflammation in the vaginal wall.
3. Experimental group 2 (EG 2, n = 20) - excess hydrogen sulfide + inflammation in the vaginal wall.
4. Experimental group 3 (EG 3, n = 20) - hydrogen sulfide deficiency + inflammation in the vaginal wall.
5. Experimental group 4 (EG 4, n = 20) - inflammation in the vaginal wall + intravaginal administration of clindamycin phosphate in the form of suppositories.
6. Experimental group 5 (EG 5, n = 20) - excess hydrogen sulfide + inflammation in the vaginal wall + intravaginal administration of clindamycin phosphate in the form of suppositories.
7. Experimental group 6 (EG 6, n = 20) - hydrogen sulfide deficiency + inflammation in the vaginal wall + intravaginal administration of clindamycin phosphate in the form of suppositories.

Groups with intravaginal administration of clindamycin phosphate in the form of suppositories were included in the study because this antibiotic is the drug of choice and an integral part of the comprehensive treatment of inflammatory diseases of the vagina [12,13]. Although clindamycin phosphate does not have a local irritant or anti-inflammatory effect [14], its effect on the vaginal wall in conditions of excess or deficiency of hydrogen sulfide requires careful study.

The data obtained during the study of the effect of excess and deficiency of hydrogen sulfide on intact vaginal tissues, as well as in the conditions of additional intravaginal administration of clindamycin phosphate are described by us in the previous work [15], and therefore are not given here.

To synchronize estrous cycles, all test animals were injected subcutaneously with 17α-hydroxy-6α-methylprogesterone (Pfizer Inc., USA), diluted in Ringer's solution lactate at a dose of 12 mg per test animal, 7 and 3 days before the study date [16].

The dose of 12 mg per experimental rat was determined by recalculating the dose of the drug for mice. The dose was recalculated according to the method proposed by Anroop B. Nair and Shery Jacob [17].

The synchronization of estrous cycles was checked immediately before the start of a set of manipulations to study the condition of the vaginal wall, by microscopy of smears from the vagina of experimental animals according to the criteria described by Fu X.-Y. and co-authors [18].

Excess hydrogen sulfide in animals was created by intraperitoneal administration of the donor H_2S

- sodium hydrosulfide (Sigma-Aldrich, USA) at a dose of 1.5 mg/kg on 0.1 M phosphate buffer (pH 7.4), as a freshly prepared aqueous solution at the rate of 0.1 ml per 100 g of rat weight, once a day for 5 days immediately preceding the date of the study of the condition of the vaginal wall [19].

Hydrogen sulfide deficiency was created by intraperitoneal administering a specific inhibitor of cystathionine-γ-lyase - D, L-propargylglycine (Sigma-Aldrich, USA) at a dose of 50 mg/kg as a freshly prepared 5% aqueous solution at the rate of 0.1 ml per 100 g of rat weight. 1 time per day for 5 days immediately preceding the date of the study of the vaginal wall [19].

Clindamycin phosphate (Pfizer Inc., USA) was administered to rats intravaginally as micro-suppositories. The dose of the drug according to the conversion tables was 1.5 mg [18]. Given that the suppository contains 100 mg of active substance and weighs 2.5 grams, and based on the fact that the active substance is distributed evenly in the suppository, to provide an equivalent dose (1.5 mg of clindamycin phosphate) we formed micro-suppositories weighing 37.5 mg.

Inflammation of the vaginal wall was simulated by intravaginal administration of 4% nonoxynol-9 (Amcapharm GmbH, Germany) in the form of micro-suppositories. The dose of the drug according to the conversion tables was 1.8 mg [18]. Given that the suppository contains 120 mg of active substance and weighs 3.0 grams, and based on the fact that the active substance is distributed evenly in the suppository, to provide an equivalent dose (1.8 mg of nonoxynol-9) we formed micro- suppositories weighing 45 mg.

The study of the condition of the vaginal wall in the control group was performed once, and in all experimental groups was performed in dynamics - after 10 min, 4, 8 and 24h, according to the method of cervicovaginal toxicity and inflammation of local intravaginal dosage forms described by Catalone and co-authors [16] and adapted by us for research on laboratory rats.

In each group and at each time point, the condition of the vaginal wall was studied in 5 rats.

To assess the condition of the vaginal wall, the levels of TNF-α and IL-1β in the tissue homogenate were determined.

Blood sampling to determine the level of hydrogen sulfide was performed by percutaneous puncture of the heart under ketamine anesthesia at the rate of 0.22 ml of ketamine per 100g of body weight of the experimental animal. The content of hydrogen sulfide in the serum was determined by spectrophotometric method in the reaction between sulfide anion and para-phenylenediamine hydrochloride in an acidic environment in the presence of iron ions (III) [19].

After blood collection, rats were removed from the experiment by translocation of the cervical vertebrae.

The vagina was removed and washed from the remnants of blood in saline, followed by careful drying of the tissues with a sterile napkin. The removed drug was cut in the longitudinal direction into 4 equal parts. One fragment was homogenized by adding 1 ml of phosphate buffer in an automatic tissue disaggregator Medimax (CTSV, Italy) using disposable Medicon cartridges (Becton Dickinson, USA) with pyramidal blades of 35 μm. The tissue homogenate was then filtered through Filcon filters (Becton Dickinson, USA) with a pore diameter of 30 μm.

The levels of TNF-α and IL-1β in the vaginal tissue homogenate filtrate were investigated by enzyme-linked immunosorbent assay using the Rat TNF-α ELISA kit and the Rat IL-1β ELISA Kit (CUSABIO, China).

The obtained data were processed using the statistical software package SPSS 20.0 for Windows.

3. RESULTS

In all groups and at all times of the study, no behavioral changes were observed in experimental animals. All rats maintained normal motor activity. Consumption of feed and water met the standards for this species.

Evaluation of the synchronization of estrous cycles showed the presence of the same changes in the microscopic examination of vaginal swabs. The smears showed a small number of cells, with a predominance of small epitheliocytes with nuclei and almost complete absence of neutrophils, large epitheliocytes with nuclei and non-nuclear keratinized epitheliocytes. According to the criteria described by Fu X.-Y.

and co-authors of [18] in our study identified this picture as that characteristic of proestrus.

Levels of serum hydrogen sulfide in groups of experimental animals are shown in Fig. 1.

The level of serum hydrogen sulfide in the control group was 76.08 ± 4.94 μMol/L. In EG 1 and 4, where the excess or deficiency of hydrogen sulfide was not modeled, the levels of hydrogen sulfide did not differ significantly (*P*= .89 and *P*= .99, respectively) from the control group.

In EG 2 and 5, where an excess of hydrogen sulfide was artificially created, this indicator significantly (*P*= .006) increased by 16.3% and 16.9%, respectively, compared with the control group. In EG 3 and 6 (hydrogen sulfide deficiency) there was a significant (*P*=.002) decrease in serum H_2S by 22.08% and 21.6%, respectively. There was no significant difference between experimental groups 2 and 5, as well as between experimental groups 3 and 6.

The levels of TNF-α and IL-1β in the control group, as well as the dynamics of changes in these indicators in experimental groups 1, 2 and 3 are shown in the diagrams (Figs. 2 and 3).

Figs. 2 and 3 show that in all groups there was a similar dynamics of changes in the studied indicators with their increase and peak at 4 hours and subsequent decline. Thus, in all groups for 4 hours of the study there was a significant increase in the levels of TNF-α and IL-1β, compared with similar indicators 10 min after simulation of inflammation in the vaginal wall (*P*= .01 and *P*= .005 for EG 1; *P* = .002 and *P* = .002 for EG 3, *P* = .04 and *P*= .02 for EG 2, for TNF-α and IL-1β respectively).

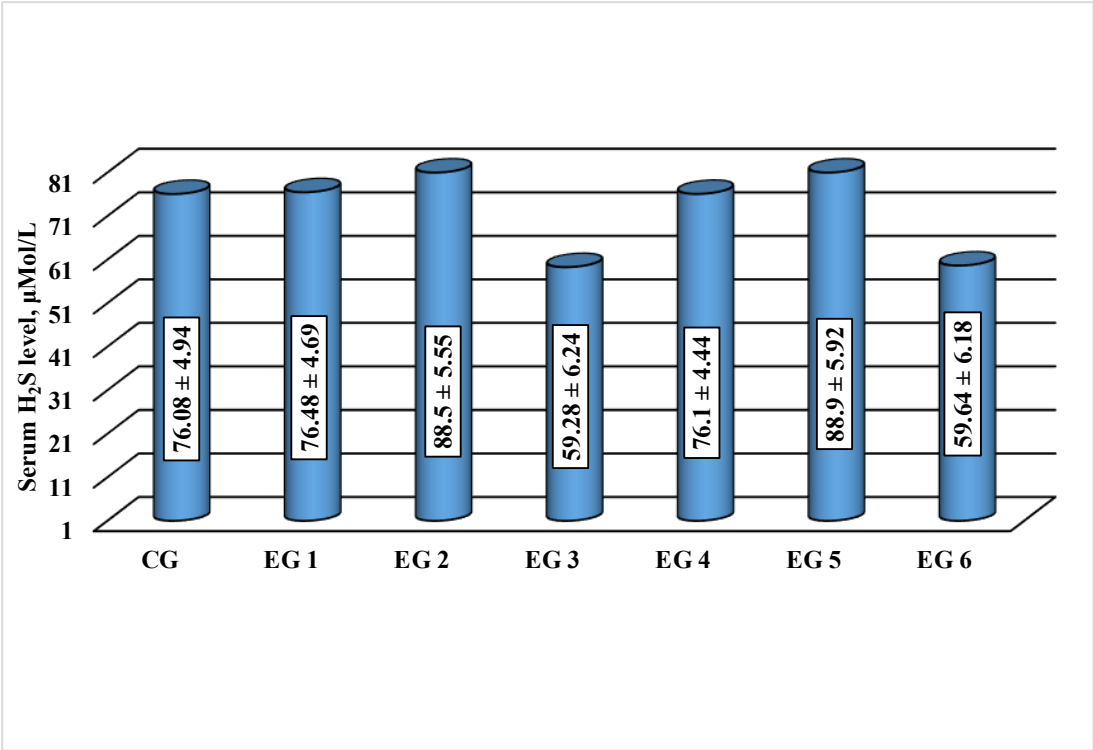

Fig. 1. Serum hydrogen sulfide levels in groups of experimental animals

CG - Control group (n=5); EG 1 - Experimental group 1 (n=5); EG 2 - Experimental group 2 (n=5); EG 3 - Experimental group 3 (n=5); EG 4 - Experimental group 4 (n=5); EG 5 - Experimental group 5 (n=5); EG 6 - Experimental group 6 (n=5).

P-value:

CG compared to EG 1 – P=.899; CG compared to EG 2 – P=.006; CG compared to EG 3 – P=.002; CG compared to EG 4 – P=.995; CG compared to EG 5 – P=.006; CG compared to EG 6 – P=.002; EG 1 compared to EG 2 – P=.006; EG 1 compared to EG 3 – P=.001; EG 1 compared to EG 4 – P=.899; EG 1 compared to EG 5 – P=.006; EG 1 compared to EG 6 – P=.001; EG 2 compared to EG 3 – P=.001; EG 2 compared to EG 4 – P=.005; EG 2 compared to EG 5 – P=.915; EG 2 compared to EG 6 – P=.001; EG 3 compared to EG 4 – P=.001; EG 3 compared to EG 5 – P=.001; EG 3 compared to EG 6 – P=.929; EG 4 compared to EG 5 – P=.005; EG 4 compared to EG 6 – P=.001; EG 5 compared to EG 6 – P=.001

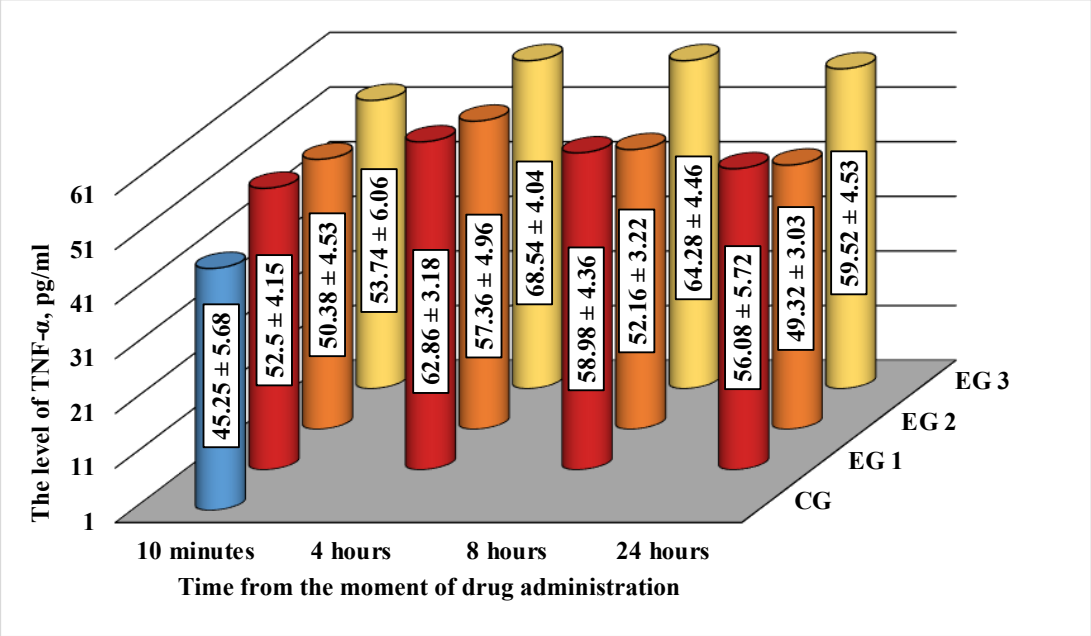

Fig. 2. Dynamics of changes in TNF-α levels in the vaginal wall of intact rats and rats with local inflammation under conditions of different levels of serum hydrogen sulfide

CG - Control group (n=5 in each time point); EG 1 - Experimental group 1 (n=5 in each time point); EG 2 - Experimental group 2 (n=5 in each time point); EG 3 - Experimental group 3 (n=5 in each time point); EG 4 - Experimental group 4 (n=5 in each time point); EG 5 - Experimental group 5 (n=5 in each time point); EG 6 - Experimental group 6 (n=5 in each time point).

P-value:

For EG 1: 10 min compared to 4 hrs – P=.002; 10 min compared to 8 hrs – P=.043; 10 min compared to 24 hrs – P=.29; 4 hrs compared to 8 hrs – P=.147; 4 hrs compared to 24 hrs – P=.108; 8 hrs compared to 24 hrs – P=.649.

For EG 2: 10 min compared to 4 hrs – P=.049; 10 min compared to 8 hrs – P=.494; 10 min compared to 24 hrs – P=.675; 4 hrs compared to 8 hrs – P=.085; 4 hrs compared to 24 hrs – P=.023; 8 hrs compared to 24 hrs – P=.184.

For EG 3: 10 min compared to 4 hrs – P=.002; 10 min compared to 8 hrs – P=.014; 10 min compared to 24 hrs – P=.126; 4 hrs compared to 8 hrs – P=.152; 4 hrs compared to 24 hrs – P=.02; 8 hrs compared to 24 hrs – P=.171

For 10 min time point: CG compared to EG 1 – P=.05; CG compared to EG 2 – P=.153; CG compared to EG 3 – P=.052; EG 1 compared to EG 2 – P=.462; EG 1 compared to EG 3 – P=.715; EG 2 compared to EG 3 – P=.35.

For 4 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.007; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.07; EG 1 compared to EG 3 – P=.039; EG 2 compared to EG 3 – P=.004.

For 8 hrs time point: CG compared to EG 1 – P=.003; CG compared to EG 2 – P=.045; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.023; EG 1 compared to EG 3 – P=.094; EG 2 compared to EG 3 – P=.001.

For 24 hrs time point: CG compared to EG 1 – P=.017; CG compared to EG 2 – P=.195; CG compared to EG 3 – P=.002; EG 1 compared to EG 2 – P=.048; EG 1 compared to EG 3 – P=.323; EG 2 compared to EG 3 – P=.003.

Although, in all groups there was a progressive decrease in the number of studied markers of inflammation over time, but statistically significant differences in each indicator between adjacent study periods (4 and 8 hours; 8 and 24 hours) were not confirmed. At the same time, in all groups there were differences between the indicators for 4 and 24 hours of the study.

When comparing the levels of TNF-α and IL-1β in experimental groups 1, 2, 3 with each other at each time point, as well as with the control group, the following data were obtained.

After 10 minutes after modeling the inflammatory process in the vaginal wall, there was a slight increase in the levels of the studied indicators in all groups, but no significant differences between the groups were found.

After 4 h, in all groups there was a peak growth of TNF-α and IL-1β in the vaginal wall. The indicators of the experimental groups significantly exceeded the indicators of the control group (*P*< .001 for EG 1 and 3; *P*= .007 for EG 2). Although at this time of the study, the indicators of EG 2 and 3 were statistically significantly different (*P*=

.005), but significant differences with EG 1 were not demonstrated.

8 h after modeling of inflammation in all experimental groups there were elevated values relative to the control group (*P*=.003 for EG 1; *P* = .04 for EG 2; *P*< .001 for EG 3). At this time of the study, the statistical differences between EG 2 and 3 were even greater *P*< .001. It should be noted that at this time of the study there were significant differences between the indicators of EG 2 and EG 1 - the indicators of EG 2 were significantly lower (*P*= .04) than the indicators of EG 1. For indicators of EG 3 such a trend was not observed.

24 h after modeling of inflammation in EG 1 and 3, elevated values were maintained relative to the indicators of the control group (*P*= .02 for EG 1; *P*= .002 for EG 3). The indicators of EG 2, although slightly higher than those of the control group, but statistically the differences between

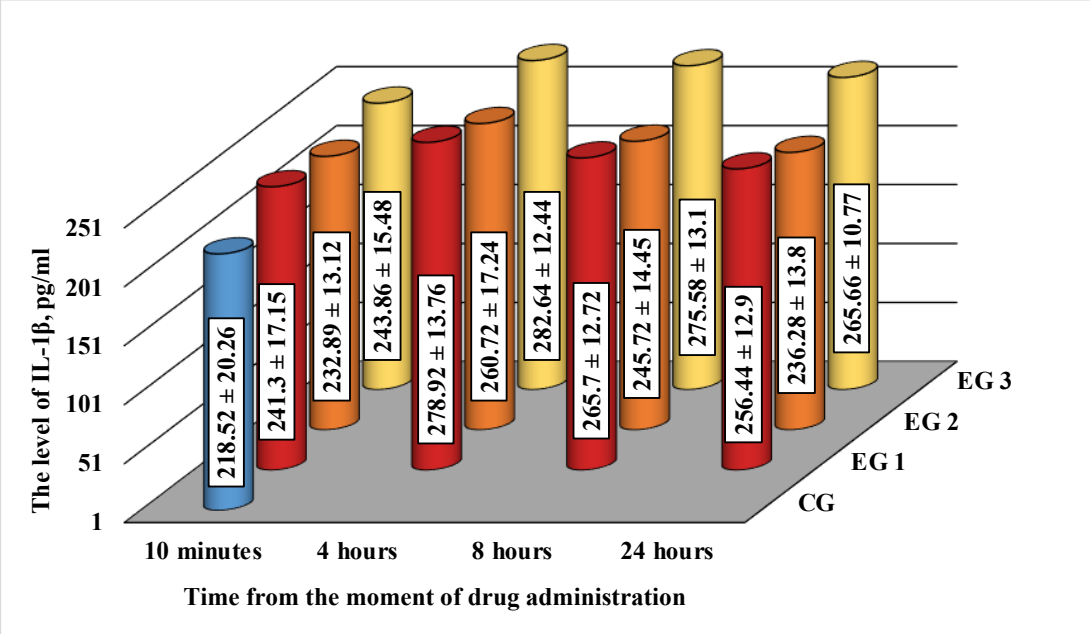

Fig. 3. Dynamics of changes in IL-1β levels in the vaginal wall of intact rats and rats with local inflammation under different levels of serum hydrogen sulfide

CG - Control group (n=5 in each time point); EG 1 - Experimental group 1 (n=5 in each time point); EG 2 - Experimental group 2 (n=5 in each time point); EG 3 - Experimental group 3 (n=5 in each time point); EG 4 - Experimental group 4 (n=5 in each time point); EG 5 - Experimental group 5 (n=5 in each time point); EG 6 - Experimental group 6 (n=5 in each time point).

P-value:

For EG 1: 10 min compared to 4 hrs – P=.005; 10 min compared to 8 hrs – P=.034; 10 min compared to 24 hrs – P=.153; 4 hrs compared to 8 hrs – P=.153; 4 hrs compared to 24 hrs – P=.025; 8 hrs compared to 24 hrs – P=.196.

For EG 2: 10 min compared to 4 hrs – P=.021; 10 min compared to 8 hrs – P=.18; 10 min compared to 24 hrs – P=.701; 4 hrs compared to 8 hrs – P=.174; 4 hrs compared to 24 hrs – P=.086; 8 hrs compared to 24 hrs – P=.59.

For EG 3: 10 min compared to 4 hrs – P=.002; 10 min compared to 8 hrs – P=.008; 10 min compared to 24 hrs – P=.032; 4 hrs compared to 8 hrs – P=.408; 4 hrs compared to 24 hrs – P=.103; 8 hrs compared to 24 hrs – P=.363.

For 10 min time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.409; EG 1 compared to EG 3 – P=.811; EG 2 compared to EG 3 – P=.261.

For 4 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.102; EG 1 compared to EG 3 – P=.666; EG 2 compared to EG 3 – P=.05.

For 8 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.049; EG 1 compared to EG 3 – P=.261; EG 2 compared to EG 3 – P=.009.

For 24 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.044; EG 1 compared to EG 3 – P=.255; EG 2 compared to EG 3 – P=.006

the groups were not confirmed. The previously described relationships between EG 1 and experimental groups 2 and 3 continued to persist - EG 1 significantly exceeded EG 2 (*P*= .04) and did not differ from EG 3. At this time of the study also disappeared significant differences between experimental groups 2 and 3.

The dynamics of TNF-α and IL-1β levels in rats with local inflammation and the use of clindamycin phosphate at different levels of serum hydrogen sulfide are shown in the diagrams (Figs. 4 and 5).

Trends and dependencies between control group and study groups 4, 5 and 6 completely repeat the trends and dependencies described for control group and study groups 1, 2 and 3. In addition, we did not find any statistical differences between study groups 1 and 4, 2 and 5, as well as 3 and 6.

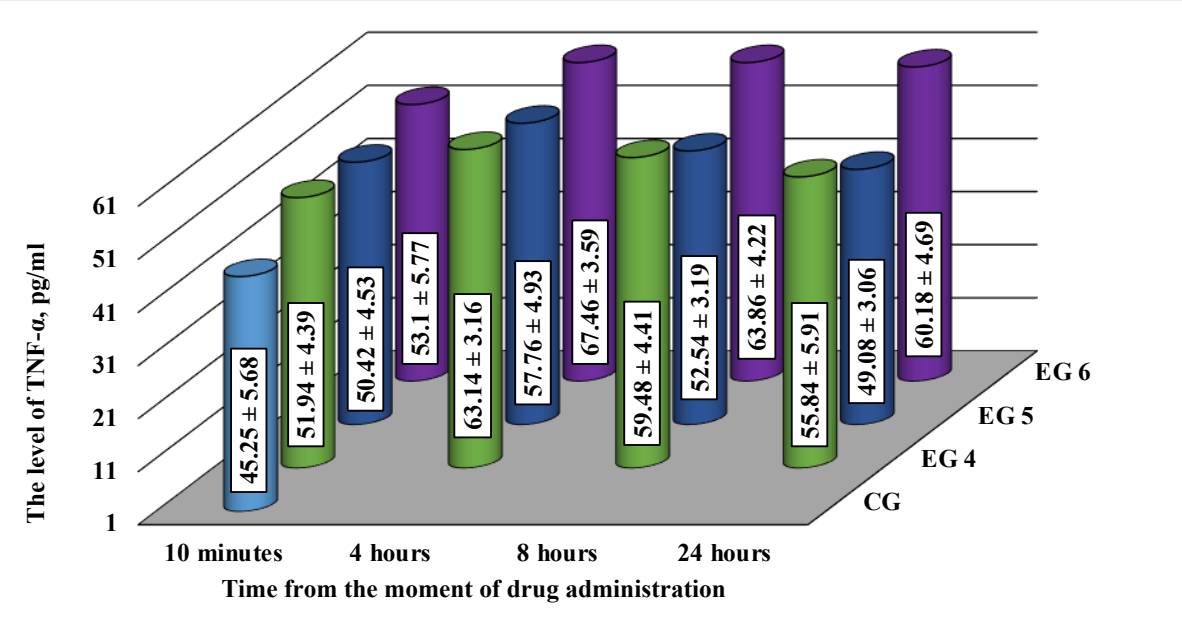

Fig. 4. Dynamics of changes in TNF-α levels in the vaginal wall of intact rats and rats with local inflammation and the use of clindamycin phosphate under conditions of different levels of serum hydrogen sulfide

CG - Control group (n=5 in each time point); EG 1 - Experimental group 1 (n=5 in each time point); EG 2 - Experimental group 2 (n=5 in each time point); EG 3 - Experimental group 3 (n=5 in each time point); EG 4 - Experimental group 4 (n=5 in each time point); EG 5 - Experimental group 5 (n=5 in each time point); EG 6 - Experimental group 6 (n=5 in each time point).

P-value:

For EG 1: 10 min compared to 4 hrs – P=.002; 10 min compared to 8 hrs – P=.027; 10 min compared to 24 hrs – P=.27; 4 hrs compared to 8 hrs – P=.17; 4 hrs compared to 24 hrs – P=.091; 8 hrs compared to 24 hrs – P=.516.

For EG 2: 10 min compared to 4 hrs – P=.04; 10 min compared to 8 hrs – P=.417; 10 min compared to 24 hrs – P=.598; 4 hrs compared to 8 hrs – P=.082; 4 hrs compared to 24 hrs – P=.018; 8 hrs compared to 24 hrs – P=.13.

For EG 3: 10 min compared to 4 hrs – P=.001; 10 min compared to 8 hrs – P=.01; 10 min compared to 24 hrs – P=.066; 4 hrs compared to 8 hrs – P=.184; 4 hrs compared to 24 hrs – P=.044; 8 hrs compared to 24 hrs – P=.278.

For 10 min time point: CG compared to EG 1 – P=.071; CG compared to EG 2 – P=.15; CG compared to EG 3 – P=.062; EG 1 compared to EG 2 – P=.604; EG 1 compared to EG 3 – P=.73; EG 2 compared to EG 3 – P=.438.

For 4 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.006; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.074; EG 1 compared to EG 3 – P=.078; EG 2 compared to EG 3 – P=.007.

For 8 hrs time point: CG compared to EG 1 – P=.002; CG compared to EG 2 – P=.037; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.021; EG 1 compared to EG 3 – P=.147; EG 2 compared to EG 3 – P=.001.

For 24 hrs time point: CG compared to EG 1 – P=.02; CG compared to EG 2 – P=.221; CG compared to EG 3 – P=.002; EG 1 compared to EG 2 – P=.053; EG 1 compared to EG 3 – P=.234; EG 2 compared to EG 3 – P=.002

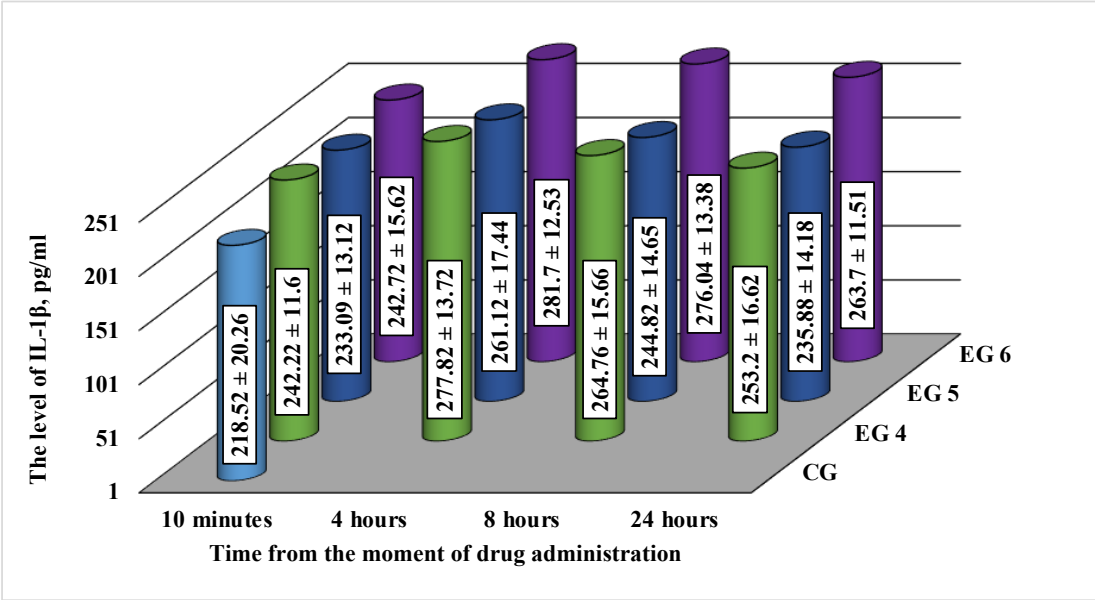

Fig. 5. Dynamics of changes in IL-1β levels in the vaginal wall of intact rats and rats with local inflammation and the use of clindamycin phosphate at different levels of serum hydrogen sulfide

CG - Control group (n=5 in each time point); EG 1 - Experimental group 1 (n=5 in each time point); EG 2 - Experimental group 2 (n=5 in each time point); EG 3 - Experimental group 3 (n=5 in each time point); EG 4 - Experimental group 4 (n=5 in each time point); EG 5 - Experimental group 5 (n=5 in each time point); EG 6 - Experimental group 6 (n=5 in each time point).

P-value:

For EG 1: 10 min compared to 4 hrs – P=.002; 10 min compared to 8 hrs – P=.032; 10 min compared to 24 hrs – P=.26; 4 hrs compared to 8 hrs – P=.198; 4 hrs compared to 24 hrs – P=.025; 8 hrs compared to 24 hrs – P=.193.

For EG 2: 10 min compared to 4 hrs – P=.021; 10 min compared to 8 hrs – P=.219; 10 min compared to 24 hrs – P=.755; 4 hrs compared to 8 hrs – P=.148; 4 hrs compared to 24 hrs – P=.082; 8 hrs compared to 24 hrs – P=.631.

For EG 3: 10 min compared to 4 hrs – P=.002; 10 min compared to 8 hrs – P=.007; 10 min compared to 24 hrs – P=.042; 4 hrs compared to 8 hrs – P=.509; 4 hrs compared to 24 hrs – P=.095; 8 hrs compared to 24 hrs – P=.268.

For 10 min time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.277; EG 1 compared to EG 3 – P=.956; EG 2 compared to EG 3 – P=.322.

For 4 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.131; EG 1 compared to EG 3 – P=.653; EG 2 compared to EG 3 – P=.065.

For 8 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.071; EG 1 compared to EG 3 – P=.256; EG 2 compared to EG 3 – P=.008.

For 24 hrs time point: CG compared to EG 1 – P=.001; CG compared to EG 2 – P=.001; CG compared to EG 3 – P=.001; EG 1 compared to EG 2 – P=.114; EG 1 compared to EG 3 – P=.279; EG 2 compared to EG 3 – P=.009

4. DISCUSSION

At present, one of the most studied and substantiated mechanisms of protective effect of hydrogen sulfide in the inflammatory process is the blockade of the signaling pathway of mitogen-activated protein kinase p38 (MAPK) [20,21]. Regulation of MAPK activity mediates inflammation and/or oxidation processes, thus exacerbating tissue damage. Hydrogen sulfide, by inhibiting the activation of the MAPK signalling pathway, indirectly protects tissues from inflammation. The expression of MAPK and ERK 1/2 (kinases regulated by extracellular signals) was reduced when using hydrogen sulfide in a model of inflammation and hypoxic damage in cell cultures [22].

In addition to protein kinases, nuclear factor kappa-B (NF-κB) and nuclear factor 2 associated with erythroid 2 (Nrf2) play an active role in the development of the inflammatory process [23]. NF-κB is responsible for the transcription of many genes involved in inflammation and is

activated in many acute and chronic inflammatory diseases, such as sepsis, inflammatory bowel disease, arthritis, asthma. Nrf2 belongs to a family of proteins that regulate endogenous antioxidant protection and promotes the transcription of a set of detoxification genes that encode protein synthesis (such as enzymes, drug transporters, antiapoptotic proteins and proteasomes) involved in the regulation of physiological and pathophysiological cellular responses to oxidants and xenobiotics.

Benedetti and co-authors have demonstrated that hydrogen sulfide not only inhibits NF-κB activation and nuclear translocation by reducing transcription of proinflammatory genes, but also enhances Nrf2 function by activating a cascade of enzymes such as hemoxygenase-1 (HO-1) and superoxide dismutase-1 (SOD1) [23].

The main substrates for endogenous hydrogen sulfide in tissues are sulfur-containing amino acids - L-cysteine and L-homocysteine, its main enzymes-producers are pyridoxal phosphate-dependent enzymes cystathionine-β-synthase, cystathionesaninamine-γ and cysteine aminotransferase [24].

The main reactions that ensure the formation of hydrogen sulfide in animal and human tissues include [24]:

1) Desulfurization of L-cysteine to pyruvate with cystathionine-γ-lyase.
2) Condensation of L-homocysteine with L-cysteine and desulfurization of L-cysteine to L-serine with the participation of cystathionine-β-synthase.
3) Transamination of L-cysteine with α-ketoglutarate with the participation of cysteine aminotransferase with the formation of 3-mercaptopyruvate, from which further H_2S is released with the participation of 3-mercaptopyruvate sulfurtransferase.

The introduction of sodium hydrosulfide as a donor of hydrogen sulfide and propargylglycine as a selective inhibitor of cystathionine-γ-lyase synthesis (key enzyme-producer of H_2S) allows to significantly change the levels of serum hydrogen sulfide and modulate a wide range of physiological and pathophysiological processes [6,7,8].

The methods used by us in the study [19] allowed us to create a statistically significant excess and deficiency of hydrogen sulfide in experimental rats.

The dynamics of local levels of TNF-α and IL-1β in all groups had a similar trend and was characterized by the rapid development of the inflammatory process from its simulation to 4 hours of study, followed by gradual attenuation of inflammation and almost complete normalization of the studied indicators for 24 hours. In general, a similar dynamic was described by Catalone B.J. and co-authors with the local intravaginal application of 4% nonoxynol-9 using morphological research methods [16].

It should be noted that the data obtained by us indicate that the previous serial introduction of sodium hydrosulfide as a donor of hydrogen sulfide, allowed not only to reduce the manifestations of the inflammatory process but also to achieve faster normalization of the studied indicators. At the same time, the artificially created deficiency of serum hydrogen sulfide (previous serial administration of propargylglycine) prolonged the duration and increased the studied indicators of inflammation in the vaginal wall.

This picture, in our opinion, is explained by the fact that hydrogen sulfide affects various parts of the inflammatory process while reducing the production of inflammatory mediators. A similar effect of excess and deficiency of serum hydrogen sulfide on the course of the inflammatory process, both general and local, has been repeatedly described in the scientific literature. Thus, other authors have demonstrated similar data in the study of the hippocampus, kidneys, heart, lungs [20,21,25,26,27].

In addition, both in conditions of deficiency and in conditions of excess hydrogen sulfide, clindamycin phosphate when applied topically did not create any statistically significant effect on the level of markers of inflammation in the vaginal wall.

In our opinion, the absence of differences in the dynamics of the studied parameters between the groups without clindamycin phosphate and with its use can be explained by the gentle effect of clindamycin phosphate on the vaginal mucosa and the absence of local irritant or anti-inflammatory effects. The absence of similar effects of the local intravaginal application of clindamycin phosphate investigated by us

coincides with earlier data of other researchers [14,28].

5. CONCLUSION

1. Preliminary serial introduction of sodium hydrosulfide as a donor of hydrogen sulfide, allows to reduce the degree of inflammation and achieve faster normalization of laboratory markers (TNF-α and IL-1β) and microscopic signs of inflammation in the experiment.
2. Preliminary serial administration of propargylglycine, as a specific inhibitor of cystathionine-γ-lyase, in order to reduce the serum level of hydrogen sulfide, leads to a prolongation of the duration and intensification of the inflammatory process in the vaginal wall of experimental rats.
3. Irrespective of the background level of hydrogen sulfide, clindamycin phosphate when administered intravaginally in the form of suppositories does not create additional local irritant or anti-inflammatory effects on the condition of the vaginal wall of experimental rats.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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