Evaluation of embryotoxicity and fetotoxicity of Clindamycin phosphate under normal and elevated levels of serum Hydrogen sulfide in rats

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Introduction

Treatment of patients with drugs from the group of antibiotics requires a balanced and professional approach, which is part of the concept of rational use of drugs. In turn, the rational use of antibiotics is an important part of the Ministry of Health's postulates, which should improve the prognosis of patients' recovery and reduce the length of hospital stay. However, despite the titanice efforts of the WHO and family doctors, the problem of controlling the circulation of antibiotics and their use by patients themselves remains. The main ones are uncontrolled intake, unauthorized discontinuation of antibiotics, self-increase or decrease in dose of drugs and, ultimately, the use of these potentially dangerous drugs without urgent need [8, 10, 19, 28].

All the principles of rational antibiotic therapy are divided into two major groups:
1. a group of principles and rules from the doctor and the manufacturer of the antibiotic;
2. a group of rules on the part of the patient.

If the second group can be little influenced by specialists, then the first group must be thoroughly studied, improved and create new approaches to antibiotic therapy.

In view of this, in recent years in the world literature there is information about the action of a number of endogenous factors that change the body's response to inflammation and septic conditions, which often use antibiotics. Such factors include the levels of vasoactive molecules, among which the most important is Hydrogen sulfide [9, 12, 13, 29, 31].
One of the most important issues in assessing the toxic effects of antibiotics on the human body is to determine the indicators of fetotoxicity and embryotoxicity.

One of the antibacterial agents widely used in gynecological practice, including for pregnant women, is a member of the group of lincosamides - the drug clindamycin [14, 15, 17, 21, 23, 25]. In the scientific literature there are reports of possible adverse effects of this drug on the course of labor and the condition of the fetus [1]. That is why, in our opinion, it is relevant both from a scientific and practical point of view to study the effect of Hydrogen sulfide on the reproductive toxicity of antibiotics, in particular clindamycin.

The aim of the research was to study the effect of Hydrogen sulfide levels on the indicators of embryotoxicity and fetotoxicity of Clindamycin phosphate under conditions of oral and intravaginal administration.

**Materials and methods**

The experimental study was performed on 60 pregnant female rats weighing 200-240 grams (219.7±11.1 grams) and under 1 year of age.

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 the provisions of 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".

Next, the experimental animals were divided into experimental groups as follows:

1 group (n=10) - control group - animals that received a solution of phosphate buffer.

Group 2 (n=10) - animals, which created an excess of serum Hydrogen sulfide.

Group 3 (n=10) - animals treated with Clindamycin phosphate intravaginally.

Group 4 (n=10) - animals injected with Clindamycin phosphate intravaginally on the background of excess serum Hydrogen sulfide.

Group 5 (n=10) - animals administered Clindamycin phosphate orally.

Group 6 (n=10) - animals administered Clindamycin phosphate orally on the background of excess serum Hydrogen sulfide.

All drugs were administered throughout pregnancy.

The phosphate buffer solution was administered orally through a 0.5 ml tube once a day.

Oral Clindamycin phosphate (Union Quimico Farmaceutica, S.A., Spain) was administered on a 1% starch gel via a tube once a day. The dose of the drug was equivalent to the maximum daily dose and according to the conversion tables was 500 mg/kg [22].

Intravaginally, Clindamycin phosphate (Pfizer Inc., USA) was administered to rats as micro-suppositories once daily.

The number of corpora lutea in the ovaries, the number of implantation sites in the uterus, the number of live and dead fetuses were recorded. Based on the obtained data, the term of fetal death was determined - before or after implantation.

Preimplantation (PreIL) and postimplantation (PostIL) mortality were determined by the formulas:

1. PreIL=(C- (A + B))/C*100%;
2. PostIL=B/(A + B)*100%,

where A is the number of live fetuses, B is the number of dead fetuses.

Throughout the experiment, the general condition of the rats, behavior and dynamics of weight gain were observed. On days 7, 14 and 20, the weight gain of pregnant rats relative to baseline values was assessed.

On day 20, half of the 7 rats from each group were removed from the experiment by translocating the cervical vertebrae under ketamine anesthesia at the rate of 0.22 ml of ketamine per 100 grams of body weight of the experimental animal. A laparotomy was performed, after which the pregnant uterus was examined (Fig. 1).

The number of corpora lutea in the ovaries, the number of implantation sites in the uterus, the number of live and dead fetuses were recorded. Based on the obtained data, the term of fetal death was determined - before or after implantation.

![Fig. 1. Pregnant uterus removed into a laparotomy wound. 1 - cervix; 2, 3 - right and left uterine horns with embryos.](image-url)
dead (resorbed) fetuses, C is the number of corpora lutea of pregnancy. 

Fetuses were examined for visible mutations, sex, weight, and cranio-caudal size were determined. After that, part of the fetuses was fixed in Buén’s solution to study the condition of the internal organs on serial sections. The rest of the fetuses was immersed in a solution of 96% ethanol and stained by the Dawson method to assess ossification points.

The rest of the rats were observed before birth. After birth, the number of newborn males and females was counted, and the weight of rats was measured at 5, 15, 30, and 50 days. In addition, for 30 days, 7 males and 7 females were randomly selected from rats born in each group, which were assessed for anxiety and mental development of offspring on the “open field” model.

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

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.

The levels of serum Hydrogen sulfide in the groups of experimental animals are shown in Fig. 2.

In all groups where the excess of serum Hydrogen sulfide was artificially simulated, there was a significant increase (p<0.01) of this indicator by 11-12% compared to the groups that did not have Hydrogen sulfide donors.

When assessing the dynamics of weight gain in pregnant female rats, the following data were obtained. During the first week, there was almost the same weight gain in all experimental animals.

In the second group (excess Hydrogen sulfide), the animals gained weight significantly faster than in the control group. Thus, the weight gain was higher compared to the control group by 15.35% in the second week and by 15.22% in 15-20 days of the study.

The studied indicator in the group with intravaginal administration of Clindamycin phosphate did not differ significantly from the control group at all terms of the study. At the same time, rats with intravaginal administration of Clindamycin phosphate on the background of excess Hydrogen sulfide gained weight in almost the same way as rats from group 2 - weight gain exceeded the control group by 14.47% in the second week and 14.33% in 15-20 days of research, respectively.

Although in the group with oral Clindamycin phosphate the numerical values of weight gain were slightly lower than the value of this indicator in the control group, but statistically significant differences between the groups were not confirmed at any time in the study. Similarly, no statistically significant difference was found between the indicators of group 6 and the control group, although the numerical values in group 6 were slightly higher. In this case, both on the 14th and on the 20th day of the study, weight gain in rats of group 6 significantly exceeded the same indicator of group 5.

Detailed values of weight gain of experimental animals in all study groups are shown in Table 1.

The indicators we evaluated on the 20th day of the study are shown in Table 2.

At visual inspection of fetuses visible mutations were not defined by us, ossification points in groups 2-6 did not differ from group 1 (control group).

Fetuses had the largest mass and cranio-caudal size in groups 2 and 4, the smallest - in group 5, and the differences were statistically significant. In groups 1, 3 and 6 these indicators were almost identical.

The number of corpora lutea, as well as the number of live fetuses in the groups did not differ significantly. At the same time, the number of resorbed fetuses was significantly lower in groups 2 and 4. The largest number of resorbed fetuses was in group 5, although not statistically significantly higher than in the control group.

Regarding the indicators of intrauterine mortality, preimplantation mortality did not differ significantly in all groups. Postimplantation mortality was highest in the group with oral Clindamycin phosphate, although no significant differences from the control group were confirmed. The lowest rates of postimplantation mortality occurred in the groups with additional administration of sodium hydrosulfide, and the indicators of groups 2 and 4 were statistically significantly higher than the groups with the usual background level of Hydrogen sulfide.

The next stage of our study was to establish the effect of the studied substances on the physical and mental development of the offspring.

No fatalities were reported during the 50-day study. All rats were active, behavioral reactions, timing of hair, weight gain met age standards.

The dynamics of weight gain in rats are shown in Table 3.

In male rats born from animals of group 2, on the 15th and 30th day of life, body weight significantly exceeded those of groups 1 and 5, and on the 50th day - only a similar indicator of group 5. In female rats born from animals of group 2, body weight significantly exceeded that in groups 1 and 5 on days 15, 30 and 50 of the study.
Table 1. Estimation of weight gain of pregnant rats (M±m).

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Initial weight, grams</th>
<th>1-7 days</th>
<th>8-14 days</th>
<th>15-20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (Phosphate buffer)</td>
<td>219.0±14.9</td>
<td>15.20±3.77</td>
<td>22.80±3.43</td>
<td>33.50±5.32</td>
</tr>
<tr>
<td>2. NaHS</td>
<td>219.6±12.7</td>
<td>16.20±1.62</td>
<td>26.30±2.77</td>
<td>38.60±3.27</td>
</tr>
<tr>
<td>3. Clindamycin phosphate intravaginally</td>
<td>221.6±10.5</td>
<td>15.00±2.16</td>
<td>22.50±2.37</td>
<td>33.10±4.23</td>
</tr>
<tr>
<td>4. Clindamycin phosphate intravaginally + NaHS</td>
<td>221.9±10.3</td>
<td>15.10±1.79</td>
<td>26.10±2.51</td>
<td>36.30±3.40</td>
</tr>
<tr>
<td>5. Clindamycin phosphate orally</td>
<td>221.6±10.5</td>
<td>15.00±2.16</td>
<td>22.50±2.37</td>
<td>33.10±4.23</td>
</tr>
<tr>
<td>6. Clindamycin phosphate orally + NaHS</td>
<td>221.8±12.5</td>
<td>15.20±3.55</td>
<td>23.80±3.16</td>
<td>36.50±4.22</td>
</tr>
</tbody>
</table>

Notes (hereinafter): * - statistically significant difference compared with the control group. The numbers indicate the group number, in comparison with which the differences are significant.

Table 2. Evaluation of the effect of the studied compounds on the embryonic development of rats (M±m).

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Fetus weight, grams</th>
<th>Fetus size, cm</th>
<th>Number of corpora lutea of pregnancy</th>
<th>Number of live fetus</th>
<th>Number of resorbed fetus</th>
<th>PreIL</th>
<th>PostIL</th>
</tr>
</thead>
</table>
| 1. Control (Phosphate buffer) | 3.086±0.367 | 2.871±0.350 | 13.43±1.27 | 10.86±1.07 | 1.429±0.787 | 8.456±4.971 | 11.43±6.26
| 2. NaHS | 3.457±0.207 | 3.214±0.168 | 13.29±0.95 | 11.29±1.11 | 0.571±0.535 | 10.53±6.86 | 4.817±4.571
| 3. Clindamycin phosphate intravaginally | 3.100±0.231 | 2.886±0.204 | 13.86±1.21 | 10.57±1.27 | 1.571±0.535 | 12.48±3.82 | 12.93±4.21
| 4. Clindamycin phosphate intravaginally + NaHS | 3.429±0.189 | 3.186±0.135 | 12.71±1.50 | 10.71±1.25 | 0.714±0.488 | 9.810±5.487 | 6.294±4.330
| 5. Clindamycin phosphate orally | 2.800±0.289 | 2.614±0.261 | 13.14±1.77 | 9.857±2.193 | 1.857±0.900 | 11.35±5.36 | 16.22±9.00
| 6. Clindamycin phosphate orally + NaHS | 3.200±0.306 | 3.014±0.313 | 13.43±1.27 | 11.00±1.41 | 0.857±0.690 | 11.68±3.76 | 7.306±5.847|

Notes: PreIL - preimplantation mortality. PostIL - postimplantation mortality.

Table 3. Dynamics of weight gain of rats from 5 to 50 days of life (M±m).

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Sex</th>
<th>Day of life</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1. Control (Phosphate buffer)</td>
<td>Males</td>
<td>11.37±0.89</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>11.44±0.85</td>
</tr>
<tr>
<td>2. NaHS</td>
<td>Males</td>
<td>12.24±1.10</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>12.30±1.13</td>
</tr>
<tr>
<td>3. Clindamycin phosphate intravaginally</td>
<td>Males</td>
<td>11.87±0.71</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>12.01±0.74</td>
</tr>
<tr>
<td>4. Clindamycin phosphate intravaginally + NaHS</td>
<td>Males</td>
<td>11.61±0.95</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>11.61±0.95</td>
</tr>
<tr>
<td>5. Clindamycin phosphate orally</td>
<td>Males</td>
<td>11.26±1.12</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>11.41±0.92</td>
</tr>
<tr>
<td>6. Clindamycin phosphate orally + NaHS</td>
<td>Males</td>
<td>11.34±0.86</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>11.56±0.71</td>
</tr>
</tbody>
</table>
An "open field" test was used to assess the behavioral responses of the offspring. The test results are shown in Table 4.

Thus, when evaluating the effect of the studied compounds on the offspring of rats, according to the "open field" test, we did not register any negative changes. All rats showed a sufficient level of resistance to stress, and indicators of interest (ambulation, ringing) and anxiety (grooming) did not go beyond the range of normal values.

Discussion

In the body, Hydrogen sulfide acts as a signaling molecule, a gas transmitter for which no specific receptors have been found. H2S molecular targets are various ion channels, receptors, enzymes and proteins that regulate a wide range of biochemical and physiological processes [13].

The content of Hydrogen sulfide in the body often changes as a result of pathological conditions and the use of pharmacological drugs. Thus, Hydrogen sulfide deficiency is associated with ischemic heart and brain disorders, mental retardation, atherosclerosis, hyperhomocysteinemia, etc. [6, 30, 31]. On the other hand, excessive production of Hydrogen sulfide is involved in the pathogenesis of inflammatory diseases, septic shock, stroke, etc. [5, 31].

This involvement of Hydrogen sulfide in the pathogenesis of various pathological conditions is due to the fact that it is involved in the regulation of a wide range of physiological and pathophysiological processes, such as vascular tone, neuromodulation, cytoprotection, inflammation, apoptosis and others [9, 12, 13, 29, 31].

No less interesting is the ability of Hydrogen sulfide to stimulate angiogenesis by stimulating the proliferation of endothelial cells [19, 32].

In this study, by serial administration of sodium hydrosulfide, we were able to achieve an increase in the background level of Hydrogen sulfide in all experimental animals, as in our previous studies [27].

Clindamycin phosphate when administered intravaginally did not create toxic effects on the mother and the embryo. The absence of toxic effects was confirmed by similar to the control group indicators of weight gain of pregnant rats, as well as indicators of weight and size of embryos on the 20th day of the study. In addition, the number of live and resorbed fetuses, as well as pre-implantation and post-implantation mortality did not differ statistically from the control group. There were also no changes in body weight gain and mental development of rats born to females who received Clindamycin phosphate in the form of suppositories during pregnancy.

Administration of Clindamycin phosphate to pregnant female rats during pregnancy had a negligible adverse effect on females and embryos. However, it was not possible to prove the statistical significance of differences with the control group.

The creation of excess Hydrogen sulfide significantly improved the studied parameters in both rats with intravaginal administration of Clindamycin phosphate and in rats that were not administered the drug. This was manifested by a significant increase in body weight gain of pregnant rats and rats born from them, an increase in anthropometric indicators of embryos, as well as a decrease in the number of resorbed fetuses and post-implantation mortality. Mental development of rats was also characterized by better performance.

Additional administration of sodium hydrosulfide to rats treated with Clindamycin phosphate orally counteracted the slight adverse effects of the drug and significantly improved the studied parameters.

Such results of our researches can be explained by increase in a placental blood-groove and increase in trophism of embryos. On the one hand, the reason for this effect is the vasodilating effect of Hydrogen sulfide on the vessels of the placental circulation, which was previously described in the scientific literature [4, 11, 20]. On the other

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Sex</th>
<th>Ambulations</th>
<th>Grooming</th>
<th>Rening</th>
<th>Defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (Phosphate buffer)</td>
<td>Males</td>
<td>16.14±0.69</td>
<td>7.286±1.113</td>
<td>3.429±1.272</td>
<td>0.714±0.488</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15.57±0.98</td>
<td>8.857±1.069</td>
<td>3.571±1.397</td>
<td>1.000±0.577</td>
</tr>
<tr>
<td>2. NaHS</td>
<td>Males</td>
<td>15.86±0.90</td>
<td>7.429±1.134</td>
<td>3.143±1.345</td>
<td>0.857±0.690</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15.43±1.27</td>
<td>9.143±0.690</td>
<td>2.571±0.976</td>
<td>0.571±0.535</td>
</tr>
<tr>
<td>3. Clindamycin phosphate intravaginally</td>
<td>Males</td>
<td>15.71±0.76</td>
<td>8.000±1.155</td>
<td>3.143±1.215</td>
<td>0.714±0.756</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15.29±0.95</td>
<td>8.143±1.069</td>
<td>2.857±1.215</td>
<td>1.143±0.690</td>
</tr>
<tr>
<td>4. Clindamycin phosphate intravaginally + NaHS</td>
<td>Males</td>
<td>16.29±0.76</td>
<td>7.571±1.134</td>
<td>2.571±0.787</td>
<td>0.571±0.535</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15.86±0.69</td>
<td>8.714±0.951</td>
<td>3.000±1.528</td>
<td>1.143±0.690</td>
</tr>
<tr>
<td>5. Clindamycin phosphate orally</td>
<td>Males</td>
<td>16.14±0.90</td>
<td>7.714±1.254</td>
<td>3.143±0.690</td>
<td>0.857±0.900</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>16.29±0.95</td>
<td>8.571±1.134</td>
<td>3.286±1.254</td>
<td>0.714±0.756</td>
</tr>
<tr>
<td>6. Clindamycin phosphate orally + NaHS</td>
<td>Males</td>
<td>16.43±0.79</td>
<td>8.286±1.113</td>
<td>3.714±1.380</td>
<td>0.429±0.535</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15.71±1.50</td>
<td>8.429±1.397</td>
<td>3.143±0.900</td>
<td>0.857±0.378</td>
</tr>
</tbody>
</table>
hand, previous studies demonstrate the presence of hydrogen sulfide in the ability to stimulate angiogenesis by stimulating the proliferation of endothelial cells [7, 18, 29, 32].

Further experimental studies will expand our understanding of the effects of hydrogen sulfide on the morphological structure and development of the placenta, as well as a clearer understanding of its involvement in the pathogenesis of pathological conditions associated with placental blood flow.

Regarding the genotoxicity of Clindamycin phosphate against the background of excess hydrogen sulfide, we did not conduct such studies for technical reasons. Literature data indicate the absence of genotoxicity in both oral and intravaginal forms of Clindamycin phosphate, which was confirmed by the results of the Ames mutation test for Salmonella typhimurium, as well as the micronucleus test in rats [1]. As for hydrogen sulfide, as an exogenous agent, it has a genotoxic effect [2, 3]. At the same time, as an endogenous gas transmitter, hydrogen sulfide has a protective and reparative effect on DNA [16, 24, 26, 28].

Conclusions

1. Artificial increase in the background level of hydrogen sulfide in pregnant rats leads to an increase in maternal weight gain, increased anthropometric indicators of embryos, as well as a decrease in the number of resorbed fetuses and postimplantation mortality and eliminates the small negative impact of high doses of Clindamycin phosphate administered orally.

2. Rats born from females with elevated levels of hydrogen sulfide in the body show faster rates of weight gain and better mental development.

References


ОЦІНКА ЕМБРІОТОКСИЧНОСТІ ТА ФЕТОТОКСИЧНОСТІ КЛІНДАМІЦИНУ ФОСФАТУ ЗА УМОВИ НОРМАЛЬНОГО ТА ПІДВІЩЕНОГО РІВНЯ СИРОВАТКОВОГО ГІДРОГЕН СУЛЬФІДУ У ЩУРІВ

Таран І.В., Гребенюк Д.І., Волощук Н.І., Лозинська М.С., Назарчук О.А., Боднарчук О.В.

Широке використання антибіотиків в клінічній практиці веде до розвитку антибіотикорезистентності та спонукає до пошуку нових шляхів модуляції їх терапевтичного впливу. Одним із потенційно успішних модуляторів може бути гідроген сульфід, який впливає на ембріо- та фетотоксичність кліндаміцину фосфату за умов нормального та підвищеного рівня сироваткового гідроген сульфіду у щурів.

Мета дослідження: вивчити вплив рівня гідроген сульфіду на показники ембріотоксичності та фетотоксичності кліндаміцину фосфату за умов нормального та підвищеного рівня сироваткового гідроген сульфіду.

Результати: Експериментальне дослідження проводили на 60 взросліх щурах, які були розподілені на 6 груп в експерименті. У першу групу вводили кліндаміцину фосфат внутрішньовагінально; у другу групу - перорально. У третю групу вводили кліндаміцину фосфат внутрішньовагінально на фоні надлишку сироваткового гідроген сульфіду; у четверте групу - перорально. У п'яту групу вводили кліндаміцину фосфат перорально; у шосту групу - внутрішньовагінально.

Заключення: Ембріо- та фетотоксичність кліндаміцину фосфату збільшуються при підвищенні рівня сироваткового гідроген сульфіду у щурів. Незалежно від шляху введення кліндаміцину фосфату, його ембріотоксичність збільшується.

Ключові слова: кліндаміцину фосфат, гідроген сульфід, ембріо- та фетотоксичність.