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## EFFECT OF RESVERATROL ON NO/SH-MECHANISMS OF NEURON DAMAGE IN EXPERIMENTAL VCD-HYPOESTROGENEMIA

***Introduction.*** *It was established that in menopause there were changes not only in the urogenital system but also in the activity of the cardiovascular and nervous systems. Phytoestrogens not only eliminate the symptoms of menopause but also, unlike hormone therapy, have fewer side effects. Resveratrol is one of the phytoestrogen drugs, which has been found to affect the pathobiochemical targets of the nervous and cardiovascular systems.*

*The study aims to establish the neuroprotective and antioxidant action of vaginal gel with resveratrol in monotherapy and the combined effect of gel with resveratrol and oral administration of resveratrol.*

***Materials and research methods.*** *The studies were carried out on 30 outbred white non-linear female rats weighing 220–240 g and aged 4.5 months. The chemical substance VCD was used to simulate hypoestrogenemia in rats with intact ovaries. After modeling the pathology, one group of animals was injected with a gel with resveratrol, the other with a gel in combination with resveratrol tablets, and the third with intravaginal cream "Colpotrofin". For the study, blood was taken from the abdominal aorta and brain tissue, in which the nature of the expression of mRNA, iNOS, eNOS, and nNOS was determined by the polymerase chain reaction method. In the brain, the content of the nitrosating stress marker, nitrotyrosine, and the state of the thiol-disulfide system was also identified following the methodological recommendations.*

***Research results.*** *It has been established that during the course administration of vaginal cream "Colpotrofin", vaginal gel with resveratrol and its combination with resveratrol tablets to female rats with hypoestrogenemia, all the studied drugs show neuroprotective and antioxidant effects. The greatest effect was noted for resveratrol in dosage forms.*

***Findings.*** *With the introduction of the gel with resveratrol and its combination with resveratrol tablets, it was found that the ability of the drug to normalize the nitrosidergic system and increase the activity of the glutathione link of the thiol-disulfide system lies in the implementation of the mechanisms of the antioxidant and neuroprotective effects of resveratrol in hypoestrogenemia.*

***Key words:*** *resveratrol, hypoestrogenemia, antioxidant, neuroprotective action, nitrosidergic, thiol-disulfide system.*

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## ВПЛИВ РЕСВЕРАТРОЛУ НА NO/SH-МЕХАНІЗМИ ПОШКОДЖЕННЯ НЕЙРОНІВ ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ВКД-ГІПОЕСТРОГЕНЕМІЇ

*Встановлено, що під час клімаксу відбуваються зміни не тільки у сечостатевій системі, але і в діяльності серцево-судинної і нервової систем. Фітоестрогени не тільки усувають симптоми менопаузи, але і, на відміну від гормональної терапії, мають менше побічних ефектів. Ресвератрол є одним із фітоестрогенних препаратів, який, як було встановлено, впливає на патобіохімічні цілі нервової та серцево-судинної систем.*

***Метою дослідження*** *є встановлення нейропротекторної та антиоксидантної дії вагінального гелю з ресвератролом у монотерапії, а також комбінованого ефекту гелю з ресвератролом і пероральним введенням ресвератролу.*

***Матеріали та методи дослідження.*** *Дослідження проводилися на 30 безпородних білих нелінійних самках щурів вагою 220–240 г і віком 4,5 місяця. Для імітації гіпоестрогенемії у щурів з неушкодженими яєчниками використовувалася хімічна речовина ВКД. Після моделювання патології одній групі тварин ввели гель з ресвератролом, іншій – гель з ресвератролом в поєднанні з таблетками ресвератролу, а третій – інтравагінальний крем «Колпотрофін». Для дослідження була взята кров з черевної аорти і тканини головного мозку, в яких характер експресії мРНК, iNOS, eNOS, nNOS визначався методом полімеразної ланцюгової реакції. У головному мозку також було виявлено вміст нітрозуючого маркера стресу, нітротирозину, а також стан тіол-дисульфідної системи відповідно до методичних рекомендацій.*

***Результати досліджень.*** *Встановлено, що під час курсу введення вагінального крему «Колпотрофін», вагінального гелю з ресвератролом та його поєднання з таблетками ресвератролу самкам-щурам з гіпоестрогенемією всі досліджувані препарати проявляють нейропротекторну та антиоксидантну дію. Найбільший ефект був відзначений для ресвератролу в лікарських формах.*

***Висновки.*** *У разі введення гелю з ресвератролом і його в поєднанні з таблетками ресвератролу було встановлено, що здатність препарату нормалізувати нітрозидергічну систему і підвищувати активність глутатіонної ланки тіол-дисульфідної системи полягає в реалізації механізмів антиоксидантної і нейропротекторної дії ресвератролу у гіпоестрогенемії.*

***Ключові слова:*** *ресвератрол, гіпоестрогенемія, антиоксидант, нейропротекторна дія, нітрозидергічна, тіол-дисульфідна система.*

**Introduction.** In menopause, despite its physiological nature, persistent molecular and biochemical shifts occur, leading not only to the extinction of the reproductive function of a woman but to a deterioration in her quality of life (Ilovayskaya, 2018). During menopause, against the background of hypoestrogenemia, against the background of autonomic dysfunction, increased anxiety, cognitive impairment, the growth of cardiovascular pathologies, and adverse outcomes increase (Shestakova et al., 2015). Through this period, as shown by experimental and clinical studies, the formation of endothelial dysfunction, disturbance in the NO system, changes in lipid and carbohydrate metabolism, and increased platelet aggregation are observed.

There are experimental data that hypoestrogenemia against the background of modeling peri- and postmenopause is accompanied by an increase in the mitochondrial BAX/Bclx1 ratio, release of cytochrome C into the cytoplasm, increased caspase-3 expression, kinase activation, and initiation of apoptosis (Plaksina & Simonovskaya, 2014). On this point, there is no doubt about the importance of adequate complex treatment of menopausal problems that occur in the peri- and postmenopausal period in women. Hormone replacement therapy is a pathogenetic method for correcting menopausal disorders, which is prescribed to relieve menopausal symptoms, and urogenital diseases, prevent bone loss, the occurrence of colorectal cancer, and the development of metabolic and other disorders (Radzinskiy & Khamoshina, 2016). However, under the conditions of hormone replacement therapy in women with hypoestrogenemia, the risk of developing thromboembolism, coronary artery disease, myocardial infarction, and stroke increases. Lately, it has been determined that hormone replacement therapy does not have the desired effect on cognitive impairment in perimenopausal and menopausal women and is associated with the risk of developing major side effects (Smetnik, 2014).

Usage of newly synthesized phytoestrogens leads to decreasing side effects (Anishchenko, 2014). However, the prescription of vaginal forms of phytoestrogen preparations does not lead to the mitigation of cognitive disorders, which requires the introduction of additional neuroprotective drugs into the complex treatment of menopause (Rietjens et al., 2016). Considering the identified molecular-biochemical disorders in early menopause, the attention of pharmacologists and clinicians is drawn to drugs that reduce the intensity of oxidative stress, prevent the formation of mitochondrial dysfunction, initiate neuroapoptosis, and normalize thiol-disulfide balance (Zaichenko et al., 2017). These drugs include resveratrol, which has antioxidant and estrogen-like properties (Glisic et al., 2018).

**The study aims** to determine the neuroprotective and antioxidant effect of the vaginal gel with resveratrol (in monotherapy), as well as in the combination of the vaginal gel with resveratrol and oral administration of resveratrol (with the combined administration of phytoestrogen) in terms of the effect on the indicators of conjugated systems – NO / SH to further substantiate the optimal regimen for the treatment of hypoestrogenic conditions, that occur as a result of menopause.

**Materials and methods.** The studied resveratrol vaginal gels were developed under the direction of Ph.D. professor O.A. Ruban at the Department of Factory Technology of the National University of Pharmacy (Kharkiv). The substance of resveratrol, which contains 50% trans-resveratrol of plant origin, was obtained from Polygonum Cuspidatum from the manufacturer of pharmaceutical substances "Naturex S.P.A." (France), supplier of Euroimpex Company LLC (series No. C091/004/A16).

The composition of the vaginal gel, in addition to resveratrol, which contains many phytoestrogens, includes hyaluronic and lactic acid, which are crucial components of the vaginal environment, and have an antioxidant effect (Zaychenko et al., 2018), and also have wound healing, capillary-strengthening and antimicrobial effects (Zaychenko et al., 2021). As a reference drug for similar indications for use, the vaginal cream "Colpotrophine" Netherlands, series: 6H772, which contains 10 mg of promestriene, was selected. The studies were carried out on 30 outbred white non-linear female rats weighing 220–240 g and aged 4.5 months.

The rats were kept under standard vivarium conditions: temperature – 20–25°C, relative humidity – 50–55%, natural light, diet, recommendations for these species of animals, and drinking regime "ad libitum". Experimental studies were carried out following the main provisions of the Council of Europe Convention for the Protection of Mammals Used in Experiments and for Other Scientific Purposes (Strasbourg, 1986), et. al. The chemical substance VCD (4-vinylcyclohexene diepoxide) was used to replicate hypoestrogenemia in rats with intact ovaries (Abolaji et al., 2020). The VCD model is recommended for the study of disorders of the musculoskeletal system, the cardiovascular system, and CNS in perimenopause and menopause (Özel et al., 2020). In this work, we used VCD (Sigma-Aldrich), which was diluted with refined corn oil and injected subcutaneously every day for 15 days at a dose of 60 mg/kg. This dose destroys 80–90% of the small preantral (primordial) follicles in the ovaries of female rats, accelerating the processes of atresia and thereby causing premature development of perimenopause/menopause.

15 days after the use of the VCD chemical, to simulate hypoestrogenemia, resveratrol vaginal gel (R) (monotherapy), resveratrol vaginal gel (R), and resveratrol tablets (iHerb, USA) were administered intravaginally for 28 days (for combined therapy), as well as the reference drug cream "Colpotrophine". Cream "Colpotrophine" and gel with resveratrol (R) were administered intravaginally once a day using a dosing syringe with an atraumatic tip of 0.005 ml/kg, resveratrol tablets were administered intragastrically using a metal probe in the form of a suspension at a dose of 100 mg/kg.

Rats were taken out of the experiment under thiopental anesthesia (40 mg/kg). For research, blood was taken from the abdominal artery and the brain. Blood was quickly removed from the brain, separated from the meninges, and the resulting lobules were placed in liquid nitrogen. Then they were crushed in liquid nitrogen to a powdery state and homogenized in a 10-fold volume of a medium at (2°C) containing (in mmol): sucrose – 250, Tris-HCl buffer – 20, EDTA – 1 (pH 7.4). At a temperature (+4°C), the mitochondrial fraction was extracted by differential centrifugation on a refrigerated centrifuge Sigma 3-30k (Germany).

**Real-time PCR**

The expression pattern of nNOS mRNA, eNOS mRNA, and iNOS mRNA was determined by real-time polymerase chain reaction. The tissues were dewaxed by incubation in two consecutive baths of xylenes and 100% ethanol. After dewaxing and centrifugation, the precipitate was dried in air to remove ethanol residues.

Isolation of total RNA from rat tissue was performed using the kit "Trizol RNA Prep 100" ("ISOGEN", Russia). This kit contains the following reagents: Trizol reagent and ExtraGene E. rRNA were isolated according to the protocol of the kit.

Reversible transcription (synthesis to DNA) was performed using the Reversible Transcription Reagent Kit (OT-1) (Syntol, Russia). Amplifier CFX96 ™ Real-Time PCR Detection Systems ("Bio-Rad Laboratories, Inc.", USA) and a set of reagents for PCR-RV in the presence of SYBR Green R-402 ("Syntol», Russia) were used to determine the expression level of the studied genes. For analysis of studied and reference genes, specific primer pairs (5'–3') were selected using PrimerBlast software (www.ncbi.nlm.nih.gov/tools/primer-blast), manufactured by ThermoScientific, USA. Registration of fluorescence intensity occurred automatically at the end of the elongation stage of each cycle through the SybrGreen channel. The actin, beta (Actb) gene was used as a reference gene to determine the relative value of the change in the expression level of the studied genes (Chekman et al., 2018).

**Enzyme-linked immunosorbent assay (ELISA)**

The degree of consumption of nitrosating stress in the brain was determined by the content of the nitrosating stress marker [13] by enzyme-linked immunosorbent assay (ELISA) on a full-plate enzyme immunoassay analyzer (SIRIO S, Italy) using test systems "Nitrotirosine ELISA Kit" ("HyCult biotechnology").

**Biochemical methods**

The state of the thiol-disulfide system of the brain was assessed by the content of reduced glutathione (RG) and oxidized glutathione (OG) (Belenichev et al., 2020; Belenichev et al., 2012) fluorimetrically with ortho-phthalic anhydride on a Quantech fluorimeter (Chekman et al., 2018). The level of free SH-groups, the activity of glutathione reductase, glutathione peroxidase, glutathione transferase, and the concentration of reduced and oxidized thiol groups were measured spectrophotometrically (Shestakova et al., 2015) using a Libra S 32 PC spectrophotometer.

**Statistics**

Data are presented as an arithmetic mean and standard error of the mean (M ± m). The results of the study were processed using the statistical package of the licensed program STATISTICA® for Windows 6.0 (StatSoftInc., No. AXXR712D833214FAN5), as well as "Microsoft Excel 2010". Statistical processing was performed using Student's t-test and Mann-Whitney U-test. For all types of analysis, differences with a significance level of less than 0.05 (95%) were considered statistically significant (Gurianov et al., 2018).

**Research results**

Analyzing the data presented in table 1, characterizing the expression of mRNA eNOS, mRNA nNOS, and iNOS in the CA1-zone of the hippocampus of the brain of females with VCD hypoestrogenemia, the following was established. The expression of eNOS mRNA in the group of untreated rats (control) was 90.8% lower than in the group of intact animals. A decrease in nNOS mRNA expression by 45.1% and a significant decrease in iNOS mRNA by 5.2 times were also registered. Enzyme immunoassay revealed an increase in the concentration of nitrotyrosine in mitochondria (3.94 times) and in the cytosol of the brain (3.48 times) with VCD-hypoestrogenemia compared with healthy females of the same age. The revealed facts indicate significant disorders of the nitroxidergic system of the brain and the activation of nitrosating and oxidative stress.

A significant role in the mechanisms of neuron death in various neurodegenerative diseases belongs to NO-mediated mechanisms, which are realized by increasing the expression and activity of various NOS

isoforms (Belenichev et al., 2022). Under conditions of transmitter autocoidosis in neurons (cerebellum, hippocampus, cortex), activation of neuronal NO synthase (nNOS) and an increase in NO production are observed, which is involved in the initiation of neuroapoptosis, opening of the pore of the mitochondrion, and the formation of mitochondrial dysfunction, in the nitrosylation of SH-containing signaling molecules, and their loss functions, as well as suppression of Zn-Cu-SOD activity (Belenichev et al., 2020).

The most sinister role in neuronal damage belongs to the inducible form of NOS, the expression of which by glial cells leads to hyperproduction, not so much of NO as of its numerous cytotoxic forms – from peroxynitrite to nitrosonium ion.

The low level of reduced intermediates of the thiol-disulfide system and an increase in the concentration of pro-inflammatory cytokines, especially IL-1b, play an important role in regulating iNOS expression and activating nitrosating stress. IL-1b activates AP-1 and NF-kB, which change the cell signal under ischemia and increase the expression of other pro-inflammatory factors while stimulating iNOS expression by astrocytes (Belenichev et al., 2020).

An excess of IL-1b can negatively affect the transport of reduced glutathione, reducing its synthesis. The deficiency of intracellular glutathione, which is involved in the mechanisms of NO transport and its bioavailability, enhances the formation of ONOO- (Belenichev et al., 2012). The role of IL-1b in the modulation of HSP70 expression is known, ranging from an increase to inhibition depending on the concentration (Belenichev et al., 2020).

Course treatment (table 2) with vaginal cream Colpotrophin did not have a pronounced effect on the expression of eNOS mRNA, nNOS mRNA, iNOS, and mRNA, as well as on the concentration of nitrotyrosine and markers of oxidative modification of the protein in the brain of females with VCD – hypoestrogenemia.

The course administration of vaginal gel with resveratrol to females with VCD-hypoestrogenemia led to a significant increase (by 133%) in the expression of eNOS mRNA and nNOS mRNA (by 29.3%). At the same time, the introduction of resveratrol did not significantly affect the expression of iNOS mRNA. Also, the introduction of resveratrol gel led to a significant decrease level of nitrotyrosine by 10.3% in mitochondria and by 19.6% in the cytosol of the brain of females with hypoestrogenemia.

Table 1

**Expression pattern of eNOS mRNA, nNOS mRNA, and iNOS in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on day 29 after treatment (M ± m)**

| Experimental groups | eNOS mRNA, c.u. | mRNA nNOS, c.u. | mRNA iNOS, c.u. |
|---|---|---|---|
| Intact control (IC) | 1000±0.008 | 1.000±0.0032 | 1.000±0.0112 |
| Control pathology (CP) | 0.0918±0.0001 | 0.549±0.0178 | 5.194±0.0922 |
| CP + gel with R | 0.214±0.008* | 0.710±0.0057* | 5.8765±0.0875* |
| CP + gel with R and tablets with R | 2.1506±0.0033*[1,2] | 1.155±0.011*[1,2] | 2.3008±0.0764*[1,2] |
| CP + "Colpotrophine" cream | 0.093±0.001 | 0,.6011±0.012 | 7.0543±0.0765* |

*Note: * – p <0.05 in relation the control pathology (CP)*

*[1] – p <0.05 in relation to the group CP + "Colpotrophine" cream*

*[2] – p <0.05 in relation to the group CP + gel with Resveratrol*

Table 2

**The concentration of nitrotyrosine in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on the 29th day after treatment (M ± m)**

| Experimental group | Nitrotyrosine, cytosol pg/ml | Nitrotyrosine, mitochondrial pg/ml |
|---|---|---|
| Intact control (IC) | 6.000±0.572 | 2.43±0.23 |
| Control pathology (CP) | 20.9±0.638 | 9.59±0.44 |
| CP + gel with R | 16.8±0.72* | 8.60 ±0.36* |
| CP + gel with R and tablets with R | 10.4±0.70*[1,2] | 5.96 ±0.29 *[1,2] |
| CP + "Colpotrophine" cream | 18.7 ± 0.41* | 9.19±0.39 |

*Note: * – p <0.05 in relation the control pathology (CP)*

*[1] – p <0.05 in relation to the group CP + "Colpotrophine" cream*

*[2] – p <0.05 in relation to the group CP + gel with Resveratrol*

The course combined use of the gel and tablets of resveratrol led to a significant increase in the expression of eNOS mRNA by 23 times and nNOS mRNA by 2.1 times, as well as to a decrease in iNOS mRNA expression by 55.7%. In the CA1 hippocampus of females with hypoestrogenemia. The combined administration of resveratrol gel and tablets to females with VCD-hypoestrogenemia significantly reduced the level of nitrotyrosine by 37.8% in mitochondria and by 50.2% in the cytosol of the brain of females with hypoestrogenemia (table 3). In terms of the degree of influence on the studied parameters, the combined administration of resveratrol gel and tablets significantly exceeded monotherapy with resveratrol gel and colpotrophine gel. Modulation of the expression of various NOS isoforms, aimed at their normalization and inhibition of nitrosating stress, under the influence of resveratrol at various routes of its administration in various dosage forms can be explained by the following fact. Due to its chemical structure, Resveratrol can regulate the activity of two nuclear factor-κB transcription factors (p65 / RelA and p50) (Zaichenko et al., 2017).

Resveratrol can also work as a direct antioxidant – due to the phenol group, it binds reactive oxygen species, and thereby inhibits the ROS-dependent activation mechanisms of IL-1b and iNOS.

Also, the antioxidant effect of resveratrol can be explained by the fact that an increased concentration of estradiol under its action leads to E2-dependent activation of the expression of mitochondrial Mn-SOD (Chekman et al., 2018).

Increased expression of Mn-SOD significantly reduces the fluxes of neurotoxic superoxide produced by mitochondria (Belenichev et al., 2012) and thereby inhibits ROS-dependent activation mechanisms of IL-1b and iNOS (Chekman et al., 2018).

Modeling of VCD hypoestrogenemia led to significant disturbances in the thiol-disulfide system of the brain of female rats, especially its glutathione link – a decrease in the pool of its reduced forms and a decrease in the activity of GPR and GR cytosolic fraction compared with intact animals. Glutathione is a crucial component of neuron protection, increases its resistance to hypoxia, limits NMDA hyperexcitability, acts as a reserve of cysteine in the cell, and regulates synthesis and stability of HSP70, involved in NO- and IL-1b-dependent mechanisms of apoptosis (Belenichev et al., 2022).

Table 3

**Indicators of the non-enzymatic link of the thiol-disulfide system in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on the 29th day after treatment (M ± m)**

| Experimental group | SH-groups, μm/g protein | SS-groups, μm/g protein | Glutathione restored, μg/protein | Glutathione oxidized, μg/protein |
|---|---|---|---|---|
| Intact control (IC) | 20.9±1.59 | 2.99 ±0.33 | 4.57±0.49 | 0.24±0.032 |
| Control pathology (CP) | 8.9±0.82 | 5.58±0.56 | 1.57±0.18 | 0.73±0.08 |
| CP + gel with R | 11.1±1.66* | 5.02±0.59 | 2.01±0.18* | 0.59±0.05* |
| CP + gel with R and tablets with R | 18.1±2.9*[1.2] | 3.56±0.28 *[1.2] | 3.3±0.22*[1.2] | 0.37±0.03*[1.2] |
| CP + "Colpotrophine" cream | 9.95±1.13 | 4.98±0.81 | 1.72±0.26 | 0.67±0.08 |

*Note: * – p <0.05 in relation the control pathology (CP)*

*[1] – p <0.05 in relation to the group CP + "Colpotrophine" cream*

*[2] – p <0.05 in relation to the group CP + gel with Resveratrol*

Table 4

**Parameters of the enzymatic link of the thiol-disulfide system in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on the 29th day after treatment (M ± m)**

| Experimental group | GR, μm/g protein/min | GPR, μm/g protein/min |
|---|---|---|
| Intact control (IC) | 29.3±3.7 | 61.4±4.0 |
| Control pathology (CP) | 8.31±0.64 | 39.5±3.7 |
| CP + gel with R | 12.2±2.7* | 41.2±4.4 |
| CP + gel with R and tablets with R | 22.8±1.9*[1.2] | 51.2±6.6 *[1] |
| CP + «Colpotrophine» cream | 9.48±1.4 | 39.1±3.1 |

*Note: * – p <0.05 in relation the control pathology (CP)*

*[1] – p <0.05 in relation to the group CP + "Colpotrophine" cream*

*[2] – p <0.05 in relation to the group CP + gel with Resveratrol*

In experiments in vitro (table 4), conducted under the guidance of professor I.F. Belenichev, were determined that deprivation of the GSH level in neurons leads to a drop level of HSP70 as well under conditions of brain ischemia, as well as under the influence of toxic concentrations of steroids, nitrosamines, neurotransmitters, a correlation was found between the severity of neurological disorders and GSH deficiency and HSP70 in the brain of animals (Pavlov & Belenichev, 2014). It has been shown that an increase in the concentration of the oxidized form of glutathione leads to increased production of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8, which, in turn, can increase the deficiency of reduced glutathione due to disruption of its transport into the cell and enhance the mechanisms of neurodestruction (Glisic et al., 2018).

Increased concentration of oxidized intermediates of the thiol-disulfide system suppresses the expression of eNOS (Belenichev et al., 2020) and increases the production of ROS due to the release of arachidonic acid from platelets, inhibits GPR and GR, and also stimulates many pathways of intracellular signaling, including neuroapoptosis (Dinger et al., 2016). Course treatment with vaginal cream Colpotrophine did not have a pronounced effect on the performance of the thiol-disulfide system. Course treatment with vaginal gel with Resveratrol led to a significant increase in reduced thiol groups by 24.7% against the background of an increase in the reduced form of glutathione by 27.3% and a decrease in its oxidized form by 19.1% in the cytosol of the brain of premenopausal females.

In groups of animals with experimental hypoestrogenemia during treatment with a gel with Resveratrol, a significant increase in GR activity by 46.8% in the cytosolic fraction of the brain was observed compared with untreated animals. The introduction of the gel with Resveratrol did not affect the activity of GPR (Pavlov & Belenichev, 2014). The course combined use of the gel with resveratrol and resveratrol tablets led to a significant increase in GR activity by 174% and GP by 29.6% against the background of an increase in the level of reduced glutathione by 110.2% and a decrease in its oxidized form by 49.3%, as well as an increase in the content of reduced thiols by 103.4% and a decrease in oxidized SS-groups by 36.2% in the cytosol of the brain of female rats with VCD-hypoestrogenemia compared with the group of untreated animals (Belenichev et al., 2012).

Increasing the functionality of the glutathione system, as well as increasing the activity of GSH enzymes, not only leads to increased protection of the brain from neurotoxic products of oxidative and nitrosative stress (reduction of nitrotyrosine in mitochondria and cytosol) but can also cause GSH-dependent mechanisms of endogenous neuroprotection due to increased expression of HSP70 (Xue et al., 2014).

Increasing the functionality of the thiol-disulfide system in VCD hypoestrogenemia and increasing its recovery of intermediates, under the influence of vaginal and intraventricular resveratrol, can contribute to an increase in the bioavailability of NO due to the formation of nitrosotiols and an increase in the expression of mRNA eNOS, as well as a decrease in the conversion of NO into cytotoxic peroxynitolol. In previous studies, we have shown the possible effects of resveratrol on HSP70-dependent mechanisms of the endogenous neuroprotector (Zaychenko et al., 2021).

It is possible that resveratrol, modulating the expression of the subtotal transcription factor NF-kappa-B, can increase the concentration of HSP70 and GSH in the brain and inhibit neuroapoptosis through the Fas/Apo-1 trigger mechanism (Roach et al., 2015).

Resveratrol, due to its biochemical properties and features of its chemical structure, can suppress ROS/IL-1b-dependent mechanisms of iNOS expression, as well as the accumulation of nitrotyrosine and markers of oxidative protein modification, thereby reducing the shift in the thiol-disulfide system of the brain towards its proapoptotic oxidative forms (Li et al., 2018).

**Conclusions**

**1. We have established for the first time that the modeling of hypoestrogenemia in female rats by 15-day administration of VCD leads to disruption in the conjugated systems of the brain – thiol-disulfide and nitroxidergic**

**2. Evaluation of the neuroprotective and antioxidant effects of a course of 28-day administration of vaginal gel Colpotrophine, vaginal gel with Resveratrol, as well as a combination of gel and resveratrol tablets in female rats with hypoestrogenemia revealed the presence of the above effects only when resveratrol was administered in dosage forms. The most pronounced effect was observed with the combined use of tablets and gel with Resveratrol.**

**3. In the mechanism of the neuroprotective and antioxidant action of resveratrol is its ability to normalize the nitroxidergic system – to reduce the expression of iNOS mRNA and increase the expression of eNOS mRNA, inhibiting nitrosating stress, and also to increase the activity of the glutathione unit of the thiol-disulfide system (the level of reduced glutathione and GR activity) in the brain**

**4. The obtained results confirm the expediency of developing a new vaginal gel with a combined composition of resveratrol and hyaluronic acid as an alternative to hormone-containing drugs for the prevention and treatment of pathological hypoestrogenic conditions that rise against the background of estrogen deficiency.**

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