THE EFFECT OF A NEW VAGINAL GEL WITH THE HOP EXTRACT ON THE CARDIOVASCULAR SYSTEM IN SPAYED FEMALE RATS

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Menopause in women is usually accompanied by physiological changes in different systems and organs due to fading and cessation of the ovarian function. One consequence of this process is development of the cardiovascular system (CVS) diseases [3, 11].

It is known that receptors for estradiol are present in the endothelium, smooth muscle cells of blood vessels and in the myocardium [14]. Interacting with endothelial receptors estradiol intensifies production of nitric oxide and blocks vasoconstriction induced by acetylcholine; it also intensifies regeneration of endothelial cells; enhances neovascularization by increasing migration, proliferation and differentiation of vascular endothelial cells [11]. Several studies have found that the presence of endothelial dysfunction in postmenopausal women is a risk factor for atherosclerosis, early coronary heart disease (CHD), hypertension, and peripheral artery lesions. According to the Framingham Study (1986) the incidence of acute coronary events increases by 12 times in postmenopausal women compared to women of the reproductive age [1].

Early natural menopause increases the risk of coronary heart disease by 3 times, while surgical menopause – by 7 times [10].

Currently the problem of prevention of cardiovascular complications caused by hypoestrogen states and in the menopause period in women is of particular relevance. The basis of prevention and treatment of CVD in menopause is the hormonal menopausal therapy [4, 5, 16]. It should be noted that in recent years more and more attention is paid to alternative therapies, particularly herbal medicine with the use of phytoestrogens as an active ingredient manufactured by Organon company, the Netherlands.

The new combined vaginal gel containing the hop extract as the main active ingredient, as well as lactic acid and excipients was developed at the Department of Professor Vishnevskaya L.I. (St. Petersburg, Russia) in collaboration with prof. Vishnevskaya L.I.

Materials and Methods

The experimental studies were conducted on 30 females of white outbred nonlinear rats weighing 190-195 g. They were divided into 5 groups, 6 animals in each group: intact control; sham-operated female rats; control pathologic; spayed rats treated with the vaginal gel with the hop extract; spayed animals treated with the reference drug – “Ovestin” vaginal suppositories containing estradiol as an active ingredient manufactured by Organon company, the Netherlands.

The new combined vaginal gel containing the hop extract as the main active ingredient, as well as lactic acid and excipients was developed at the Department of Chemist’s Technology of Drugs named after D.P.Salo of the National University of Pharmacy under the supervision of prof. Vishnevskaya L.I.

In animals simulation of the state close to menopausal symptoms in women was caused by bilateral spaying according to Kirshenblat Y.D. [6]. Sham-operated animals were subjected to laparotomy and wound suturing without removal of ovaries. Starting with the 35th day after spaying females were introduced vaginally the drugs studied within 28 days, namely the gel with the hop extract in the dose of 0.06 mg/kg, estradiol suppositories – 0.03 mg/kg.

After completing the treatment animals were taken out from the experiment by decapitation under chloroform anesthesia. The aorta, coronary vessels, and the myocardium of female rats were morphologically studied.
The test material was fixed in 10% formalin solution, dehydrated in alcohols with the increasing concentration, poured to celloidin-paraffin, and sections were stained with hematoxylin-eosin [9]. Microslides were examined using the Granum microscope; the microscopic pictures were taken by a Granum DSM 310 digital video camera. Pictures were processed with Pentium 2,4GHz PC using ToupView software.

The studies were conducted in compliance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) [7].

Results and Discussion

The results of the studies conducted show that the histostructure of the aorta, coronary arteries, the myocardium and intramural arteries of female rats of the intact control corresponded to the normal physiological state being typical for these animals [12, 13, 15].

The aorta of ovarioectomied female rats showed sticking of red blood cells on the surface of media; b – loosening of the intima and superficial media. Hematoxylin-eosin. x250. (Fig. 1, 2)

Coronary arteries and media also showed loosening of the extracellular collagen matrix, and in some places there was tiny thickening of the intimal surface (Fig. 3a). One of the females had large accumulation of fat in this wall thickening under the endothelium in the disorganized superficial layers of media (Fig. 3b). Probably, there were atherosclerotic “plaques” at various stages of formation.

The cardiac muscle of the spayed female rats showed changes of the myofibril apparatus, fibre fragmentation accompanied by edema, the loss of striated myofibrils. The vascular wall of intramural arteries is often thickened, swollen, loosened, and sometimes impregnated with a protein liquid. Endothelial cells were proliferated with the picket-fence arrangement. In some cases we managed to trace the appearance of vacuoles in the intima probably of the lipid nature. Vacuoles were also present in media (Fig. 4).
Fig. 5. The intramural artery (a – the endothelial layer defect, its thickening into the lumen of the vessel, Immersion) and the myocardium (b – perivascular cellular infiltrates. x200) of the spayed female rats. Hematoxylin-eosin.

Fig. 6. The myocardium of the spayed female rat. The spasm of blood vessels of different caliber: a – thin-walled vessel; b – large artery. Hematoxylin-eosin.

Fig. 7. Different parts of the myocardium of the spayed female rats (cross section): a – normal cardiomyocytes; b – hypertrophic cardiomyocytes nearby vessels in the state of spasm. Hematoxylin-eosin. x200.

Fig. 8. The myocardium of the spayed female rat after using the gel with phytoestrogens: a – cardiomyocytes with the normal size (cross section); b – the normal state of the vascular wall of the intramural artery. Hematoxylin-eosin. x200.

Fig. 9. The vascular wall: a – abdominal aorta; b – coronary artery of the female rat after vaginal application of the gel with phytoestrogens. The absence of changes in the intima and media. Hematoxylin-eosin. x250.

Fig. 10. The vascular wall: a – coronary vessels; b – abdominal aorta of the female rat after application of estriol suppositories. The normal state of the intima and media of blood vessels. Hematoxylin-eosin. x250.
In one thin-walled artery there was a focal defect of the endothelial layer, namely its thickening into the lumen of the vessel. Sometimes there is moderate perivascular round-cell infiltration was observed (Fig. 5).

Many blood vessels of different caliber were in the state of spasm (Fig. 6). Cardiomyocytes were visually hypertrophied preferably nearby vessels in the state of a distinct spasm (Fig. 7).

The signs of necrotic changes and myocardial cell infiltration were not found. The histological picture described shows development of the complex of morphological changes in the cardiovascular system of the spayed female rats that are typical for deficiency of endogenous estrogens and formation of endothelial dysfunction in them, and the literature confirms this fact [8].

The use of the vaginal gel with phytoestrogens prevents development of degenerative changes of the myofibrillar apparatus of cardiac muscle (myocytolysis, the loss of striated myofibrils) manifestations of endothelial dysfunction such as changes in the state of the vascular wall of intramural arteries, coronary vessels and aorta, hypertrophy of cardiomyocytes (Fig. 8, Fig. 9).

There were no apparent disorders of the intima and media in microscopic examination of the abdominal aorta wall and coronary vessels of female rats treated with the reference drug – suppositories with estriol on the background of estrogen deficiency (Fig. 10).

In the cardiac tissue the state of fibres, intramural arterial vessels visually corresponded to the norm (Fig. 11).

CONCLUSIONS
1. Bilateral oophorectomy in female rats leads to degenerative changes of the myofibrillar apparatus of cardiac muscles by the myocytolysis type, focal fragmentation of fibres, small foci of myocardial damage. The complex of morphological changes (vasospasm, thickening, swelling, loosening of the vascular wall, focal proliferation, destruction, the picket-fence arrangement of endothelial cells, small foci of fat accumulation in the intima and media) is typical for endogenous estrogen deficiency. This can be interpreted as manifestations of endothelial dysfunction.

2. After application of the vaginal gel with the hop extract in the female rat cardiac muscles there were no changes in the myofibrillar apparatus; the distinct signs of reduction or the absence of endothelial dysfunction was observed.

3. By its effect on the morphological condition of the cardiovascular system the gel with the hop extract under study is not inferior to the reference drug – suppositories with estriol.

4. The vaginal gel with the hop extract is promising for further study for the purpose of its use in complex therapy of cardiovascular diseases associated with hypoestrogenia in women during the menopause.

REFERENCES

ВЛИЯНИЕ НОВОГО ВАГИНАЛЬНОГО ГЕЛЯ С ЭКСТРАКТОМ ХМЕЛЯ НА СОСТОЯНИЕ СЕРДЕЧНО-СОСУДИСТОЙ СИСТЕМЫ ОВАРИОЭКТОМИРОВАННЫХ САМОК КРЫС
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Ключевые слова: овариоэктомированные самки крыс; дефицит эстрогенов; сердечно-сосудистая система; гель с экстрактом хмеля; фитоэстрогены
Приведены результаты исследования морфоструктуры аорты, коронарных артерий, миокарда и интрамулярных артерий сердца у самок крыс, которым была проведена биплательная овариэктомия, а также после лечения их новым вагинальным гелем с экстрактом хмеля и препаратом сравнения – суппозиториями «Овестин», содержащими эстрол. Показано, что в результате стойкого эстрогендефицитного состояния в сердечно-сосудистой системе самок крыс сформировался комплекс морфологических изменений, которые можно оценить как эндотелиальную дисфункцию. После терапии изучаемым фитопрепаратом у животных отсутствовали изменения в миофibriлярном аппарате миокарда, отмечено уменьшение или отсутствие признаков эндотелиальной дисфункции.