

INFLUENCE OF RALOXIFENE AND 17 β -OESTRADIOL ON RATS' ORAL MUCOSAL STRUCTURE

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Abstract

The aim of this study was to determine histological changes in rats' oral mucosa at induced postmenopausal oestrogen decrease and after administration of raloxifene and 17 β -oestradiol. Wistar female rats were ovariectomized and divided into experimental groups; basal – after ovariectomy without drugs - BOV, taking raloxifene - RA and oestradiol – OVH (10 rats per group). Additionally, 10 rats without ovariectomy and drug supplementation made up control group – CL. Specimens of oral buccal mucosa were stained with haematoxylin and eosin. While the oral mucosa in BOV group had a slight epithelium atrophy, mucosa in rats taking raloxifene did not differ much from that in control group without ovariectomy. Oral mucosa in group taking oestradiol did not differ significantly from that in the group without ovariectomy except characteristic changes in reproductive layer of mucosa epithelium. Lack of oestrogen in experimental menopause caused changes in oral mucosa i.e. shortening of epithelial ridge. These changes were inhibited in different degree after administration of raloxifene and oestradiol.

Key words: rats, raloxifene, oestrogen, oral mucosa.

In postmenopausal period the endogenous oestrogen level decreases. This primary change provides for many characteristic alterations in almost all the body. Oestrogen acts through two intracellular receptor proteins ER α and ER β (2). Leimola-Virtanen reported in her study on the presence of ER also in oral buccal mucosa, minor salivary glands and parotid and submandibular glands (8).

Ovaries are the main source of natural oestrogens. Their dysfunction decreases oestradiol level in blood and at the same time it's stimulatory function in target tissues. Presence of oestrogen receptors in cells of soft tissues causes changes evoked by lack of natural oestrogen (3, 8). Drugs used in the prevention of postmenopausal osteoporosis protect body against alterations caused by hypooestrogenism. Prevention of postmenopausal hypooestrogeny can be based not only

on simple supplementation with missing hormone, but also drugs modulating oestrogen receptors can be used, e.g. raloxifene (6, 4). Changes caused by oestrogen deficiency are manifested in the vascular, urogenital and skeletal systems. There are many studies about structural and functional changes in the urogenital system in women. In short, mucosal changes in postmenopausal period concerning this system can be named atrophical.

The aim of this study was to show changes in rats' oral mucosa in experimental postmenopausal hypooestrogenic model. The histological alterations in oral mucosa in postmenopausal period have not been finally explained. However, hormonal background of menopause is well known, the influence of hormone replacement therapy (HRT) with 17 β -oestradiol and raloxifene on oral mucosa histological pattern also has not been completely investigated.

Material and Methods

The experiment was conducted according to the animal experimental guidelines approved by the Animal Experiment Committee, Skubiszewski Medical University of Lublin.

Wistar female rats, aged 6 weeks, were used in this study. After two-week adaptation to diet and new environment, the rats were divided into 4 experimental groups, 10 animals in each group. Rats were fed a specially prepared chow with low corn content. The animals were given free access to the feed and water with diluted *calcium lactogluconicum* (Calcium- Polfa) (20 mg/100ml).

At the beginning of the experiment 30 animals were bilaterally ovariectomized by laparotomy under general anaesthesia with thiopental sodium. These ovariectomized rats were divided into 3 experimental groups; basal - BOV, receiving raloxifene (Evista- Lilly) – RA, and receiving oestrogen (Oestradiolum benzoicum-Jelfa Jelenia Góra) - OVH. Group of 10

animals without ovariectomy established control group - CL.

Each animal in the RA group was given 0.25 mg of Evista by a stomach-tube (in special water-oil prepared mixture) each day, in group OVH 12.5 μ g of 17 β -oestradiol was administered intramuscularly each day for 120 days. After experiment period rats were anaesthetized with thiopental sodium. Animals were decapitated and samples of buccal mucosa were taken. The specimens were immediately immersed in 10% neutral buffered formalin and microscopic specimens were stained with haematoxylin and eosin. Specimens were investigated under an optical microscope and compared between particular groups. After surveying each sample of rat mucosae, the selected pictures were photographed with magnification from 80 to 200x.

After a detailed analysis of the samples and photographs the assessment criteria were established. On the basis of the photographs characteristic for each group of animals, the changes appearing in the structure of mucosa of the examined animals were presented.

Results

Normal microscopic appearance of the rat buccal epithelium is demonstrated in the mucosa structure of animals from the control group (CL). No histopathological changes in the structures of examined tissues were observed (Fig. 1).

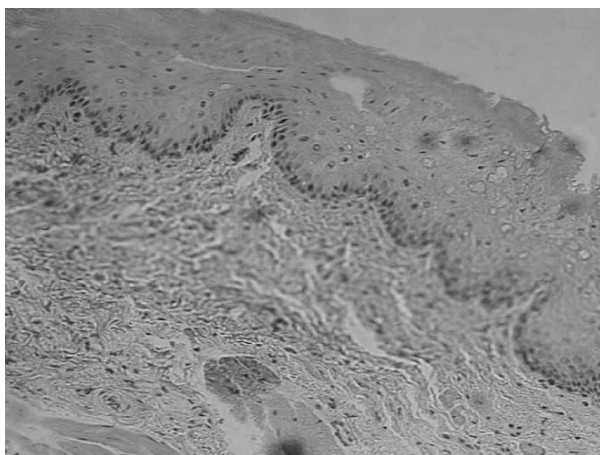


Fig. 1. Group CL: control without ovariectomy and drug administration. H-E, 100x.

In the group of rats after ovariectomy without any supplementation, the BOV group, shortening of the epithelial ridge with reduction of basal and granular layer of epithelium (Fig. 2), and decreasing in excretion contents in small salivary glands of mucosa were observed (Fig. 3).

In the group of rats after ovariectomy receiving 17 β -oestradiol, OVH group, basal cell layer was strongly developed, and thick keratin layer was observed (Fig. 4).

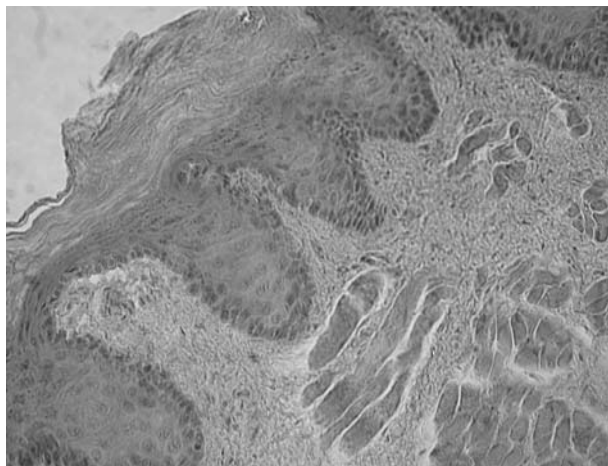


Fig. 2. Group BOV: shortening of the epithelial ridge, reduction of granular layer of epithelium. H-E, 100x.

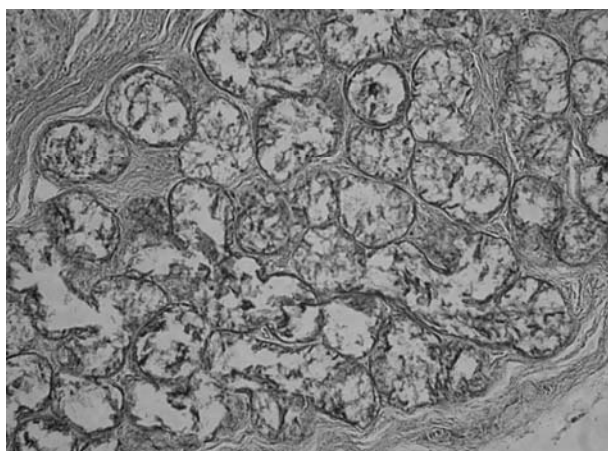


Fig. 3. Group BOV: decreasing in excretion contents in small salivary glands of mucosa. H-E, 200x.

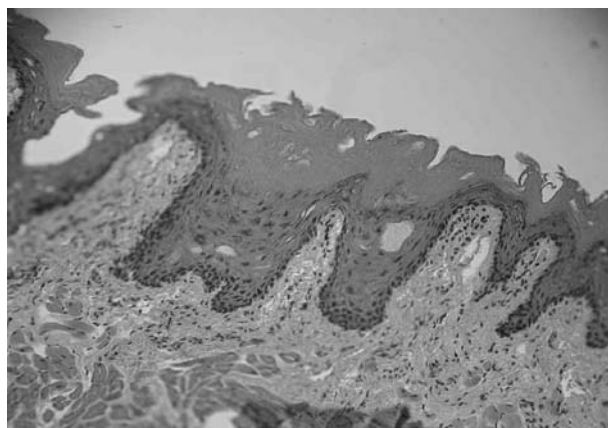


Fig. 4. Group OVH: basal and granular cell layer strongly developed, thick keratin layer. H-E, 100x.

After the raloxifene therapy in the RA group, there were no remarkable histopathological changes comparing with the control group, except a slight shortening of the epithelial ridge and increasing in number of a small salivary glands in the lamina propria presented in Fig. 5.

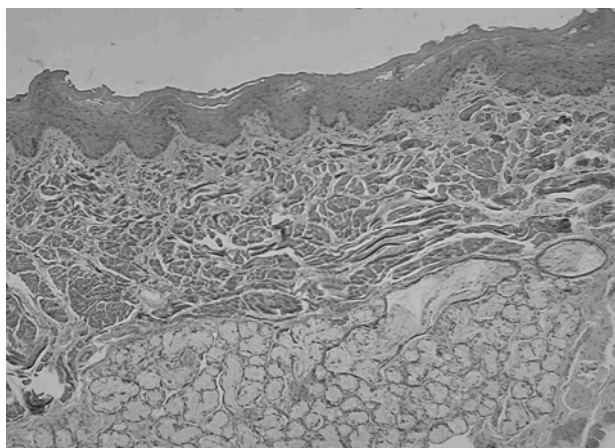


Fig. 5. Group RA: a slight shortening of the epithelial ridge and increasing in number of small salivary glands in lamina propria in comparison with CL group. H-E, 80x.

Discussion

Low oestrogen level after ovariectomy reflects in many organs and tissues. Atrophical changes in the vagina in menopause can be compared with those in oral mucosa. Thompson in his study compared the structure of oral and vaginal mucosa of postmenopausal women. He concluded that vaginal epithelium can be used as a substitute for buccal epithelium *in vitro* (11). Forabosco (5) suggested that oral discomfort in postmenopausal women may be related to steroid hormone withdrawal and that replacement therapy may improve the clinical picture and cytologic features in this group of patients. The burning mouth syndrome (BMS) is frequently connected with peri- and postmenopausal period in women. Symptoms of BMS have been reported in 10 to 40 percent of women presenting for treatment of menopausal symptoms (1, 13).

In literature there are studies about suggested aetiologies, beginning from psychological dysfunction, local and systemic factors (e.g. nutrition deficiencies), and hormonal changes, especially in menopause. Also hyposalivation have been associated with BMS but at the same time, oral mucosa seems to be clinically normal (7). Histological changes in oral mucosa can be a prime background of BMS.

Based on structural changes between investigated groups, it is concluded that lack of endogenic oestrogen and HRT supplementation cause changes in oral mucosa. Reduction of epithelial ridge in the BOV group after ovariectomy, probably due to oestrogen deficiency, was inhibited after supplementation of raloxifene and oestrogen. Reproductive potential of epithelium was recovered after supplementation of both drugs. Basal and granular layer of epithelium improved visibly stronger in the OVH group than in the RA group. Evista acts as oestrogen on bone and serum lipids but also as an oestrogen antagonist in the breast and endometrium (12). This feature manifested itself in our study. In the group taking oestrogen the increase of reproductive

potential of epithelium was even higher than in the control group without ovariectomy, but in the group taking raloxifene we did not observe significant differences in comparison with control without ovariectomy. Although in this experimental study performed on animals it was not possible to examine and compare sensations associated with BMS, structural alterations in mucosa gave a reason for its hormonal background. Raloxifene can help preserve normal structure of oral mucosa, nevertheless oestrogen also prevents atrophical alterations of mucosa, and in our study the reproductive potential of epithelium seemed to outgrow that in the group without ovariectomy and group taking raloxifene.

Administration of raloxifene after ovariectomy reduced changes in oral mucosa structure. Scully (10) observed that dry mouth symptoms are strongly related to age of patients, and number of drugs taken permanently, especially antimuscarinic agents and some sympathomimetic agents, as well agents affecting serotonin and noradrenalin uptake, may produce subjective dry mouth. He concluded that drugs mentioned above are the most common cause of reduced salivation. In microscopic pattern of specimens we observed reduction of small salivary glands excretion content after ovariectomy in BOV group, we also noticed apparent normalizing of oral small salivary glands structure in the group taking raloxifene.

It is to be concluded that HRT with oestradiol reduces changes in rats' oral mucosa evoked by hypoestrogenism after ovariectomy but simultaneously increases reproductive potential of basal layer of epithelium. A new generation drug, raloxifene, reduces pathological changes in oral mucosa and helps approaching its structure to normal.

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