Effect of Eplire Ultraphonophoresis on Fibrosclerotic and Adhesive Alternations in Uterine Appendages

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Ultraphonophoresis of eplire in combination with antibacterial therapy during experimental inflammation of uterine appendages activates fibroclasts and macrophages, prevents hyperplasia of connective ovary stroma and the development of fibrosclerotic alterations and adhesive process.

Key Words: *eplire; inflammation; ovaries; oviducts*

The inflammatory diseases of uterine appendages are characterized by long-term relapsing course and the development of adhesions and fibrosclerotic alterations of the connective tissue in small pelvis in 35-37% women [8-10], which is the major cause of tuboperitoneal infertility, extrauterine pregnancy, and pelvic pain syndrome. The new drug preparation eplire, an extract of high-polar lipids of silt sulfide mud, was developed at Research Petrochemical Institute, Siberian Division of the Russian Academy of Sciences (Tomsk). It exhibits antioxidant, membrane-stabilizing, and pronounced antiinflammatory effects [1,7]. Beneficial therapeutic effect of eplire in experimental adnexitis (EA) was demonstrated [2].

Our aim was to study the effect of eplire ultraphonophoresis (EUP) in combination with antibacterial therapy on fibrosclerotic and adhesive alterations in uterine appendages during EA.

MATERIALS AND METHODS

Experiments were carried out on 98 outbred female rats weighing 180-200 g divided into four groups. Intact rats served as the control (n=18), while in other rats EA was modeled [5]. To this end, the rats were narcotized with ether and *Staphylococcus aureus* culture (strain No. 209, two doses of 50 mln microbial bodies) was injected through insulin syringe into each uterine horn near oviducts. The test group 1 rats (n=25) received EUP for 10 days starting from day 5 of EA against the background antibacterial therapy (20,000 U/kg penicillin administered from day 3 of EA). Before physiotherapy the skin was depilated. Eplire (0.5 ml, 1% oil solution) was applied to the skin immediately prior to EUP. Group 2 rats (n=30) received antibacterial therapy alone. Group 3 rats (n=25) was subjected to a 10-day ultrasound therapy starting from day 5 of EA against the background antibacterial therapy. Ultrasound therapy and EUP were performed with an UZT-31F apparatus with due account for species-specific features of experimental animals [6]. Both fields corresponding to projections of uterine appendages were irradiated with ultrasound according to a labile method in a pulse mode (4 m/sec, 0.05 W/cm^2 beam power, 0.3 sec pulse duration, 3-4 min exposure).

The rats were decapitated during diestrus (colpocytological control) on days 5, 10, 15, 30, 75, and 120 of EA. For electron microscopy, the oviductal and ovarian specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2), postfixed in 1% osmium tetroxide, and embedded in Araldite. The specimens were cut on LKB-III ultratome. Semithin sections were stained with toluidine blue, and ultrathin sections were contrasted with uranyl acetate and lead

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citrate. The sections were analyzed and photographed under a JEM-100 CX electron microscope. For light microscopy the ovaries were fixed in Carnoy fluid. Paraffin-free sections (5-6 μ) were stained with hematoxylin and eosin and according to Van Gieson. Specific volume of the connective tissue of ovarian stroma was determined using Avtandilov's eyepiece graticule. The results were statistically analyzed using Student's *t* test.

TABLE 1. Specific Volume of Ovarian Tissues on Day 75 of Experiment (*M*±*m*)

Group	Tissue specific volume, cm^3/cm^3		
	interstitial	glandular	fibrous
Control	0.372±0.03	0.121±0.012	0.251±0.026
Group 2	0.452±0.047*	0.076±0.02	0.358±0.030*
Group 1	0.359±0.045	0.117±0.022	0.234±0.036

Note. *$p<0.05$ compared to the control.

RESULTS

The early period (5-15 days) of EA was characterized by pronounced ultrastructural changes in the ovarian and oviducal blood vessels predominantly in the endothelial layer against the background of antibacterial therapy. We observed destruction of follicular epitheliocytes and ovocytes, irregular thickening of the basal membrane in mature and growing follicles and their enhanced atresia. Oviductal epithelium was characterized by decreased number of mirovilli and cilia and ultrastructural disorganization of common organelles. On day 15, multiple adhesions were formed between ovaries and oviducts with surrounding peritoneum. There was no surface ovarian epithelium in sites of adhesions. Fibrous tissue developed in adhesions was presented by thick oxyphilic collagen fibers (Fig. 1, *a*).

On day 30, we observed an enlargement of fibrous component and degeneration of the connective tissue in the *lamina propria* of oviductal mucosa and in the medullar and cortical ovarian substance. The most part of compactly arranged collagen fibers were clearly cross-striated. Some fibers were irregularly contrasted, and in some regions their striation was blurred. Fibroblasts in dense connective tissue were hyperchromatic and pyknotic. Their cytoplasm was characterized by a decreased volume and looked like a narrow rim with fine electron dense ultrastructure, while the perinuclear space was enlarged. Chromatin was hypercondensed, karyoplasm was characterized by high electron density, the fibrillar component in nucleoli dominated over the granular one (Fig. 2, *a*). On days 30, 75, and 120, perivascular fibrosclerotic alterations of the ovarian connective tissue were most pronounced.

Ultrasound applied against the background antibacterial therapy (group 3) produced little effect on the pathomorphology of inflammatory process and did not prevent its chronization and development of fibrosis of the connective tissue.

EUP in combination with drug therapy (group 1) induced a pronounced cell reaction in the connective tissue and alterations in the fibrous component of the intercellular substance. On days 15-30, mature fibroblasts with well-developed granular endoplasmic reticulum were seen in some regions of medullar and cortical substance in the adhesions between the ovaries, oviducts, and surrounding peritoneum. Here we also observed macrophages with well-developed lysosomes and vacuolar apparatus and phagosomes of different size. These cells were surrounded by solitary

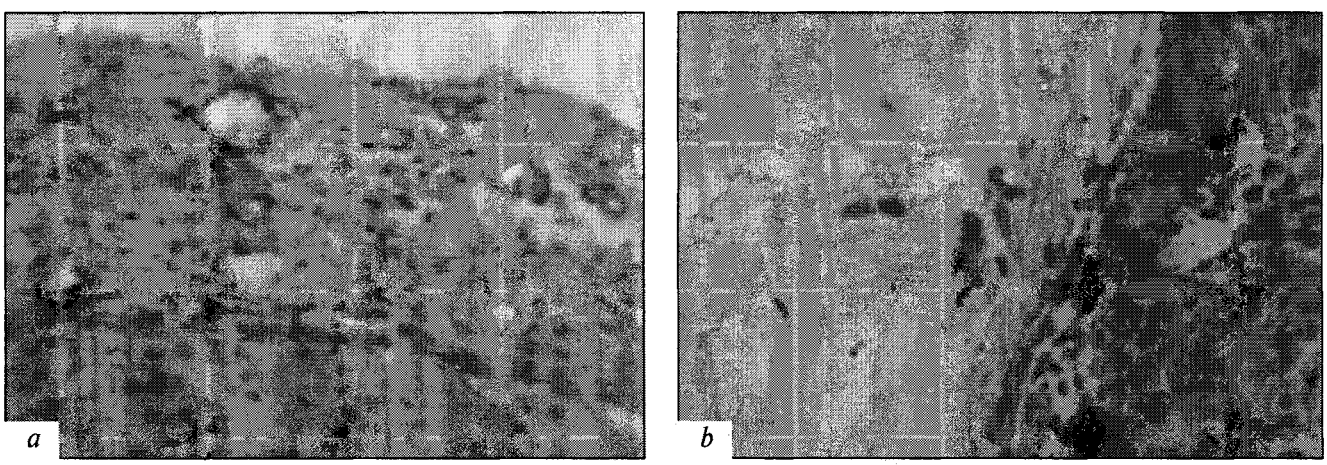

Fig. 1. Adhesion alterations in uterine appendages during experimental inflammation and balneotherapy. *a)* rough fibrous adhesions with ovary on inflammation day 15 in group 2 rats; *b)* thin adhesion with preserved surface epithelium on inflammation day 15 in group 1 rats. Here and in Fig. 2: hematoxylin and eosin staining, ×370 (*a*), ×530 (*b*).

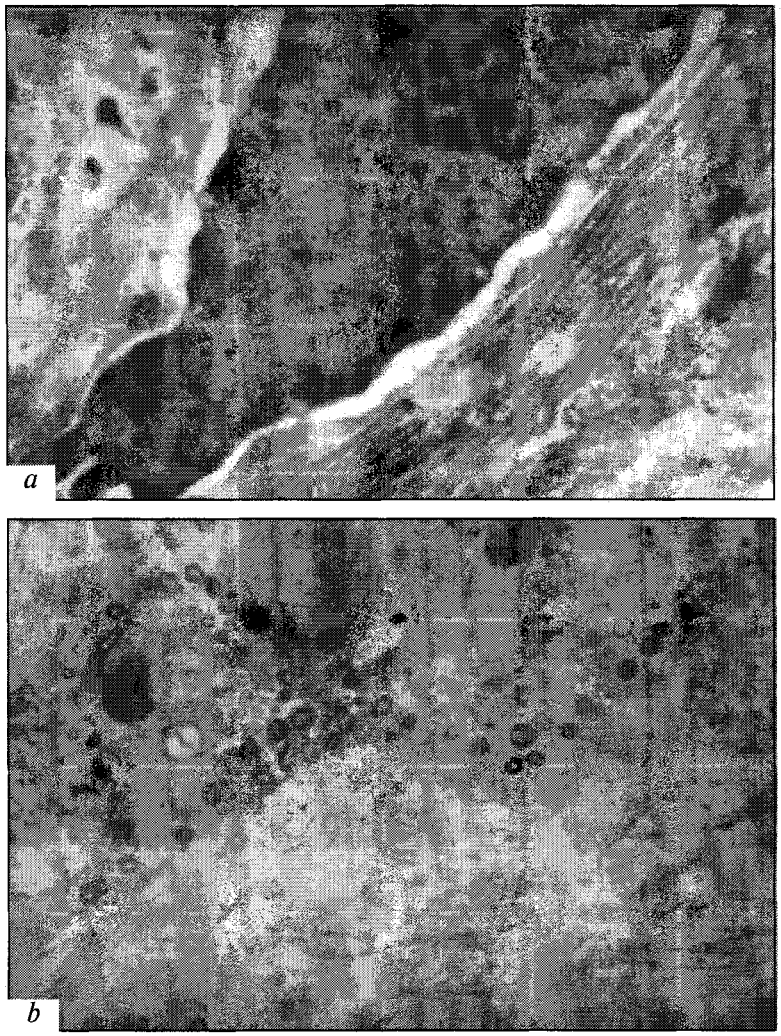

Fig. 2. Alterations in ovarian connective tissue during experimental adnexitis and balneotherapy. *a*) abundant compactly arranged collagen fibers, pyknotic fibroblast in the cortical ovarian substance on inflammation day 30 in group 2 rat; *b*) a fragment of fibroclast cytoplasm with multiple lysosomes and phagosomes in *tunica albuginea* on inflammation day 15 in group 1 rat.

collagen fibers in the state of lysis. Cells of the fibroblast lineage often seen in these regions ultrastructurally were presented by typical fibroclasts. They had projections and elongated nucleus with low chromatin condensation. Their cytoplasm contained few cisternae of the granular endoplasmic reticulum, free ribosomes, and mitochondria. Multiple primary and large secondary lysosomes were seen in the body and projections of these fibroclasts. Additionally, the cytoplasm often contained membrane-enclosed cavities with fibrillar or fine-grained substance with low electron density (lyzed collagen fibrillae, Fig. 2, *b*). We also observed phagocytosis of collagen bundles by these cells. No pronounced fibrosclerotic alterations were observed in the uterine appendages during inflammation after EUP. Appendage-peritoneal adhesions were thin, and in large fragments of adhesions the ovarian surface epithelium was preserved (Fig. 1, *b*).

On day 75, the specific volume of ovarian interstitial tissue in group 2 increased (Table 1), while the volume of the hormone-producing tissue in the interstice decreased. Therefore, the enlargement of the interstitial tissue during inflammation proceeds due to fibrous connective tissue. After EUP, there were no pronounced changes in specific volume of the interstitial glandular and connective tissues.

Thus, EUP applied against the background of antibacterial therapy of EA activates fibroclasts and macrophages regulating the balance between collagen synthesis and resorption [3,4] and prevents the increase in specific volume of the interstitial connective tissue and the development of fibrosclerotic alterations and adhesions.

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