EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Heterogeneity of Serum Activities of Matrix Metalloproteinases in Chronic Endometritis

G. T. Sukhikh, G. M. Soboleva, E. S. Silantyeva, E. A. Shagerbieva, and V. N. Serov

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Matrix metalloproteinases belong to the key molecules of tissue remodeling involved in physiological and pathological processes of the female reproductive system. Adequate levels of their expression in the endometrium are essential for effective implantation and uneventful pregnancy. Chronic inflammatory process in the endometrium is associated with low tissue expression of metalloproteinase-9. Histologically verified chronic endometritis is associated with low serum activities of metalloproteinases 2 and 9, which are restored after combined etiotropic therapy. We measured serum levels of metalloproteinases in patients with chronic endometritis concomitant with sterility and its changes during the first days after magnetotherapy.

Key Words: *matrix metalloproteinases; chronic endometritis*

Extracellular matrix (ECM) proteins are a group of families of polyfunctional proteins with related structure and located mainly in the basal membrane and stroma of the viscera and tissues and performing structural and regulatory functions (participation in morphogenesis and angiogenesis processes, cell transformation, apoptosis, tissue regeneration) [15]. This large group includes the family of structural proteins (collagens, elastin, laminins), matrix metalloproteinases (MMP), tissue inhibitors of matrix metalloproteinases (TIMP), and proteoglycans. The MMP family, in addition to participation in ECM degradation, is involved in activation of bioactive molecules (other proteases, some TIMPs, growth factors, cytokines, receptors, peptide hormones) [6,10,15]. MMP-2 and MMP-9 are the most prevalent representatives of this family expressed in virtually all tissues [6,10].

Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence***: soboleva_galina@hotmail.com. G. M. Soboleva

Increased activities of MMP-2 and MMP-9 are characteristic of invasive growth (angiogenesis, implantation, carcinogenesis) [6,10,15,16], while reduction in tissue content of these proteins is associated with final phases of tissue regeneration (wound healing, fibrosis, sclerosis) [11]. Due to very low molecular weight and solubility in biological fluids, they penetrate into the serum in amounts proportional to tissue expression. A correlation of tissue and serum activities of MMP-2 and MMP-9 was shown for neurological, cardiovascular, skin diseases, cancer, and some infections [8,9,12-14]. Low level of their expression in the endometrium is associated with sterility and habitual abortions [1,5], reduction of their serum activity is characteristic of chronic endometritis associated with sterility, while normalization of these parameters reflects clinical recovery [3].

Chronic endometritis is a clinical and morphological syndrome characterized by defective cyclic transformation of the endometrium in the presence of inflammatory process, disorders in desquama-

tion and regeneration of the functional layer. Clinical variants of chronic endometritis are determined by common and tissue reactivity, etiological factor, disease duration, presence of exacerbations and their severity [2]. Etiotropic and metabolic therapy is prescribed with consideration for the pathogenesis. On the other hand, physical factors normalizing regional hemodynamics and promoting recovery of the tissue structure, are traditionally used in the treatment [4]. We studied the effects of magnetotherapy on the time course of serum activities of MMP-2 and MMP-9 in patients with chronic endometritis.

MATERIALS AND METHODS

The study was carried out in 16 women aged 34.23± 5.36 years suffering from chronic endometritis concomitant with sterility. Control group consisted of 3 healthy women. Blood serum was collected on days 7-8 of the cycle before therapy by "running" pulsed magnetic field and on days 7-8 of the first cycle after therapy. In healthy women, the blood was collected on days 7-9 of the cycle.

Serum activities of MMP-2 and MMP-9 were evaluated by substrate electrophoresis in 8% PAAG [3]. The sera were diluted in a buffer for sample (62.5 mM Tris-HCl, 2% sodium dodecylsulfate, 10% glycerol, pH 6.8), applied onto 8% acrylamide/bis-acrylamide gel (37.5:1), 0.125 M Tris-HCl (pH 6.8), 0.1% sodium dodecylsulfate, and run at direct current of 30 mA. The gel was then washed 2×30 min at ambient temperature in incubation buffer I (5 mM Tris-HCl, 2.5% Triton X-100, pH 7.5), incubated for 16 h at 37°C in incubation buffer II (5 mM Tris-HCl, pH 7.5), stained with 2% Coomassie Blue, and washed in solution containing 20% acetic acid and 40% methanol. The gel was then exposed for 2 h at ambient temperature in distilled water and dried.

RESULTS

Comparison of serum activities of MMP-2 and MMP-9 in patients with chronic endometritis before therapy and in healthy women prompted us to divide all examined women into 3 groups (Fig. 1). In group 1 activities of MMP-2 and MMP-9 were normal (5 patients, 31.25%), in group 2 MMP-2 activity was normal, while MMP-9 was reduced (6 patients, 37.5%), and in group 3 activities of MMP-2 and MMP-9 were reduced (5 patients, 31.25%).

In one patients reduction of serum metalloproteinase activities was detected on days 7-8 of the cycle following magnetotherapy. Normalization of MMP-9 level was observed in 2 patients of group 2. In other 14 patients including two patients receiving placebo treatment (one patient from group 2 and one from group 3) serum metalloproteinase activity did not change.

Heterogeneity of metalloproteinase activities in patients with chronic endometritis can indicate different intensity of the pathological process in the endometrium. In this case surface involvement corresponds to normal levels of MMP-2 and MMP-9 (group 1). The progress of changes in the endometrium seems to be associated with reduction of expression of at first MMP-9 (group 2), which restored as soon as by the menstrual cycle directly following magnetotherapy in 2 of 5 patients (1 of 6 patients in group 2 received placebo treatment). More profound involvement of the endometrium was associated with reduction of both MMP-9 and MMP-2 activities (group 3).

Heterogeneous distribution of serum activities of MMP-2 and MMP-9 in endometritis is presumably associated with peculiarities of the pathological process. Decreased metalloproteinase activity is usually associated with the development of fibrosis and sclerosis of different location [11], and hence, low levels of MMP-9 and particularly MMP-2 expression can reflect predominance of the fibrosing component with a trend to the development of endometrial degeneration. Higher expression of MMP is often linked with hyperplastic processes [8,16]. Normal activity of MMP-2 and MMP-9 in the presence of chronic inflammatory process in the endometrium is presumably associated with liability to pro-

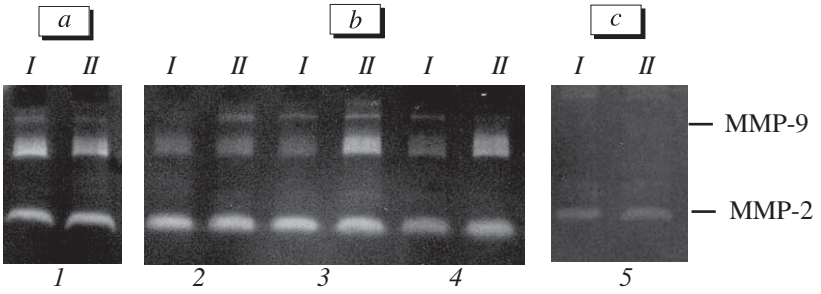

Fig. 1. Gels after electrophoresis of sera from patients from groups 1 (*a*), 2 (*b*), and 3 (*c*) before (*I*) and after (*II*) magnetotherapy. Light bands: active MMP-2 and MMP-9 in substrate gel. *1*) patient from group 1; *2-4*) patients from group 2; *5*) patient from group 3.

liferative processes in the reproductive organs (a history of endometriosis, adenomyosis, endometrial polyp) and of extragenital location. Our results indicate pathogenetic heterogeneity of processes in the endometrium, united by the clinical diagnosis of "chronic endometritis". In our next studies we plan to clear out the mechanisms of relationship between MMP-2 and MMP-9 activities and clinical morphological variants of chronic endometritis; we intend to trace the time course of the recovery of serum levels of MMP-2 and MMP-9 after magnetotherapy.

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