ROLE OF ESTRADIOL IN THE DEVELOPMENT OF FIBROSIS IN CYSTIC SCLERODERMA (POSSIBLE SEX-LINKED PREDISPOSITION TO THE DISEASE)

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Most research workers now consider that the development of fibrosis, which gives systemic scleroderma (SSD) its specific features, is linked with increased biosynthesis of collagen proteins by fibroblasts [10, 13]. The fibroblasts of patients with SSD are characterized by an excessively high level of procollagen-mRNA of types I and III and by a coordinated increase in the synthesis of the corresponding proteins [6, 8]. The principal factors specifically affecting the synthesis of particular proteins are steroid hormones (SH).

It has been shown that synthesis of type I procollagen by fibroblasts is controlled by glucocorticoids (GC) and that the degree of depression of protein synthesis is determined by the concentration of GC receptors in the cells [12]. Estradiol (E_2), one of the principal female sex SH, also has been shown to inhibit collagen synthesis in the skin [14]. According to data obtained on cultures of aortic cells E_2 depresses the synthesis of type III procollagen [2]. E_2 receptors have been found in the skin of various animals [4, 5]. These observations indicate that collagen synthesis can be regarded as an E_2-dependent process, a conclusion which has attracted particular attention in connection with the stronger predisposition to SSD of women.

This paper gives the results of a study of the E_2-receptor apparatus and the character of action of the hormone on type III procollagen synthesis by cultures of skin fibroblasts from normal individuals and patients with SSD.

EXPERIMENTAL METHOD

Cultures of fibroblasts (3rd-4th passages, stationary phase of growth), obtained from skin biopsy specimens from the forearm of 17 clinically healthy persons (one man, the rest women, ages from 20 to 36 years) and 17 patients with SSD (all women, from 16 to 46 years old, duration of the disease 1-6 years). The disease followed an acute course in two patients, subacute in seven, and chronic in eight.

The cell cultures used in all the experiments were obtained from patients undergoing treatment in the Institute of Rheumatology, Academy of Medical Sciences of the USSR. Culture of the fibroblasts and determination of specific binding of E_2 by whole cells (in suspension) were carried out by the method in [1]. Specific binding of E_2 by a homogenate of fibroblasts was determined [5] with a final E_2 concentration in the incubation medium of 80 nM.

In experiments using Scatchard plot analysis fibroblasts were cultured until a monolayer was formed (7th-8th days of culture) in flasks with a bottom surface area of about 5 cm^2. The culture medium was changed 24 h before the experiment for similar medium, but containing 1% serum. Each determination was made on three or four flasks. The cells were incubated, using a culture medium (with 1% serum) containing 10-100 nM 2,4,6,7-(n)^{3}H-E_2, with specific radioactivity of 140 Ci/mmole (Amersham, England). In parallel tests a 200-fold excess of unlabeled hormone was added Incubation proceeded for 30 min at 37°C with constant shaking. The process was arrested by rapid removal of the incubation medium. The cell layer was washed 3 times

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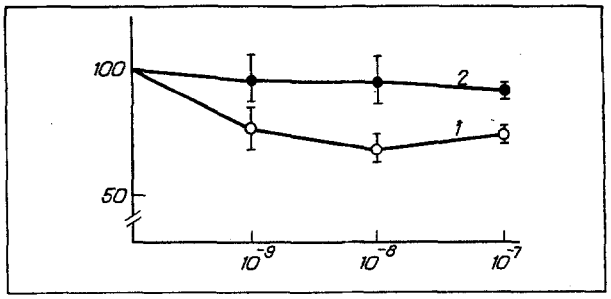

Fig. 1. Effect of E_2 on production of type III procollagen by skin fibroblasts of normal individuals (n = 4) and patients with SSD (n = 5). Abscissa, concentration of E_2 (in M); ordinate, production of type III procollagen (in % of control).

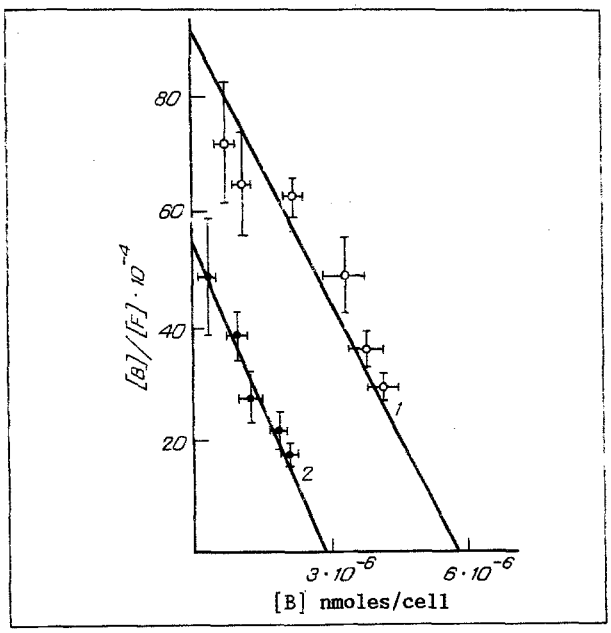

Fig. 2. Scatchard plot for binding of ^3H-E_2 by skin fibroblasts from normal individuals (1) and patients with SSD (2).

to remove radioactive unbound hormone with physiological saline The cells were next lysed by the addition of 200 μl of 0.4% Na deoxycholate and the radioactivity of the samples was determined in the following scintillation mixture: methylcellosolve B 4 ml, toluene, containing 0.5% 2,5-diphenyloxazole, 5 ml.

The results of determination of specific binding of E_2 in the homogenate, whole cells, or monolayer culture were comparable: under normal conditions (irrespective of the method used) specific binding of E_2 amounted to 15-30% of the total.

The quantity of type III procollagen synthesized by fibroblasts was assessed on the basis of accumulation of the amino-terminal propeptide type III procollagen, whose concentration was measured by radioimmunoassay [11]. E_2 was added to the cultures 24 h before the experiment in concentrations of 10^{-9}, 10^{-8}, or 10^{-7} M. The equivalent quantity of ethanol was added to the control cultures. Assistance with the technique and with some of the experiments was given by the Finnish research workers L. and J. Risteli, based in the Department of Clinical and Medical Chemistry, University of Oulu (Finland).

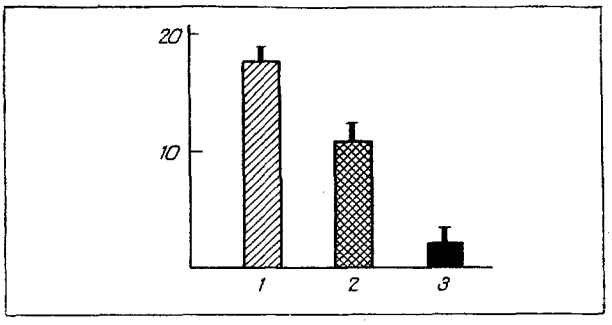

Fig. 3. Specific binding of E_2 by skin fibroblasts from patients with SSD depending on type of course of the disease. 1) Normal individuals (n = 16), 2) chronic SSD (n = 5), 3) acute and subacute SSD (n = 8) Ordinate, binding of E_2 (in % of total).

EXPERIMENTAL RESULTS

Data on the effect of E_2 on production of type III procollagen by fibroblasts from normal individuals and patients with SSD are given in Fig. 1. Under normal conditions E_2 depressed production of type III procollagen: if the cells were cultured in the presence of 10^{-9} M E_2 the concentration of type III procollagen in the culture medium was 77.5 ± 10.3% of the control (n = 4), in the presence of 10^{-8} M E_2 it was 68.6 ± 6.6% (n = 4), and in the presence of 10^{-7} M E_2 it was 80.0 ± 3.1% (n = 3). Under these circumstances, inhibition of type III procollagen production was observed in all the strains studied, and in the presence of all three concentrations of the hormone. Incidentally, these results are in agreement with data in the literature indicating that E_2 inhibits synthesis of type III procollagen by vascular cells in culture [2].

In SSD the effect of the hormone was virtually undetectable: during cell culture in the presence of 10^{-9} M E_2 the concentration of type III procollagen in the culture medium was 96.2 ± 9.9% (n = 4) of the control (i.e., the same as during culture of the cells without E_2), in the presence of 10^{-8} E_2 it was 97.3 ± 10.4% (n = 5), and in the presence of 10^{-7} M E_2 it was 92.5% (n = 2). Inhibition of type III procollagen production under normal conditions compared with that in SSD was significant ($p < 0.05$) if concentrations of 10^{-8} and 10^{-7} M E_2 were used.

The results are thus evidence that under normal conditions synthesis of type III procollagen by fibroblasts is an E_2-dependent process. Weakening of the effect of the hormone on this parameter can be regarded as an important factor in the hyperproduction of type III collagen and the development of fibrosis in SSD.

It is now considered that the physiological responses of the cells to the action of SH are mediated by receptors. In particular, it has been shown that the quantity of type I procollagen synthesized by fibroblasts depends on the concentration of GC receptors [12] and that activation of the synthesis of this protein in SSD is evidently due to reduction of receptor binding of GC in this disease [9].

Experiments accordingly were carried out to determine the number of E_2 receptors in skin fibroblasts of healthy donors and patients with SSD. Scatchard plot analysis (Fig. 2) shows that the number of E_2 receptors in the patients' fibroblasts was 1.96 times ($p < 0.02$) less than in normal subjects. The affinity of the hormone for the receptor was unchanged in SSD, however, as shown by the value of the binding constant K_b, which was $8 \cdot 10^{10}$ M^{-1} in normal individuals and $8.5 \cdot 10^{10}$ M^{-1} in SSD Consequently, in the modern view, weakening of the effect of E_2 on the basic function of fibroblasts in patients with SSD can evidently be attributed to a decrease in the number of receptors of the hormone in these cells.

The results of four experiments on normal material and three on material from SSD patients are given in Fig. 2. Determination of binding of the hormone, using only one point on the Scatchard plot (specific binding) made it possible to compare receptor binding of E_2 in a large group of healthy individuals (n = 16) and of patients with SSD (n = 13). In eight strains from SSD patients receptor binding was absent or was at a very low level, and in the remaining five cases it did not exceed 50-60% of the normal level, A low level of specific binding of E_2 (13% of normal, $p < 0.01$) was observed in strains from patients with a less favorable type of course of the disease (acute or subacute). Binding of the hormone in cells from patients with chronic SSD (Fig. 3) was found in all strains tested, and was on average depressed by about half ($p < 0.02$). These results show that the degree of decrease in the number of E_2 receptors corresponds to the severity of the pathological process. The

possibility cannot be ruled out that an unfavorable course of the disease process may be due to loss of hormone receptors not only by skin fibroblasts, but also by cells of other types of connective tissue.

Considering that smooth-muscle cells and vascular endothelial cells synthesize relatively large quantities of type III procollagen [3], control of the synthesis of this isoform by E_2 is evidently particularly important. Damage to blood vessel walls, characteristic of SSD, may be connected with weakening of the inhibitory effect of E_2 (coupled with loss of receptors), resulting in excessive accumulation of type III collagen in blood vessel walls. In addition, an excess of type III collagen may be a factor in the development of vascular lesions, for ability to induce platelet aggregation is a feature of this isoform to a greater degree than of others [7].

This investigation demonstrated the importance of the principal female sex hormone E_2 in the regulation of fibroblast function. E_2 is known to be present also in the male, but in a much lower concentration. Dependence of tissues in the female on E_2 must evidently be greater than in the male. Lowering of the blood E_2 level in women with SSD was found in our previous investigation [1], and weakening of the regulatory action of E_2 on connective tissue cells ought to be manifested mainly in women, and this to a certain extent explains the linkage between sex and the incidence of SSD.

In conclusion, the authors are grateful to L and J. Risteli for help with the technique of determination of type III procollagen, and for providing reagents, and also to Professor K. Kivirikko for interest in the work and providing facilities for it in the Department of Clinical and Medical Chemistry, University of Oulu (Finland).

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