Comparison of the effects of dexamethasone and 13-cis-retinoic acid on connective tissue biosynthesis in human skin fibroblasts

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Summary. The effects of glucocorticoids and retinoids on connective tissue biosynthesis were studied in cultured human skin fibroblasts (HSFs). More specifically attention was paid to the effects of dexamethasone and 13-cis-retinoic acid (RA) on total protein and collagen synthesis and on collagen and fibronectin mRNA levels. The results indicated that dexamethasone reduced the relative collagen synthesis and collagen mRNA levels in HSFs and increased the total incorporation of proline into proteins, the latter effect being due to increased activity in the intracellular proline pool. 13-cis-RA did not affect collagen synthesis at the concentration studied (10⁻⁷ M) but it did reduce the corresponding mRNA levels. Simultaneous addition of both dexamethasone and 13-cis-RA or etretinate resulted in the largest decrease in type I and type III procollagen mRNA levels, indicating that retinoids do not oppose the effect of glucocorticoids on collagen synthesis in cultured HSFs. For comparison the effects of dexamethasone and 13-cis-RA on the mRNA levels of another extracellular matrix component, fibronectin, and of a constitutive enzyme, glyceraldehyde-3-phosphate dehydrogenase, were also studied. The results indicated that dexamethasone treatment did not alter fibronectin mRNA levels in HSFs, while 13-cis-RA did so to a marked extent. Both dexamethasone and 13-cis-RA also reduced the mRNA level of glyceraldehyde-3-phosphate dehydrogenase, indicating that glucocorticoids and retinoids have both similar and different effects on gene expression in HSF.

Key words: Dexamethasone — 13-cis-Retinoic acid — Human skin fibroblasts — mRNA

Glucocorticoids, which are extensively used for treating various skin diseases [15, 32], may cause atrophy of the skin [30]. Most experimental studies using animal models or fibroblast cultures have suggested that glucocorticoids reduce the synthesis of collagen [6, 11, 17, 19, 21, 25, 29], the major component in the connective tissue of the dermis [1], this being due to a decrease in the level of the corresponding mRNA suggesting pretranslational regulation [10, 17, 21, 29]. Several studies have nevertheless reported either no changes or even increased collagen synthesis after glucocorticoid treatment employing skin fibroblasts [3, 13]. These apparently conflicting results regarding the action of glucocorticoids on collagen synthesis are due to variations in the experimental conditions, type of steroid used, or even due to variation in cell types. In addition the failure to measure the specific activity of intracellular proline pools in several earlier studies may have resulted in incorrect results.

Clinically glucocorticoids and retinoids are sometimes used simultaneously. There exists experimental evidence that retinoids could oppose the effects of glucocorticoids on collagen production [7]. For these reasons the action of dexamethasone and 13-cis-retinoic acid (13-cis-RA) on collagen synthesis and intracellular proline pools, and the effect of dexamethasone and etretinate on collagen messenger RNA levels were studied in human skin fibroblasts.

Methods

Collagen synthesis studies

Human skin fibroblasts (HSFs; altogether three cell lines were used in the study) were preincubated for 4—48 h in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with dialyzed 5% fetal calf serum, 50 µg/ml ascorbate, 210 µg/ml l-glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml) in the presence of dexamethasone, etretinate, and 13-cis-RA (Hoffman-La Roche, Basel) as indicated. A solvent (ethanol) alone was added to control samples. The maximum ethanol concentration in all the experiments was less than 0.1%. The cells were then labelled with [¹⁴C]proline for 4—16 h. Thereafter, total radioactivity
and [14C]hydroxyproline were assayed from medium and cell fractions as described [12]. Part of the cell fractions were used for the assay of DNA after sonication [4]. Culture medium proteins were precipitated with ammonium sulfate. Aliquots of the precipitates were reduced and fractionated by electrophoresis on 6% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) and radioactive peptides were visualized by fluorography [2]. The relative amounts of fibronectin and procollagen were determined by densitometric scanning.

**RNA extraction and Northern blotting**

Total RNA was extracted using the method of Chirgwin et al. [5] from the human skin fibroblasts preincubated for 48 h in the presence of dexamethasone and retinoids as indicated. The cell cultures were washed with phosphate buffered saline and dissolved in 5.7 M guanidine isothiocyanate for RNA isolation; 10-μg aliquots of total RNA were denatured with glyoxal and dimethylsulfoxide, fractionated on 1% agarose gels, and blotted onto Pall Biodyne transfer membranes as suggested by the manufacturer of the membrane (Pall Process Filtration, Portsmouth, UK) [33]. After prehybridization and hybridization, the filters were washed under stringent conditions and the bound probe detected by autoradiography at -70°C using X-ray films and intensifying screens. As a control parallel gels were stained with ethidium bromide and ribosomal RNA was quantified by densitometer.

**Hybridization probes**

The following cDNA probes were used: pHCAL1 for human proα1(I) collagen mRNA [16, 34], pHFS3 for human proα1(III) collagen mRNA [28], pHF1 for human fibronectin [14], and pRGAPDH-13 for rat glyceraldehyde-3-phosphate dehydrogenase [9]. For the Northern hybridization, whole plasmids were nick-translated using 32P-dCTP. The amount of specific mRNAs present were estimated by densitometric scanning of multiple exposures of the X-ray films, and specific mRNAs were calculated per corresponding ribosomal RNA, which was measured as described above.

**Other assays**

The intracellular-specific activity of [14C]proline was measured by preincubating the cells in the presence of dexamethasone or retinoids for 48 h and labelling them for 4 h with [14C]proline. They were then rinsed with PBS and scraped into 20% (w/v) sulfosalicylic acid. Free proline was separated on a Jeol-AH amino acid analyser. Radioactivity was determined by liquid-scintillation counting, and the quantity of proline in the corresponding peak was determined by the ninhydrin reaction.

For the statistical analyses Student’s t-test was used.

**Results**

**Effects of dexamethasone alone or with 13-cis-RA on protein and collagen synthesis**

The first set of experiments examined the effects of dexamethasone on total protein and collagen synthesis in human skin fibroblasts by labelling the cells with [14C]proline as described. Dexamethasone in concentration of 10^-9 and 10^-7 M slightly inhibited the proliferation of fibroblasts, the decrease in DNA being 7.2 and 5.7%, respectively. Total [14C] proline incorporation increased by about 50%—70% in the presence of various concentrations of dexamethasone. After preincubation, the cells were labelled for 16 h with [14C]proline and total nondialyzable [14C]radioactivity and [14C]hydroxyproline assayed from cellular and medium fractions as described. A Total [14C]radioactivity in cells plus medium. B [14C]hydroxyproline ([14C]HOP) in cells plus medium. C Relative synthesis of [14C]hydroxyproline. The values are means from duplicate plates and are expressed per μg cellular DNA.