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13-*Cis* Retinoic Acid and Dexamethasone Modulate the Gene Expression of Epidermal Growth Factor Receptor and Fibroblast Proteoglycan 40 Core Protein in Human Skin Fibroblasts

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The effects of 13-*cis* retinoic acid (RA) and dexamethasone on the levels of epidermal growth factor (EGF) receptor and fibroblast derived proteoglycan core protein (PG40) mRNAs were studied in human skin fibroblasts. The EGF receptor is involved in the regulation of cellular proliferation and the synthesis of matrix proteins, and proteoglycan 40 is important for cell attachment and interaction with collagen and fibronectin. 13-*cis*-RA at a concentration of 10^{-7} M markedly reduced the levels of the EGF receptor and PG40 mRNAs, the decreases being 33 and 56 %, respectively. Dexamethasone reduced these mRNAs markedly less. Simultaneous treatment of the fibroblasts with 13-*cis*-RA and dexamethasone resulted in similar decreases in EGF receptor and PG40 mRNAs as with 13-*cis*-RA alone. Surprisingly, the proliferation rate of the fibroblasts was increased in the presence of dexamethasone under conditions similar to those which caused slight decrease in the EGF receptor mRNA levels. This indicates that glucocorticoids also affect the cellular growth by mechanisms which do not involve EGF receptors.

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Glucocorticoids and retinoids are extensively used for treating dermatological diseases (1, 2), and recent investigations into their mechanisms of action indicate that both bind to specific intracellular receptors, and further, that retinoid or steroid receptor complexes interact with chromatin (3–6).

Both glucocorticoids and retinoids modulate the growth of various cell types and the synthesis of matrix proteins (7–11). Thus glucocorticoids and retinoids have been shown to inhibit the synthesis of collagen and to reduce collagenase activity (8, 11). The effects of glucocorticoids on the proliferation of cells are more controversial, and increased, decreased or unaltered rates of proliferation have been detected (9). Retinoids suppress the proliferation of malignantly transformed cells (10).

Stimulus of the cells by growth factors is of great importance for cellular proliferation, so that the growth of both epidermal and mesenchymal cells is increased by growth factors such as epidermal growth factor (EGF) (12). Both retinoids and glucocorticoids have been shown to modulate the response of cells to EGF, perhaps by modulating its receptors of EGF (13–19). For these reasons the effects of retinoids and glucocorticoids on the gene expression of EGF receptors were studied here by measuring the messenger RNA of EGF receptors in human skin fibroblasts. The effects of EGF receptor mRNA were then compared with those on the mRNA of the major fibroblastic proteoglycan, PG40 core protein (20). This proteoglycan interacts with other matrix components such as collagen and fibronectin and participates in cell attachment.

The results indicate that retinoids and glucocorticoids reduce EGF receptor mRNA, and that they are also capable, either separately or simultaneously, of reducing the messenger RNA for PG40 core protein.

MATERIAL AND METHODS

Cell cultures

Human skin fibroblasts at passage 7 of subcultivation (obtained from healthy subjects) were preincubated for 48 h in Dulbecco's modified Eagle's medium (DMEM) supplemented with dialysed 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 (Sigma Chemical Co, Kingston-upon-Thames, Surrey, England), and 13-*cis*-RA (Hoffman, La Roche, Basle, Switzerland), as indicated at dark. A solvent (ethanol) alone was added to control samples. The maximum ethanol concentration in all the experiments was less than 0.1 %.

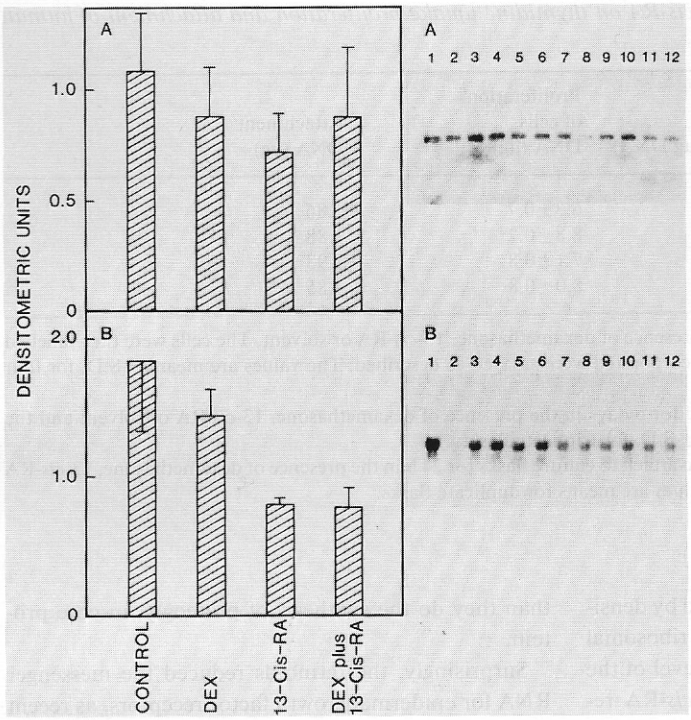

Fig. 1. Effects of dexamethasone and 13-*cis* retinoic acid on the levels of mRNAs for EGF receptors and core protein of proteoglycan 40 in human skin fibroblasts assayed by Northern blotting. The fibroblasts were incubated in the presence of dexamethasone (10^{-7} M), 13-*cis*-RA (10^{-7} M), or dexamethasone (10^{-7} M) plus 13-*cis*-RA (10^{-7} M) for 48 h. Total RNA was then extracted from the cultures, and 10 μg aliquots of RNA were fractionated on 1 % agarose gels, transferred to nylon membranes and hybridized with cDNA probes for EGF receptors (*A*) and proteoglycan 40 (*B*). Total RNAs extracted from three parallel cultures in each group were loaded for Northern blotting (panel *A* and *B* on right) as follows. Lanes *1–3,* control (sample 2 is partially degraded in panel *B* and is not included in the left column); lanes *4–6*, dex; lanes *7–9*, 13-*cis*-RA; lanes *10–12*, dex plus 13-*cis*-RA. Messenger RNA for EGF receptor is expressed in one band (see panel *A*, on right) and that for PG40 in two closely migrating bands (panel *B*, on right). The levels of the mRNAs for EGF receptors and PG40 after densitometric quantification are expressed in the columns on the left in (*A*) and (*B*) (mean ± SD.)

RNA extraction and Northern blotting

Total RNA was extracted *ad modum* Chirgwin et al. (21) from the human skin fibroblasts preincubated for 48 h in the presence of dexamethasone and 13-*cis*-RA, 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 dimethylsulphoxide, fractionated on 1 % agarose gels and blotted onto Pall Biodyne transfer membranes in the manner recommended by the manufacturer (Pall Process Filtration, Porthsmouth, Hants, England) (22). To quantify ribosomal RNA content, parallel gels were stained with ethidium bromide, photographed, and the amount of ribosomal RNA was quantified densitometrically from the negatives.

Hybridization procedures

A small chondroitin/dermatan sulphate fibroblast proteoglycan (PG40) mRNA levels were measured using 1.1 kb human specific cDNA clone as a probe (20). A 634 bp EcoR I–BamH I fragment of viral erbB gene (a gift from Dr Kari Alitalo) was used to measure EGF-receptor mRNA levels (23). This probe corresponds to 3′ end of the coding region of human EGF-receptor mRNA and is sufficiently homological for specific detection (24).

After prehybridization, the filters were hybridized with isolated inserts, which were nick-translated using ^{32}p-dCTP. The amounts 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, measured as descibed above.

Assay of DNA synthesis, proliferation and attachment of fibroblasts

DNA synthesis activity was estimated by determining the incorporation of [^{3}H]thymidine into the trichloracetic acid-precipitable material, as described elsewhere (25). To study the attachment and proliferation of fibroblasts, the same number of fibroblasts were inoculated into cell culture wells and numbers of cells and amounts of DNA assayed after 24 h and 6 days.

Student's *t*-test was used for the statistical analyses.

RESULTS

Effects on mRNA levels of EGF receptor and proteoglycan 40

The effects of dexamethasone and 13-*cis*-RA on the levels of the EGF receptor and proteoglycan 40 mRNAs were studied by hybridizing the total RNA with specific cDNAs for EGF receptors and PG40. Hybridization with EGF receptor cDNA revealed one major transcript: in the control cells and in those treated with either dexamethasone, 13-*cis*-RA, or dexamethasone plus 13-*cis*-RA, while hybridization with PG40 showed two closely migrating transcripts (Fig. 1). Thus no qualitative alteration in the sizes of the mRNAs could be observed following treatment with glucocorticoid or retinoid.

Table I. *Effects of dexamethasone and 13-cis-RA on thymidine uptake proliferation and attachment of human skin fibroblasts*

Treatment	Thymidine[a] (DPM×10^{-3}/μg DNA)	Proliferation[b] of cells DNA (μg)	Attachment[c] DNA (μg)
Control	42.7±3.5	6.3±0.7	1.86
Dex 10^{-7}M	52.2±13.4	8.8±0.2[d]	1.88
13-*cis*-RA 10^{-7}M	48.0±13.7	7.1±0.8	1.93
Dex 10^{-7}M+13-*cis*-RA 10^{-7}M	51.4±20.8	8.0±0.8	1.85

[a] Fibroblasts were preincubated for 48 h in the presence of dexamethasone, 13-*cis*-RA or solvent. The cells were then labelled with [^{3}H]thymidine, and the amounts of label incorporated were assayed as described. The values are means ± S.D. for four parallel wells.
[b] The same numbers of fibroblasts were cultured for 6 days in the presence of dexamethasone, 13-*cis*-RA or solvent and the amount of DNA assayed. The values are means ± S.D. for triplicate flasks.
[c] The same numbers of fibroblasts were allowed to attach to culture flasks for 24 h in the presence of dexamethasone, 13-*cis*-RA or solvent and the DNA content assayed. The values are means for duplicate flasks.
[d] Statistically different from control; $p<0.005$.

The specific mRNAs were then quantified by densitometer scanning and expressed relative to ribosomal RNA. Dexamethasone alone reduced the level of the EGF receptor mRNA by 18%, while 13-*cis*-RA reduced it by 33% ($p<0.2$). Dexamethasone plus 13-*cis*-RA reduced the level by 18% (Fig. 1). The levels of PG40 mRNA were reduced more extensively, the largest decrease being noted in the presence of 13-*cis*-RA (56%, $p<0.050$). Dexamethasone alone reduced the level of PG40 mRNA by 21%.

Effects on proliferation and attachment of fibroblasts

Dexamethasone stimulated proliferation rate by 40% ($p<0.005$, Table I). The attachment of fibroblasts was not altered in the presence of dexamethasone or 13-*cis*-RA (Table I).

DISCUSSION

The results indicate that glucocorticoids and retinoids modulate the gene expression of the epidermal growth factor receptor and of extracellular matrix proteoglycan core protein (PG40). 13-*cis* retinoic acid caused the largest decrease in both EGF receptor and PG40 mRNA levels, at a concentration, which is clinically relevant. In contrast, dexamethasone only slightly reduced the EGF receptor and PG40 messenger RNA levels. Glucocorticoids have previously been shown to reduce the synthesis of glycosaminoglycans (GAG) in fibroblasts (26). It is possible that they may affect the GAG synthesis of proteoglycans more markedly than they do the synthesis of proteoglycan core protein.

Surprisingly, the retinoids reduced the messenger RNA for epidermal growth factor receptors, as recent experiments employing mouse embryo palatal mesenchymal cells suggest that retinoic acid increases the number of EGF receptors (13). The discrepancy remains unexplained, although the different models used (human skin fibroblasts in this case) and the presence of growth factors in fetal calf serum used for cell culturing could contribute to the different results. In any case, the regulation of cellular growth by retinoids might be mediated partially through EGF receptors.

In order to correlate the results obtained regarding EGF receptor and PG40 messenger RNA levels, the proliferation rate and attachment of the fibroblasts were studied. Surprisingly, the proliferation rate and thymidine uptake increased in the presence of dexamethasone, under conditions similar to those which caused a slight decrease in the EGF receptor mRNA concentrations. The attachment of fibroblasts was not altered in the presence of 13-*cis*-RA or dexamethasone.

It is possible that even though the amount of EGF receptor RNA decreased, dexamethasone was able to cause qualitative changes in the EGF receptors which could have induced an increased proliferation rate (16). At the same time, glucocorticoids affect other matrix proteins, notably collagen and fibronectin, which could also modulate the growth of fibroblasts (8, 9).

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