Biochemical Pharmacology, Vol. 35, No. 8, pp. 1283–1286, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

GLUCOCORTICOID INHIBITION OF FIBROBLAST CONTRACTION OF COLLAGEN GELS

PATRICIA E. VAN STORY-LEWIS* and HOWARD C. TENENBAUM†
Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20205, U.S.A.

(*Received* 12 *April* 1985; *accepted* 13 *September* 1985)

Abstract—When skin fibroblasts are grown in culture on collagen gels, the collagen gels contract. We have studied the effects of various steroids on the contraction process. Cortisol, β-estradiol and dexamethasone inhibited fibroblast-mediated gel contraction at low (10^{-8} to 10^{-9} M) concentrations whereas dihydrotestosterone was without effect. These effects were time and concentration dependent and could be reversed if the steroids were removed. This system may be useful for assaying the activities of various steroids in terms of their activities and modulating effects on connective tissue.

Collagen substrates greatly enhance the growth and development of various cells including, for example, fibroblasts and myoblasts [1–5]. This is not unexpected since these cells normally reside in tissues enriched in type I collagen [6–9]. It has also been noted that fibroblastic cells when cultured in collagen gels cause the gel to contract [10, 11]. The *in vitro* fibroblast-mediated collagen gel contraction system has many potentially important applications. This contracted, tissue-like collagen lattice has been proposed as a possible replacement for damaged skin [12–14]. Since fibroblasts at the margins of wounds contract, the *in vitro* contracting gel has potential as a model for wound healing studies [10, 15–18]. In addition, it has been used to study tooth eruption *in vitro* [19, 20].

Little is known about the mechanisms involved in fibroblast-mediated gel contraction. The rate of contraction is dependent on both the number of cells present and the presence of serum. In the absence of cells and/or sera, no contraction occurs. The cells spread in the presence of serum and contract the gels. There is an alignment of the collagen fibers in contact with the cells [21–23]. The contraction of collagen gels is prevented by inhibitors of protein synthesis, such as cyclohexamide, as well as by colchicine and by inhibitors of chronic inflammation, such as nonsteroidal anti-inflammatory agents [24]. Recently, corticosteroids at high concentrations (10^{-4} M) have also been shown to inhibit fibroblast-mediated gel contraction [25]. Due to the alleged role of matrix contraction in tooth eruption [11] and the inhibition of tooth eruption by certain steroids,‡ we became interested in determining whether steroids at near physiological levels could inhibit collagen gel contraction. We find that cortisol, β-estradiol and dexamethasone inhibit gel contraction at physiological levels.

MATERIALS AND METHODS

Materials. Human embryonic fibroblasts, strain CRL 1475, were obtained from the American Type Culture Collection, Rockville, MD. The cells were grown in Eagle's medium containing Earle's salt formula supplemented with 15% fetal calf serum (FCS), penicillin and streptomycin at 37° in an atmosphere of 5% CO_2 plus 95% air. Dexamethasone, hydrocortisone, β-estradiol and dihydrotestosterone were obtained from the Sigma Chemical Co., St. Louis, MO. All stock solutions were stored in ethanol at −20° and were added to tissue culture medium just prior to feeding the cells.

Preparation of the collagen solution. A dilution of Vitrogen 100 was made in Eagle's MEM (containing 15% FCS, $NaHCO_3$, and NaOH) to obtain a final concentration of 1.2 mg/ml. Generally, 3 ml of this material was used per 60 mm bacteriological petri dish.

Assay for gel contraction. Subconfluent cultures of fibroblasts which in some cases had been cultured for up to 24 hr previously with steroids were washed with 0.2 M $NaPO_4$, pH 7.4, containing 0.15 M NaCl (PBS). The cells were removed by incubation for 5 min with trypsin (0.25%) and (0.01 M) EDTA in Hanks' buffer, and trypsin was inhibited by the addition of 15% fetal calf serum. The cells were collected by centrifugation and resuspended in tissue culture medium; 9×10^5 cells/dish were added to the collagen solution. The cells and collagen were gently mixed by pipetting and then placed at 37° in the incubator. A solid gel containing dispersed cells formed within 1 hr. The amount of gel contraction was measured directly with a ruler after 24 hr and was found not to differ by more than 10% among triplicate samples.

* Address all correspondence to: Dr. Patricia E. Van Story-Lewis, NIH, NIDR, Bethesda, MD 20892.
† Visiting scientist from the Faculty of Dentistry, Department of Dentistry, Mount Sinai Hospital, University of Toronto, 600 University Ave., Toronto, Ontario, Canada M5G 1X5.
‡ P. E. Van Story-Lewis, manuscript in preparation.

RESULTS

Human skin fibroblasts caused a marked contraction of the collagen gel when present in the collagen gel. No contraction was observed when cells were omitted or when conditioned medium was used. We found, in agreement with others [10, 11, 26], that the amount of contraction depended on both the number of cells present (Fig. 1) and the presence of serum (data not shown).

Various steroids, added to the cells for 24 hr before the contraction assay as well as to the contraction assay, inhibited the fibroblast-mediated contraction of the collagen gels in a concentration-dependent manner (Figs. 1 and 2, Tables 1 and 2). Cortisol completely inhibited contraction, whereas β-estradiol and dexamethasone caused partial inhibition and dihydrotestosterone was essentially inactive. Cortisol caused an 80% inhibition of contraction at 10^{-9} M and 100% at 10^{-8} M. The maximum inhibitions observed with β-estradiol and with dexamethasone were 50 and 43% respectively. No inhibition was observed with dexamethasone at 10^{-7} M, due possibly to toxicity of the steroid.

We determined the length of time that the cells must be exposed to steroid in order to prevent contraction and whether the effects are reversible. When the cells were exposed to dexamethasone for 4 hr or less, no significant inhibition of contraction was observed (Table 1). These data suggest that an induction period is required for the steroids as would be expected based on their diversity of activities. The reversibility studies were carried out with cells treated for 24 hr with steroid. The cells were then trypsinized and finally replated in the presence or absence of a steroid on the collagen gels (Table 3). These experiments indicated that the effects of the steroid are reversible. Complete reversal of the effects of the steroids required a minimum of 12 hr of incubation without steroid.

Finally, we determined if the contraction of the collagen by the cells or the presence of the steroids caused a change in the size of the collagen in the gel. We isolated collagen from such cultures and compared it by sodium dodecyl sulfate (SDS) gel electrophoresis with the original material. The collagen in the gel cultures alone, with fibroblasts or without fibroblasts plus steroid, showed identical patterns, indicating that it had not been cross-linked or degraded by the cells (data not shown).

DISCUSSION

We have studied the effects of various steroids on fibroblast-mediated collagen gel contraction. Cortisol, β-estradiol and dexamethasone all inhibited contraction, whereas dihydrotestosterone was essentially inactive. Cortisol was the most potent, with 100% inhibition at 10^{-8} M. The maximum inhibitions observed with β-estradiol and dexamethasone were 50 and 43% respectively. We found that cortisol was more active than dexamethasone in our *in vitro* assay perhaps due to measurement of different activities

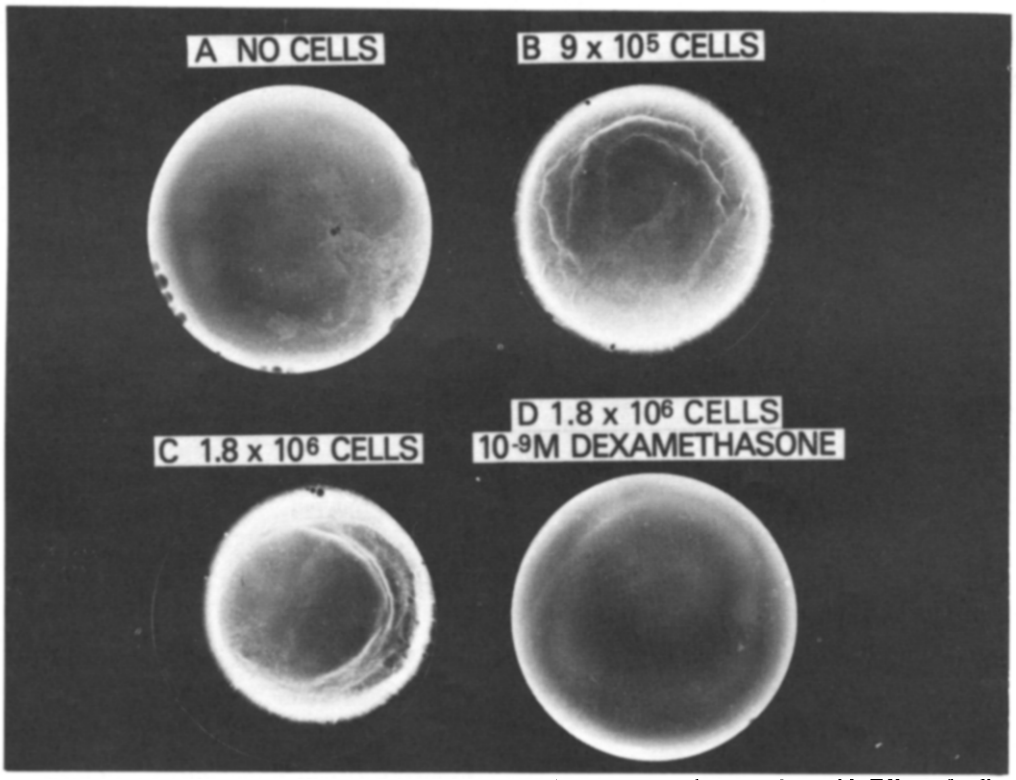

Fig. 1. Collagen gels in relation to number of cells and presence or absence of steroid. Effect of cell number on the contraction of collagen gels. Key: (A) no cells; (B) 9×10^5 cells caused partial contraction and (C) 1.8×10^6 cells caused maximal contraction; (D) 1.8×10^6 cells treated with 10^{-9} M dexamethasone did not contract the gel.

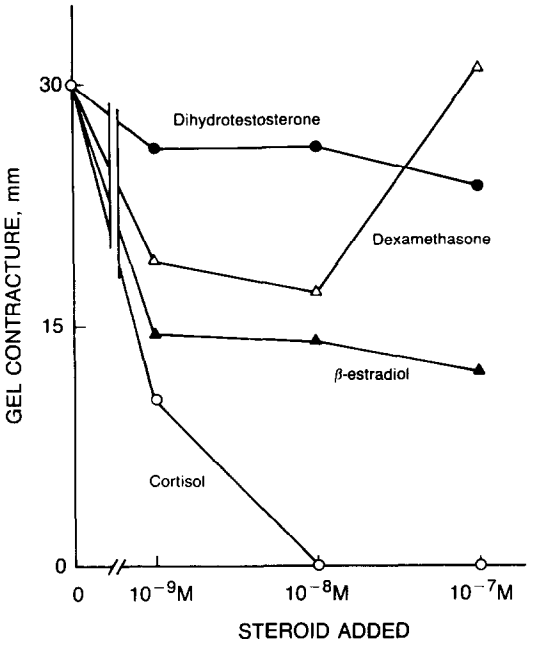

Fig. 2. Effects of various steroids upon gel contracture. Certain steroids added to the cells for 24 hr before as well as during the contraction assay inhibited the fibroblast-mediated contraction of the collagen gels in a concentration-dependent manner. Cortisol completely inhibited contraction, whereas β-estradiol and dexamethasone caused a partial inhibition and dihydrotestosterone was essentially inactive.

Table 2. Effects of steroids on collagen gel contraction

Steroid added*	Amount of contraction (mm)	Inhibition (%)
Control (no steroid)	30	0
Dihydrotestosterone	27	10
β-Estradiol	14	53
Cortisol	0	100

* All cultures were pretreated with 10^{-8} M of each steroid for 24 hr. These steroids were also present during contraction.

Table 3. Reversibility of dexamethasone effect on collagen gel contraction

Dexamethasone* (preincubation) (hrs)	Incubation† (hr)	Amount of contraction (mm)	Inhibition (%)
0‡	0	10.5	0
24§	0	0	100
24	6	0	100
24	12	7.0	33
24	24	13.5	−35‖

* All cultures were incubated with 10^{-9} M dexamethasone for 24 h at 37°.

† After 24 hr of incubation with 10^{-9} M dexamethasone, all cultures were rinsed in PBS and allowed to incubate in the presence of Eagle's medium containing 15% FCS at 37° for the specified amount of time.

‡ This was the control sample which means cells were never in the presence of dexamethasone.

§ Steroid was not present in gel assay.

‖ Negative number indicates stimulation of contraction.

(i.e. receptor levels) or metabolism of the compounds. Time–course studies showed that a preincubation period of at least 24 hr was necessary to obtain inhibition of contraction. The steroid-induced inhibition was reversible. It was found that 12 hr of recovery time (incubation in culture medium without steroid) was necessary to reverse the steroid effect.

Previous studies have employed steroid concentrations of a 3- to 4-fold greater magnitude in order to achieve a similar response [25]. The sensitivity which we achieved at physiological levels may be due to our treating the cells with steroid prior to incorporating them within the gel. It may also be possible that the gel state itself inactivates the steroid effect or desensitizes the cells while in the gel.

The mechanism of action of the steroids on the gel contraction is not known. It is possible that an inhibitory protein is elaborated by the cells or that the cells are unable to synthesize the necessary stimulatory protein. The requirement for protein synthesis

Table 1. Effect of dexamethasone pretreatment of cells on collagen gel contraction

Dexamethasone pretreatment time* (hr)	Steroid added to collagen	Amount of contraction (mm)	Inhibition (%)
0	+	21.0	0
0	−	21.0	0
1	+	18.0	14
2	+	19.0	10
3	+	22.2	−6†
4	+	23.0	−10
24	+	0	100

* All cultures were pretreated for the times indicated with 10^{-9}M dexamethasone in Eagle's medium + 15% FCS. The cells were then trypsinized and plated at 3×10^4 cells/ml/dish in collagen matrices in the presence or absence of 10^{-9} M dexamethasone.

† Negative values indicate stimulation of contraction.

is consistent with the 12 hr required to reverse the effects. Collagen may play a role in the steroidal effect. For example, it is well documented that patients on corticosteroids have impaired wound healing [27] which is due, in part, to decreased collagen synthesis [26, 28, 29]. It is also known that transformed cells and fibroblasts from patients with certain heritable dermatological conditions, where collagen synthesis is altered, do not cause contraction of the collagen gels [24, 30]. However, it is likely that other proteins are affected as well by the steroids. In addition, there appears to be no structural changes in the collagen present in the gel either with contraction or in the presence of steroids. Thus, the steroids block gel contraction through some alterations of the cells.

These studies show that steroids inhibited cell-mediated collagen gel contraction at physiological levels and that the effects were reversible. They suggest that steroids may delay wound healing through mechanisms involving cell–matrix interactions. Since corticosteroids impair wound healing and gel contraction, it is possible that this *in vitro* model system can be used to determine the mechanism of this regulatory effect on wound repair.

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