Journal of Cellular Biochemistry 92:6–15 (2004)

PROSPECT

How do Glucocorticoids Compare to Oligo Decoys as Inhibitors of Collagen Synthesis and Potential Toxicity of These Therapeutics?

Kenneth R. Cutroneo[1]* and Kenneth M. Sterling, Jr.[2]

[1]Department of Biochemistry, College of Medicine and the Cell and Molecular Biology Program, University of Vermont, Burlington, Vermont 05405
[2]Dartmouth College, Department of Physics and Astronomy 6127 Wilder Laboratory, Hanover, New Hampshire 03755-3528

Abstract This article demonstrates how glucocorticoids decrease collagen synthesis. The parameters used to assess procollagen synthesis in our laboratory will be compared to those used by others. This article will note all the pertinent literature on the molecular mechanisms of this down regulation of procollagen synthesis. For example, what are the effects of glucocorticoids at the levels of transcription and translation of collagen mRNAs? Finally, we will define a molecular mechanism to inhibit Type I collagen synthesis by decreasing the binding of the TGF-β activator protein complex to the TGF-β element in the distal promoter of the *proα1* Type I collagen gene, preventing the 2:1 ratio of α1 to α2 chains in the processed Type I collagen molecule. We will next ask "How do sense oligo decoys decrease Type I collagen synthesis at the in vivo and at the cell levels?" In primary fibrotic cell culture, the double-stranded phosphorothioate oligodeoxynucleotide decoys were more effective than their sense single-stranded counterparts. The molecular mechanism for the decrease in Type I collagen synthesis is the same as glucocorticoids, that is by decreasing the binding of the TGF-β activator protein complex to the TGF-β element in the distal promoter of the *proα1* Type I collagen gene for the transcription of the proα1 mRNAs. The reason for using sense oligo decoys as anti-fibrotic agents as compared to the anti-fibrotic glucocorticoids, is that presently marketed and FDA approved glucocorticoids have many untoward side effects which the sense oligo decoys do not have. J. Cell. Biochem. 92: 6–15, 2004. © 2004 Wiley-Liss, Inc.

Key words: TGF-β element; TGF-β activator protein complex; TGF-β1; Type I procollagen; Type III procollagen; procollagen antibodies; propeptide antibodies; Proα1 (I) collagen gene transcription; collagen; glucocorticoids; sense oligo decoys; extracellular matrix (ECM); chloramphenacol acetyltransferase (CAT)

The amelioration of the fibrotic response is to inhibit the synthesis of the extracellular matrix (ECM) components in fibrous tissues. Type I collagen is a major fibrous protein of the ECM. Therefore, the inhibition of Type I collagen synthesis would enhance the amelioration of fibrogenesis. In a previous review, Cutroneo [1993] demonstrated that glucocorticoids regulate procollagen synthesis in a number of different tissues and cells. This prospect article will predominantly focus on the effect of glucocorticoids on inhibiting collagen synthesis in fibroblasts. The studies cited will form the basis for inhibition of collagen synthesis at the in vivo, cell, and molecular levels.

We have designed phosphorothioate oligodeoxynucleotides (ODNs) decoys, either single-stranded (ssPTs) or double stranded (dsPTs), to inhibit collagen synthesis by the same molecular mechanism as glucocorticoids. These oligo decoys have the TGF-β element which binds to the TGF-β activator protein complex and inhibits its binding to the TGF-β element in the distal promoter of the *proα1* Type I collagen gene, thus inhibiting Type I collagen synthesis like glucocorticoids (Fig. 1).

The major difference between glucocorticoids and the ODNs is that glucocorticoids have many

*Correspondence to: Kenneth R. Cutroneo, Department of Biochemistry, College of Medicine and the Cell and Molecular Biology Program, University of Vermont, Burlington, VT 05405. E-mail: kenneth.cutroneo@uvm.edu

Received 19 December 2003; Accepted 23 December 2003

DOI 10.1002/jcb.20030

© 2004 Wiley-Liss, Inc.

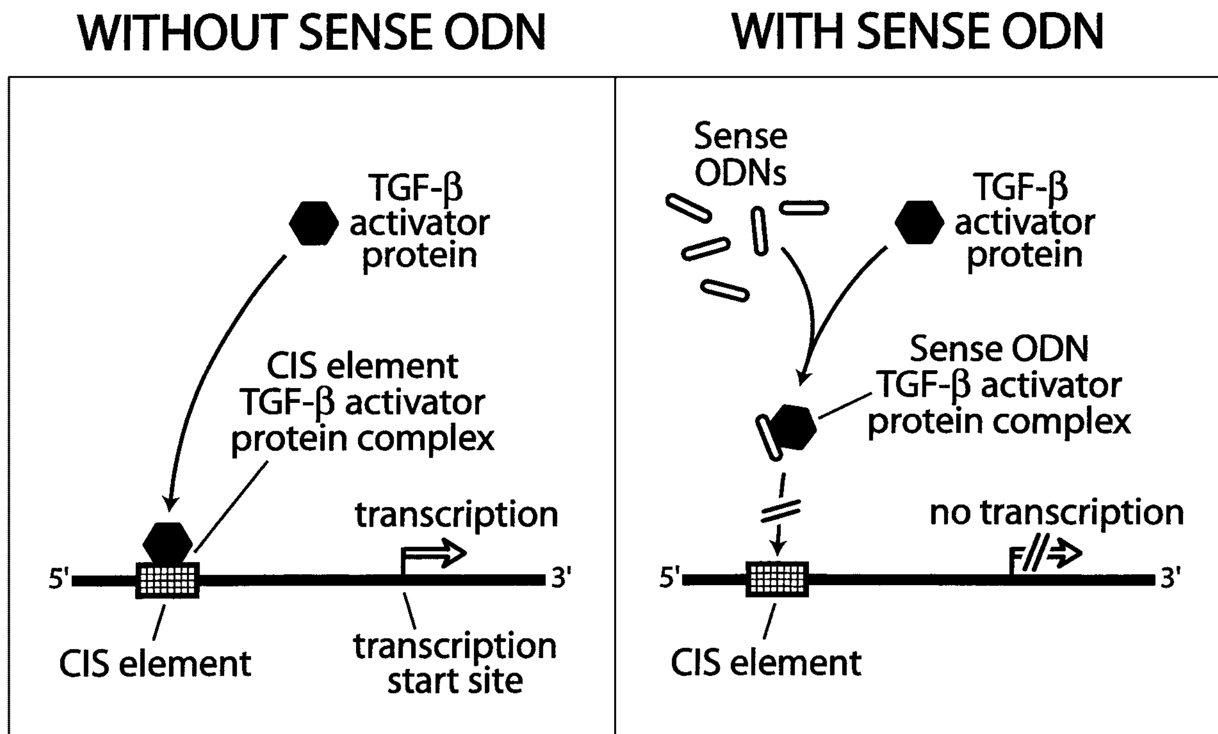

Fig. 1. Diagrammatic representation of the components and interactions of TGF-β activator protein complex with the TGF-β element 5′-TGCCCACGGCCAG-3′ in the absence of sense phosphorothioate oligodeoxynucleotides containing the TGF-β element and in the presence of sense oligodeoxynucleotides (ODN) decoy.

toxic effects, which make them contraindicated in treatment of various disorders. However, the ODNs used at below clinical doses do not possess these toxic effects. In this article, we will first present data demonstrating that glucocorticoids inhibit collagen synthesis and then focus on the ODNs as inhibitors of parameters of fibrogenesis in vivo, in cell culture, and at the molecular level.

HOW DO GLUCOCORTICOIDS INHIBIT COLLAGEN SYNTHESIS?

In 1971, the effect of glucocorticoids on granuloma tissue prolyl hydroxylase was described. The effect was an inhibitory effect on either the amount or activity of this enzyme [Cutroneo et al., 1971]. This prolyl hydroxylase activity inhibition was later shown to be temporally related in pre-existing granulomas as compared to other tissues [Counts and Cutroneo, 1979]. The inhibitory effect of glucocorticoids on prolyl hydroxylase activity was also demonstrated in various organs from animals of different ages [Cutroneo et al., 1975]. The effect of glucocorticoids on collagen synthesis was selective since collagen synthesis was decreased to a greater extent than non-collagen protein synthesis [Cutroneo and Counts, 1975]. Collagen accumulation in skin occurred in the absence of collagen degradation in the absence of glucocorticoids during normal growth and in glucocorticoid-treated rats was essentially a function of only collagen synthesis [Jeffrey et al., 1985].

In an interesting study, Cutroneo et al. [1990] demonstrated that the decrease of collagen synthesis by corticosteroids was not shown to be related to their anti-inflammatory activity as measured by the size of the sponge-induced granuloma after treatment, but related to the specific corticosteroid used to inhibit collagen synthesis in the skin. The selective inhibitory effect of glucocorticoids on collagen synthesis as compared to non-collagen protein synthesis was further demonstrated by Rokowski et al. [1981] in which these investigators isolated polysomes from lung and dermis of neonatal rats treated

with glucocorticoid and isolated mRNA by oligo-dT cellulose chromatography. The mRNA was then translated in a nuclease-treated reticulocyte lysate system in the presence of radioactive proline and collagen synthesis and non-collagen synthesis was determined by the collagenase digestion assay. Collagen synthesis was selectively decreased. Later Sterling et al. [1983] demonstrated that dexamethasone treatment decreased the amounts of Type I procollagen mRNAs in neonatal chick skin and in chick lung fibroblasts in a dose-dependent selective manner. This was later followed by the studies of Cockayne et al. [1986] in which they demonstrated that glucocorticoids decreased the synthesis of Type I procollagen mRNAs without affecting the degradation of these mRNAs. These studies also demonstrated that the beta actin content and beta actin mRNA steady-state level were not altered by glucocorticoids in chick skin fibroblasts. The inhibitory effect of glucocorticoids on procollagen synthesis was therefore related to a decrease in total Type I procollagen mRNAs. The effect of dexamethasone on Type I procollagen mRNAs were receptor-mediated as determined by pretreatment with the glucocorticoid antagonists progesterone and RU486 and with the agonist beta dihydrocortisone.

Glucocorticoids coordinately decrease procollagen Type I and procollagen Type III collagen syntheses [Shull and Cutroneo, 1983]. Type I and Type III procollagen antibodies were raised in rabbits and were made monospecific by chromatography on collagen and procollagen affinity columns. The antibodies were determined to be monospecific by direct linked and enzyme linked immunoabsorbent assays and enzyme linked immunoabsorbent assay inhibition. The coordinate decrease was seen not only in lung, but also in skin of neonatal rats treated with glucocorticoid. These results agree with the effects of hydrocortisone on human dermal fibroblasts [Russell et al., 1989]. These investigators demonstrated that Type I and Type III procollagen mRNAs were decreased to the same level in human normal dermal fibroblasts. Another study demonstrated that Type I and Type III procollagen mRNA synthesis in nuclei in vitro and gene expression were regulated to the same extent by glucocorticoids during intestinal development [Walsh et al., 1987].

Glucocorticoids have been shown to reduce collagen propeptide levels in serum of patients, in suction blisters, and in cell culture. These studies determined the propeptides of Type I procollagen and Type III procollagens. The procollagen peptides of Type I and Type III collagen have also been used to determine the effect of systemic glucocorticoids on the synthesis of Type I and Type III collagen in human skin in vivo. Type I and Type III collagen synthesis in intact skin was investigated by measuring the carboxy-terminal and amino-terminal propeptides of Type I procollagen and the amino terminal propeptide of Type III procollagen in suction blister fluid. These results indicated that systemic glucocorticoid treatment suppresses the synthesis of both Type I and Type III collagen in dermis and suggested that many of the side effects of these drugs, such as atrophy of the skin, were due to inhibition of collagen synthesis [Autio et al., 1993, 1994]. In another study by measuring the amino terminal propeptide of Type I procollagen and the amino terminal propeptide of Type III procollagen in skin blisters after glucocorticoid treatment, the order of inhibitory potency of three glucocorticoids on the propeptides was hydrocortisone less than hydrocortisone-17-butyrate and betamethasone [Haapasaari et al., 1995]. These investigators concluded that this assay of collagen propeptides from suction blisters may be used to screen various steroids with respect to their action on collagen synthesis and the side effect of dermal atrophy. One-week treatment of human skin with mometasone and betamethasone-17-valerate was determined on collagen synthesis in human skin in vivo by analyzing procollagen propeptides in suction blisters. One-week treatment had no detectable influence on skin thickness and the effect of these glucocorticoids on collagen synthesis was seen to have the same effect in human skin in vivo [Koivukangas et al., 1995].

By monitoring the amino terminal propeptides of Type I and Type III collagen in suction blisters after short-term topical corticosteroid treatment which occurred during a 2 week period prior to the start of corticoid treatment, collagen synthesis recovered to about 50% the level seen in untreated skin, indicating that collagen synthesis is not completely normalized in human skin even during a 2 week corticoid free period [Haapasaari et al., 1996]. The radioimmunoassays for the procollagen peptides of Type I and Type III procollagen were also determined in suction blisters from human

skin treated with hydrocortisone, methylprednisolone aceponate, and momethasone furonate. It was concluded that hydrocortisone decreases the concentration of procollagen peptides in human skin in males to nearly the same extent as methylprednisone aceponate and momethasone furonate [Haapasaari et al., 1997].

Other studies indicate that glucocorticoids preferentially interfere with the synthesis and degradation more dramatically of Type III collagen which is known to play a major role in the initiation of wound healing [Oishi et al., 2002]. In a study by Mintz and Mann [1990], these investigators raised antibodies to Type I and Type III procollagen peptides and showed somewhat similar effects on the synthesis of these procollagens in the culture-media of rat lung fibroblasts grown in the presence of glucocorticoids. In another study by Shull and Cutroneo [1986] glucocorticoids treatment was shown to change the ratio of Type III to Type I procollagen extracellularly by a pulse chase experiment in which the Type I and Type III procollagens were isolated by immunoprecipitation with monospecific polyclonal antibodies prepared as described [Shull and Cutroneo, 1983]. Besides this differential effect in fibroblasts in culture, we also demonstrated that the intracellular and extracellular Type I and Type III procollagens were decreased coordinately by glucocorticoid treatment. However, although the ratio of Type III and Type I procollagen was the same for control and glucocorticoid treated cells, in rat skin fibroblasts incubated with radioactive proline and chased in the presence of unlabeled proline, the ratio of Type III to Type I extracellular procollagen remained constant for glucocorticoid-treated cells and increased for control fibroblasts. Since many steps occur between the synthesis of procollagen at the translational level and the secretion of the procollagen molecule and the enzymatic cleavage of the procollagen peptides from the procollagen molecules, it would appear that measurement of the propeptides may give erroneous results compared to determining the actual synthesis of Type I and Type III procollagen after glucocorticoid treatment by monospecific antibodies and radioimmunoassay. It is also necessary when determining the glucocorticoid effects on the synthesis of procollagen types by quantifying propeptides, to measure the effects of these steroids on the procollagen peptidases. In addition, lung fibroblasts exist as subpopulations with different amounts of Type I and Type III procollagen mRNAs as shown by Breen et al. [1990] using a fluorescent based antibody method employing a cell sorter to separate the rat fibroblasts into subpopulations before isolation of Type I and Type III mRNAs and quantifying by Northern analysis. The subpopulations of fibroblasts after isolation using a fluorescent cell sorter were readily propagated for at least four passages.

The reader is directed to a recent article, which is entitled "How is Type I procollagen synthesis regulated at the gene level in tissue fibrosis?" [Cutroneo, 2003a]. This article indicates that active TGF-β is a major cytokine acting extracellularly which stimulates the transcription of the *proα1* and *proα2* Type I collagen genes during tissue fibrosis. Secondly, the signals transmitted by this extracellular profibrotic, occurring on the cellular membrane to the nucleus for the transcription of *proα1* and *proα2* collagen genes are described. Thirdly, the signaling pathways which crosstalk resulting in expression of Type I collagen genes are described. TGF-β1 affects ECM homeostasis. Nuclear factors corresponding to DNA elements are defined which are required for the promotion of the *proα1* Type I and *proα2* Type I collagen genes. This article stresses how the *proα1* Type I and *proα2* Type I collagen genes are regulated during tissue fibrosis. Finally, strategies are presented for reducing fibrosis which is a result of overexpression of coordinately regulated Type I and Type III collagen genes and TGF-β1.

CYTOKINES, WHICH INFLUENCE COLLAGEN SYNTHESIS

IGF1 maintained collagen formation in human fibroblasts is suppressed by the cytokines TNFα and IFNγ. These interactions are influenced by dexamethasone. The proinflammatory cytokines interact in a complex manner with other serum factors to modulate IGF1 stimulated ECM production and have important roles in regulating tissue repair [Bird and Tyler, 1995].

The down regulator of collagen synthesis, glucocorticoid [Cutroneo, 2002], and the upregulator of collagen synthesis, bleomycin, the fibrogenic anti-cancer drug, are regulated at the nuclear level by TGF-β through TGF-β activator

protein complex binding to the TGF-β element in the distal promoter of the *proα1* Type I collagen gene. Corticoids regulate Type I procollagen promoter activities in stably transfected cells through the TGF-β response element (Fig. 2) which is a novel mechanism for glucocorticoid regulation of the procollagen genes [Meisler et al., 1995]. Glucocorticoid treatment of fibroblasts results in their nuclear extracts having a decrease while bleomycen treatment results in an increase of TGF-β activator protein complex binding to the TGF-β element in the 5′ flanking region of the *proα1* Type I collagen gene. This causes a decrease in transcription of the collagen gene (Fig. 3) and a decrease in Type I collagen synthesis. The wild-type TGF-β element is required for the *proα1* Type I collagen gene transcription (Fig. 4). Perez et al. [1992] also demonstrated that glucocorticoids regulate the proα2 Type I procollagen promoter activity in stably transfected fibroblasts.

TGF-β is a known fibrogenic cytokine, which increases ECM protein synthesis and in particular the fibrous Type I collagen. Dexamethasone has the opposite effects on collagen synthesis, both in low passage human dermal fibroblasts in culture [Slavin et al., 1994] and in granuloma and granulation tissue fibroblasts [Meisler et al., 1997]. When used in conjunction with TGF-β1, glucocorticoids may normalize the effect of TGFβ-1 on collagen synthesis thereby reducing excessive collagen deposition and fibrosis [Parrelli et al., 1997]. TGF-β is the crossroad for glucocorticoid and bleomycin regulation of collagen synthesis [Shukla et al., 1999].

GLUCOCORTICOIDS AND FIBROSIS

In puromycin amino nucleoside fibrosis as glomerular mRNA encoding the basement membrane components and mRNAs encoding for the interstitial collagens Type I and Type III are elevated, these are markedly decreased by methylprednisolone administration which results in the amelioration of enhanced gene expression in both glomeruli and medulla in this type of necrosis [Nakamura et al., 1991]. Glucocorticoids can also serve as an effective therapeutic approach to abnormal ECM regulation at the onset of nephritis [Nakamura et al., 1992]. In humans, reoccurring keloid growth with surgical excision is accompanied by immediate treatment with triaminolone acetonide injections into the wound bed and healing of the wound is not compromised by the inhibition of Type I collagen gene expression [Kauh et al., 1997].

Bleomycin effects on collagen synthesis have been studied both in cell culture and in vivo. After intratracheal instillation of bleomycin in vivo, collagen accumulates in the lung. Collagen accumulation in the lung is associated with an increase in prolyl hydroxylase activity [Sterling et al., 1982a]. There was a twofold to

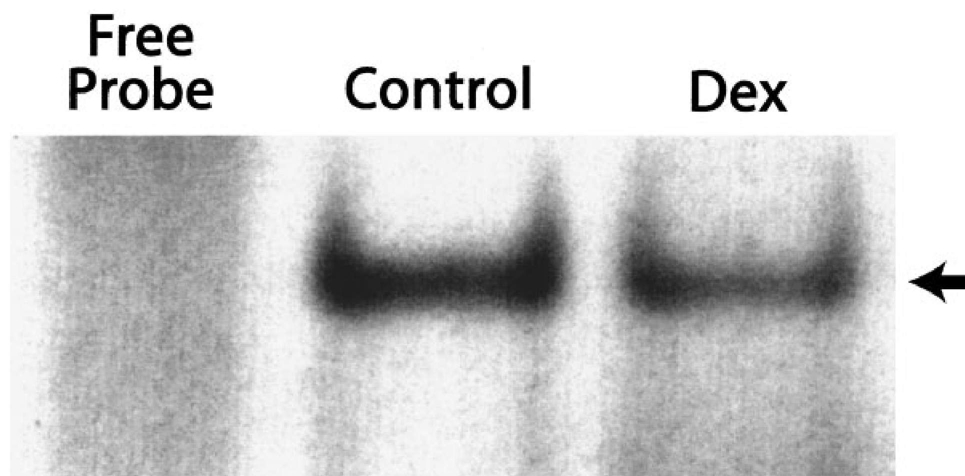

Fig. 2. Effect of dexamethasone on nuclear protein bound to the TGF-β activating element. Skin fibroblasts were treated with dexamethasone. ^{32}P-end labeled double-stranded oligonucleotide containing the TGF-β element sequence (5′-TGCCCACG GCCAG-3′) was incubated with nuclear protein extract. Reaction were separated on 6% polyacrylamide gels. Gels were air-dried and exposed to X-ray film.

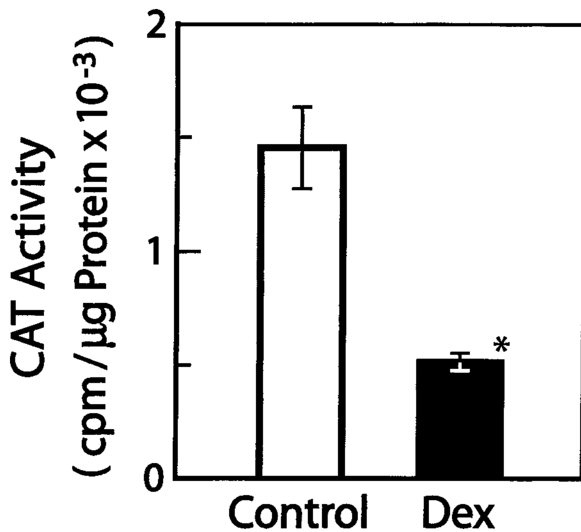

Fig. 3. Effect of dexamethasone on the proα1 (1) collagen promoter activity in skin fibroblast cells stably transfected with a plasmid containing a portion of the 5′ flanking region of the *proα (I)* collagen gene linked to CAT. Control cells and cells treated with dexamethasone were lysed and cell lysates were assayed for CAT activity. *Significantly different from control values at $P < 0.01$. Values represent the mean ± SE of data from three to four separate dishes.

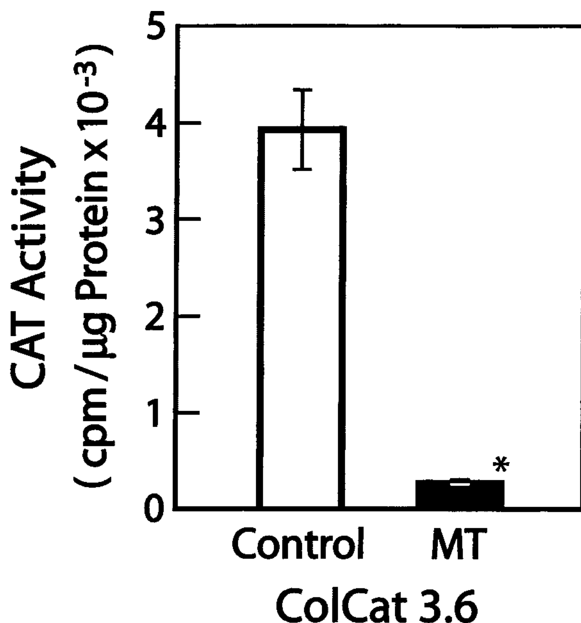

Fig. 4. The TGF-β response element is required for proα1 (I) collagen promoter activity. Skin fibroblasts were stably transfected with wild-type plasmid or a mutated TGF-β response element (MT) plasmid containing 5′-TGTGCGCGGCCCT-3′. Cells were grown to late log phase, and cell lysates were prepared and assayed for protein content and CAT activity. *Significantly different from control values at $P < 0.01$. Values represent the mean of four samples ± SEM.

fourfold of total RNA during the first week after bleomycin instillation above control. However, mRNA levels for procollagens and fibronectin were selectively elevated during the first week [Kelley et al., 1985]. When late phase human fetal fibroblasts were incubated with bleomycin for 48 h, collagen synthesis was increased selectively as compared to non-collagen protein synthesis [Sterling et al., 1982b]. Furthermore, polysomes isolated from the bleomycin-treated cells synthesized significantly more collagen in the wheat germ lysate system as compared to control polysomes.

Bleomycin regulates TGF-β1 in rat lung fibroblasts at the mRNA level, the protein level and the level of transcription [Breen et al., 1992]. At the level of transcription bleomycin stimulates proα1 Type I collagen promoter activity through transforming growth factor beta response element in the *proα1* Type I collagen gene by intracellular and extracellular signaling. In this latter study several constructs containing the rat proα1 Type I collagen promoter linked to the chloramphenacol acetyltransferase reporter gene were transfected into rat lung fibroblasts either stably or transiently. Results of both stable transfection and transient transfection were similar [King et al., 1994].

The fibrogenic effect of TGF-β1 in human IMR90 embryonic lung fibroblasts was promoted by an autoinduction of TGF-β [Kelley et al., 1993]. Therefore, the fibrogenic effect of this cytokine is enhanced once it is secreted, activated, and then reacts with receptors on the fibroblast membranes to transmit signals to the *TGF-β* genes to produce more TGF-β mRNA which results in more fibrosis. It is noteworthy that glucocorticoids down regulate TGF-β in adult rat lung fibroblasts [Shull et al., 1995]. Parrelli et al. [1998] also identified a glucocorticoid response element in the TGF-β1 gene promoter.

HOW DO OLIGO DECOYS SPECIFICALLY INHIBIT COLLAGEN SYNTHESIS?

One means of regulating gene expression is to use chemicals that alter the expression of all genes within a cell, tissue, or organism. For example, cyclohexamide blocks the peptidyltransferase reaction on eukaryotic ribosomes and acts as a general inhibitor of translation. Likewise, alpha amanitin globally blocks

mRNA synthesis by binding to eukaryotic RNA polymerase. Furthermore, actinomycin is capable of blocking RNA synthesis by intercalating guanosine, cytosine base pairs and disrupting transcription. Because all of these chemicals prevent the expression of all genes, any prolonged treatment results in the loss of critical factors needed to maintain the cells leading to irrepairable damage and cell death. To overcome this drawback, agents which self-regulate the expression of specific genes or gene families must be developed.

Another means of regulating gene expression is to activate or repress the signal transduction pathways that are responsible for regulating gene transcription. By activating or inhibiting the important steps in the pathway, for example, binding of specific molecules to receptors, entry of signaling molecules into cells or nuclei, covalent modification of enzymes or release or decrease of ions from organelles, gene expression can be activated or repressed. The release of modulator proteins from precise regulatory sites provide the strategy for gene-specific activation or repression. Thus, the art remains in need of the means for regulating expression of genes regulated by TGF-β during the process of fibrosis.

The sense decoys phosphorothioate oligodeoxynucleotides in which the sulfur is in place of one of the non-bridging oxygens in the phosphodioester bond are effective in inhibiting collagen synthesis in vivo, in cell culture and at the molecular level like glucocorticoids. In vivo Cutroneo and Chiu [2000] using sponge-induced granulomas indicated that sense single-stranded ODNs (ssPTs) containing the wild-type TGF-β response element designed to inhibit the rat *proα1* Type I collagen gene activity, resulted in anti-fibrotic activity based on their ability to reduce granuloma tissue formation and specifically inhibit collagen synthesis. The mutated ssPTs, on the other hand, or dexamethasone given at equal doses as ssPTs, failed to do so. These data suggest that the ssPTs have an anti-fibrotic effect and may be used to inhibit the development of fibrosis. The effect on collagen synthesis is specific in that collagen synthesis is decreased while non-collagen protein synthesis is not decreased by the sense ssPTs in the granuloma system unlike glucocorticoids, which have an inhibitory effect on non-collagen protein synthesis [Rokowski et al., 1981]. The mechanism by which the inhibition of collagen synthesis occurs in fibroblasts is by inhibiting the binding to the TGF-β activator protein complex thereby inhibiting the ability of the activated complex to translocate to the nucleus and bind to the TGFβ element in the distal promoter of the *proα1* Type I collagen gene thus inhibiting transcription and ultimately inhibiting the synthesis of Type I collagen (Fig. 1).

Using primary fibroblasts from liver granulomas of *Schistosoma mansoni*-infected mice, Cutroneo and Boros [2002] demonstrated that the ssPTs are less effective in inhibiting collagen synthesis than the dsPTs (Table I). Although the ssPTs inhibited collagen synthesis by 56%, the dsPTs inhibited collagen synthesis by 99%. In both cases, the ssPTs and dsPTs did not have toxic effects since non-collagen protein synthesis was not effected. These latter studies indicate that the TGFβ element containing sense ssPTs and their counterparts dsPTs may be successful therapeutic agents to inhibit hepatic fibrogenesis associated with hepatocellular carcinoma. The excessive fibrosis which occurs in abnormal wound healing is mostly mediated by persistent TGF-β and fibrosis which is an abnormal response to tissue injury. Because of the role of collagen in the wound healing process, the pharmacological control of collagen synthesis has been of paramount importance as a possible

TABLE I. The Effect of Single-Stranded and Double-Stranded Phosphorothioate Oligos Containing the TGF-β Element on Collagen and Non-Collagen Protein Synthesis in Fibroblasts Isolated From Liver Granulomas of Mice

	Percent synthesis of controls	
Treatment	Collagen	Non-collagen
ssPT (50μg/plate)	44.0 ± 16.3	115.3 ± 4.5
dsPT (50μg/plate)	0.6 ± 0.3*	109.2 ± 0.3

Fibroblasts were isolated from dispersed granulomas. Control cells and ssPT and dsPT transfected cells were incubated in AIM V media. Heat denatured cell lysates were clarified by centrifugation. The cell lysates were then submitted to protease-free bacterial collagenase digestion to determine the percent of proline incorporated into collagen and non-collagen protein. The values represent the mean ± SEM of four to six cultures in each group.
*Significantly different at $P < 0.05$. These studies were done in collaboration with Dr. Dov L. Boros in the Department of Immunology and Microbiology at Wayne State University School of Medicine.

way to abrogate abnormal wound healing and prevent irreversible fibrosis [Cutroneo and Chiu, 2000, 2003; Cutroneo, 2003b].

Considerable evidence has ensued on the importance of growth factors during regeneration for both cell proliferation and for stimulation of reparative cells to synthesize and secrete ECM proteins. During the healing process, if growth factor concentration is too high because of over-expression, abnormal wound healing, tissue fibrosis, and ultimately scarring will occur. Therefore, the growth factor concentration at the wound site has to be controlled specifically either by gene therapy or by titration of gene dosage. However, if there is a narrow window between the beneficial effects and toxic effect(s) of gene therapy, ODN decoy therapy may be used concurrently with gene therapy to control growth factor concentration(s) at the wound site. ODN decoys offer an alternate form of therapy for controlling gene dosage [Cutroneo and Chiu, 2003].

In summary, glucocorticoids have been shown to decrease collagen synthesis by decreasing the binding of the TGF-β activator protein complex to TGF-β element in the distal promoter of the *proα1* Type I collagen gene. Likewise ssPTs have been shown to decrease the binding of the TGF-β activator protein complex to the TGF-β element in the distal promoter of the *proα1* Type I collagen gene, thereby inhibiting *proα1* Type I gene transcription and Type I collagen synthesis. However, the dsPTs are more effective in inhibiting collagen synthesis than the ssPTs (Fig. 5).

Glucocorticoids have many toxic effects in vivo and are contraindicated in many types of diseases (Table II). In addition, although glucocorticoids selectively inhibit collagen synthesis we have shown consistently that these steroids also have the toxic side effect of inhibiting non-collagen protein synthesis [Cutroneo and Counts, 1975; McNelis and Cutroneo, 1978; Newman and Cutroneo, 1978; Counts et al., 1979; Rokowski et al., 1981; Sterling et al., 1983; Cockayne et al., 1986]. The ds ODNs may be excellent therapeutic agents since at doses that are clinically used they have shown no toxic effects. All toxicities associated with ds ODNs were reversible and occurred at doses well above those currently used in clinical trials [Henry et al., 1999]. There has also been no evidence of genetic toxicity and no changes in reproductive performance for fetal development.

Glucocorticoids down regulate TGF-β1 mRNA and total TGF-β activity as measured by inhibition of mink epithelial cell growth, in adult lung fibroblasts [Breen et al., 1992]. In accord with these findings, we have also identified a consensus glucocorticoid response element in the TGF-β1 gene promoter [Parrelli et al., 1998]. We have identified glucocorticoids which down regulate collagen synthesis. In addition, glucocorticoids neutralize the effect of TGF-β on the *proα1* Type I collagen gene [Meisler et al., 1995]. We have identified the relationship between bleomycin and TGF-β on the *proα1* Type I collagen gene expression. All the above findings led to the discovery of novel ODN decoys as anti-fibrotic agents which mimic the effects of glucocorticoids, however, do not have the untoward side effects of these steroids. Recently some pharmaceutical companies have made steroid derivatives which are devoid of

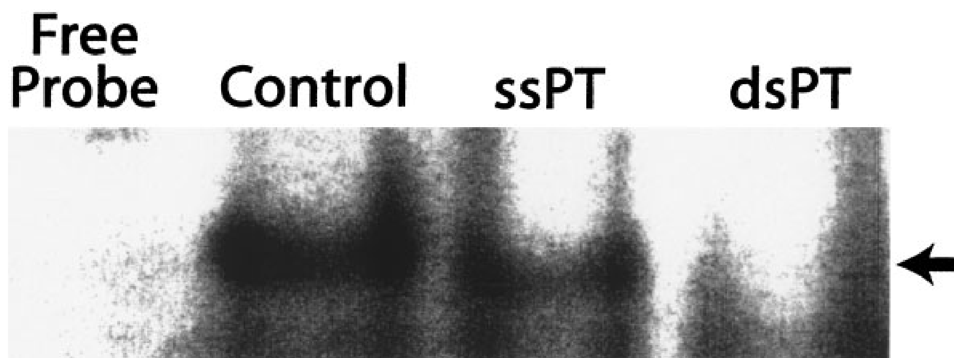

Fig. 5. In vitro competitive binding oligodeoxynucleotide experiment. Fibroblasts were grown to late log phase and the nuclear extract was prepared. The nuclear extract was incubated with ^{32}P double-stranded TGF-β oligodeoxynucleotide in the presence of either single-stranded phosphorothioate oligodeoxynucleotides (ssPTs) or double-stranded phosporothioate oligodeoxynucleotides (dsPTs). The samples were then submitted to gel mobility shift analysis.

TABLE II. Toxic Effects of Anti-Fibrotic Steroids

Skin atrophy and telangiectasia
Growth suppression
Muscle weakness
Osteoporesis
Peptic ulcers
Lymphoid tissue regression
Adrenal atrophy
Decreased plasma corticosterone
Decreased plasma ACTH
Cushing symptoms
Infection susceptibility
Glycosuria
Hyperglycemia
Electrolyte imbalance
Psychosis

many of the systemic toxic effects except osteoporesis which results, at least in part, from an inhibition of collagen synthesis. These companies are using cell culture to screen steroid derivatives for a lack or diminished effect on collagen synthesis. DiPetrillo et al. [1984] reported that two methylprednisolone derivatives neither inhibited skin collagen synthesis nor caused dermal atrophy in vivo. However, these same prednisolone derivatives when added to rat skin fibroblast cell cultures specifically inhibited collagen synthesis at the translational and transcriptional levels [Cutroneo et al., 1994].

REFERENCES

Autio P, Risteli J, Kiistala U, Risteli L, Karvonen J, Oikarinen A. 1993. Serum markers of collagen synthesis and degradation in skin diseases. Altered levels in diseases with systemic manifestation and during systemic glucocorticoid treatment. Arch Dermatol Res 285:322–327.

Autio P, Oikarinen A, Melkko J, Risteli J, Risteli L. 1994. Systemic glucocorticoids decrease the synthesis of Type I and Type III collagen in human skin in vivo, whereas isotretinoin treatment has little effect. Br J Dermatol 131:660–663.

Bird JL, Tyler JA. 1995. Tumour necrosis factor alpha, interferon gamma, and dexamethasone regulate IGF-I-maintained collagen production in cultured human fibroblasts. J Endocrinol 147:167–176.

Breen E, Falco VM, Absher M, Cutroneo KR. 1990. Subpopulations of rat lung fibroblasts with different amounts of Type I and Type III collagen mRNAs. J Biol Chem 265:6286–6290.

Breen E, Shull S, Burne S, Absher M, Kelley J, Phan S, Cutroneo KR. 1992. Bleomycin regulation of transforming growth factor-β mRNA in rat lung fibroblasts. Am J Respir Cell Mol Biol 6:146–152.

Cockayne D, Sterling KM, Shull S, Mintz KP, Illeyne S, Cutroneo KR. 1986. Glucocorticoids decrease the synthesis of Type I procollagen mRNAs. Biochemistry 25: 3202–3209.

Counts DF, Cutroneo KR. 1979. Temporal response of prolyl hydroxylase in pre-existing granuloma to glucocorticoid administration. J Pharm Pharmacol 31:780–782.

Counts DF, Rojas FJ, Cutroneo KR. 1979. Glucocorticoids decrease prolyl hydroxylase activity without the cellular accumulation of underhydroxylated collagen. Mol Pharmacol 15:99–107.

Cutroneo KR. 1993. Glucocorticoid regulation of procollagen synthesis. In: Zern MA, Reid LM, editors. Extracellular matrix. Chemistry, biology, and pathibiology with emphasis on the liver. New York, NY: Marcel Dekker, Inc. pp 351–368.

Cutroneo KR. 2002. Relationship between glucocorticoid-mediated early decrease of protein synthesis and the steady state decreases of glucocorticoid receptor and TGF-β activator protein. Int J Biochem Cell Biol 34:194–203.

Cutroneo KR. 2003a. How is Type I procollagen synthesis regulated at the gene level during tissue fibrosis? J Cell Biochem 90:1–5.

Cutroneo KR. 2003b. Gene therapy for tissue regeneration. J Cell Biochem 88:418–425.

Cutroneo KR, Boros DL. 2002. Rational basis for oligodeoxynucleotides to inhibit collagen synthesis in lung fibroblasts and primary fibroblasts from liver granulomas of *Schistosoma mansoni*-infected mice. Cancer Lett 180: 145–151.

Cutroneo KR, Chiu J-F. 2000. A sense phosphorothioate oligodeoxynucleotide containing the transforming growth factor beta regulatory element acts as a novel local nonsteroidal antifibrotic drug. Wound Rep Reg 8:399–404.

Cutroneo KR, Chiu J-F. 2003. Sense oligonucletide competition for gene promoter binding and activation. Int J Biochem Cell Biol 35:32–38.

Cutroneo KR, Counts DF. 1975. Anti-inflammatory steroids and collagen metabolism: Glucocorticoid-mediated alterations of prolyl hydroylase activity and collagen synthesis. Mol Pharmacol 11:632–639.

Cutroneo KR, Costello D, Fuller GC. 1971. Alteration of proline hydroxylase activity by glucocorticoids. Biochem Pharmacol 20:2797–2804.

Cutroneo K, Stassen FLH, Cardinale GJ. 1975. Anti-inflammatory steroids and collagen metabolism: Glucocorticoid-mediated decrease of prolyl hydroxylase. Mol Pharmacol 11:44–51.

Cutroneo KR, DiPetrillo TA, Cutroneo KR. 1990. Variation of corticosteroid-induced inhibition of collagen synthesis at equivalent anti-inflammatory doses. J Am Acad Dermatol 22:1007–1010.

Cutroneo KR, Meisler N, Counts DF. 1994. Anti-inflammatory prednisolone derivatives inhibit collagen synthesis and proα1 (I) collagen promoter activity in rat skin fibroblasts. Wound Rep Reg 2:292–296.

DiPetrillo T, Lee H, Cutroneo KR. 1984. Anti-inflammatory adrenal steroids that neither inhibit skin collagen synthesis nor cause dermal atrophy. Arch Dermatol 120:878–883.

Haapasaari KM, Risteli J, Koivukangas V, Oikarinen A. 1995. Comparison of the effect of hydrocortisone, hydrocortisone-17-butyrate, and betamethasone on collagen synthesis in human skin in vivo. Acta Derm Venereol 75:269–271.

Haapasaari KM, Risteli J, Oikarinen A. 1996. Recovery of human skin collagen synthesis after short-term topical

corticoisteroid treatment and comparison between young and old subjects. Br J Dermatol 135:65–69.

Haapasaari KM, Risteli J, Karvonen J, Oikarinen A. 1997. Effect of hydrocortisone, methylprednisolone aceponate, and mometahsone furoate on collagen synthesis in human skin in vivo. Skin Pharmacol 10:261– 264.

Henry SP, Templin MV, Gillet N, Rojko J, Levin AA. 1999. Correlation of toxicity and pharmacokinetic properties of a phoshporothioate oligonucleotide designed to inhibit ICAM-1. Toxicol Pathol 27:95–100.

Jeffrey JJ, Di Petrillo T, Counts DF, Cutroneo KR. 1985. Collagen accumulation in the neonatal rat skin: Absence of fibrillar collagen degradation during normal growth. Coll Relat Res 5:157–165.

Kauh YC, Rouda S, Mondragon G, Tokarek R, diLeonardo M, Tuan RS, Tan EM. 1997. Major suppression of pro-alpha1(I) Type I collagen gene expression in the dermis after keloid excision and immediate intrawound injection of triamcinolone acetonide. J Am Acad Dermatol 37:586–589.

Kelley J, Chrin L, Shull S, Rowe DW, Cutroneo KR. 1985. Bleomycin selectively elevates mRNA levels for procollagen and fibronectin following acute lung injury. Biochem Biophys Res Commun 131:836–843.

Kelley J, Shull S, Walsh JJ, Cutroneo KR, Absher M. 1993. Auto-induction of transforming growth factor-β in human lung fibroblasts. Am J Respir Cell Mol Biol 8:417–424.

King SL, Lichtler AC, Rowe DW, Xie R, Long GL, Absher MP, Cutroneo KR. 1994. Bleomycin stimulates pro-α1(I) collagen promoter through transforming growth factor β response element by intracellular and extracellular signaling. J Biol Chem 269:13156–13161.

Koivukangas V, Karvonen J, Risteli J, Oikarinen A. 1995. Topical mometasone furoate and betamethasone-17-valerate decrease collagen synthesis to a similar extent in human skin in vivo. Br J Dermatol 132:66–68.

McNelis B, Cutroneo KR. 1978. A selective decrease of collagen peptide synthesis by dermal polysomes isolated from glucocorticoid treated newborn rats. Mol Pharmacol 14:1167–1175.

Meisler N, Shull S, Xie R, Long GL, Absher M, Connolly JP, Cutroneo KR. 1995. Glucocorticoids coordinately regulate Type I collagen proα1 promoter activity through both the glucocorticoid and transforming growth factor β response elements: A novel mechanism of glucocorticoid regulation of eukaryotic genes. J Cell Biochem 59:376–388.

Meisler N, Keefer KA, Ehrlich HP, Yager DR, Myers-Parrelli J, Cutroneo KR. 1997. Dexamethasone abrogates the fibrogenic effect of transforming growth factor-β in rat granuloma and granulation tissue fibroblasts. J Invest Dermatol 108:285–289.

Mintz KP, Mann KG. 1990. Detection of procollagen biosynthesis using peptide-specific antibodies. Matrix 10: 186–199.

Nakamura T, Ebihara I, Fukui M, Tomino Y, Koide H. 1991. Effects of methylprednisolone on glomerular and medullary mRNA levels for extracellular matrices in puromycin aminonucleoside nephrosis. Kidney Int 40:874–881.

Nakamura T, Ebihara I, Tomino Y, Koide H. 1992. Glucocorticoid ameliorates altered gene expression of extracellular matrix components in kidneys of New Zealand black/white F1 mice. Clin Sci 83:701–709.

Newman RA, Cutroneo KR. 1978. Glucocorticoids selectively decrease the synthesis of hydroxylated collagen peptides. Mol Pharmacol 14:185–198.

Oishi Y, Fu ZW, Ohnuki Y, Kato H, Noguchi T. 2002. Molecular basis of the alteration in skin collagen metabolism in response to in vivo dexamethasone treatment: Effects on the synthesis of collagen Type I and III, collagenase, and tissue inhibitors of metalloproteinases. Br J Dermatol 147:859–868.

Parrelli JM, Meisler N, Cutroneo KR. 1997. Abrogation of the fibrotic effect of transforming growth factor-β in dermal wound healing. Wound Rep Reg 5:136–140.

Parrelli JM, Meisler N, Cutroneo KR. 1998. Identification of a glucocorticoid response element in the human transforming growth factor beta I gene promoter. Int J Biochem Cell Biol 30:623–627.

Perez JR, Shull S, Gendimenico GJ, Capetola RJ, Mezick JA, Cutroneo KR. 1992. Glucocorticoid and retinoid regulation of alpha-2 Type I procollagen promoter activity. J Cell Biochem 50:26–34.

Rokowski RJ, Sheehy J, Cutroneo KR. 1981. Glucocorticoid-mediated selective reduction of functioning collagen messenger ribonucleic acid. Arch Biochem Biophys 210:74–81.

Russell SB, Trupin JS, Myers JC, Broquist AH, Smith JC, Myles ME, Russell JD. 1989. Differential glucocorticoid regulation of collagen mRNAs in human dermal fibroblasts. Keloid-derived and fetal fibroblasts are refractory to down-regulation. J Biol Chem 264:13730–13735.

Shukla A, Meisler N, Cutroneo KR. 1999. Perspective article: Transforming growth factorbeta: Crossroad of glucocorticoid and bleomycin regulation of collagen synthesis in lung fibroblasts. Wound Rep Reg 7:133–140.

Shull S, Cutroneo KR. 1983. Glucocorticoids coordinately regulate procollagens Type I and Type III synthesis. J Biol Chem 258:3364–3369.

Shull S, Cutroneo KR. 1986. Glucocorticoids change the ratio of Type III to Type I procollagen extracellularly. Coll Relat Res 6:295–300.

Shull S, Meisler N, Absher M, Phan S, Cutroneo K. 1995. Glucocorticoid-induced down regulation of transforming growth factor-β1 in adult rat lung fibroblasts. Lung 173:71–78.

Slavin J, Unemori E, Hunt TK, Amento E. 1994. Transforming growth factor beta (TGF-beta) and dexamethasone have direct opposing effects on collagen metabolism in low passage human dermal fibroblasts in vitro. Growth Factors 11:205–213.

Sterling KM, DiPetrillo T, Cutroneo KR, Prestayko A. 1982a. Inhibition of collagen accumulation by glucocorticoids in rat lung after intratracheal bleomycin instillation. Cancer Res 42:405–408.

Sterling KM, DiPetrillo TA, Kotch JP, Cutroneo KR. 1982b. Bleomycin-induced increase of collagen turnover in IMR-90 fibroblasts: An in vitro model of connective tissue restructuring during lung fibrosis. Cancer Res 42:3502–3506.

Sterling KM, Harris MJ, Mitchell JJ, DiPetrillo TA, Delaney GL, Cutroneo KR. 1983. Dexamethasone decreases the amounts of Type I procollagen mRNAs in vivo and in fibroblast cell cultures. J Biol Chem 258:7644–7647.

Walsh MJ, LeLeiko NS, Sterling KM. 1987. Regulation of Types I, III, and IV procollagen mRNA synthesis in glucocorticoid-mediated intestinal development. J Biol Chem 262:10814–10818.