Effects of steroid hormones on human fibroblasts *in vitro*

II. Antagonism by androgens of cortisol-induced inhibition

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Harvey, W., Grahame, R., and Panayi, G. S. (1976). *Annals of the Rheumatic Diseases*, 35, 148–151. Effects of steroid hormones on human fibroblasts *in vitro*. II. Antagonism by androgens of cortisol-induced inhibition. Inhibition of dermal activity by cortisol in culture was partially reversed by two naturally occurring androgens, testosterone and dihydro-testosterone. ACTH and the androgen precursor dehydroepiandrosterone sulphate showed no such antagonistic effect. These results suggest that increased production of adrenal androgens during ACTH therapy may account for the relative absence of 'skin-thinning' and 'steroid-bruising' which are common side-effects of corticosteroid therapy.

The loss and disorganization of dermal collagen which results in reduced skin thickness (Grahame, 1969) and 'steroid bruising' are side-effects of corticosteroid therapy which have been attributed to inhibition of dermal fibroblast activity (Uitto, Teir, and Mustakallio, 1972; Harvey, Grahame, and Panayi, 1974). During long-term adrenocorticotrophin (ACTH) therapy, however, these side-effects are far less pronounced, with a relative absence of pathological bruising (West, 1961) and no further reduction of skin thickness (Grahame, 1969). As ACTH is known to stimulate the synthesis of adrenal androgens as well as of glucocorticoids (Nieschlag, Loriaux, and Lipsett, 1972), we felt it of interest to investigate the possibility of antagonism between these two classes of steroids in their effects on cultured fibroblasts. An *in vitro* fibroblast culture system was used in which the effects of two anti-inflammatory steroids (cortisol and prednisolone) has already been established (Harvey and others, 1974).

Materials and methods

The culture of human dermal fibroblasts, and the estimation of cell proliferation, DNA, and protein synthesis

were performed using standard tissue-culture techniques described in a previous paper (Harvey and others, 1974). Briefly, cell proliferation was estimated by cell counts performed on a Coulter counter following harvesting after a 3-day culture period. The rates of DNA and protein synthesis were estimated by the incorporation of ³H-thymidine and ³H-proline, respectively, into acid-insoluble fractions of the cell layer and culture medium. Porcine ACTH (Armour Pharmaceuticals) was dissolved directly in the culture medium. The steroids (obtained from Sigma) were dissolved in dimethylacetamide (DMA) before dilution in the culture medium.

The cells were cultured in the presence of ACTH, androgens, or DMA as a control, both alone and with inhibitory concentrations of cortisol (10 µg/ml for studies of cell proliferation and DNA synthesis, 50 µg/ml for protein synthesis). Five replicate cultures were employed in each case.

Results

The results shown in Figs 1–3 are representative experiments from a series of at least three for each combination of drugs. The vertical bars above and below mean values each represent one standard error of the mean. The asterisk denotes a statistically significant difference (Student's 't' test for unpaired

samples, $P < 0.05$) between experimental and control mean values; the dagger denotes a significant difference between experimental and 'cortisol alone' mean values. The rates of DNA and protein synthesis were calculated on the basis of cell numbers present at the termination of each culture.

At a concentration of $10 \mu\text{g/ml}$, cortisol consistently reduced cell proliferation by 20–40% and DNA synthesis by 40–85% of control values. Protein synthesis was inhibited approximately 30% by cortisol at $50 \mu\text{g/ml}$.

DEHYDROEPIANDROSTERONE SULPHATE (DHAS)

At concentrations of 0.1 – $10 \mu\text{g/ml}$, DHAS did not markedly affect proliferation, DNA, or protein synthesis, and did not appear to influence the inhibitory effects of cortisol (Fig. 1).

TESTOSTERONE

Testosterone produced slight increase in the rates of DNA and protein synthesis which, although consistent, were not statistically significant (Fig. 2). In

FIG. 1 Effect of dehydroepiandrosterone sulphate (DHAS) on cell proliferation, DNA, and protein synthesis, and their inhibition by cortisol. Results are expressed as the mean percentage of control values (5 replicates per dose level) $\pm$ two SEM. * Difference between experimental and control mean values is significant, $P < 0.05$

FIG. 2 Effect of testosterone on cell proliferation, DNA, and protein synthesis, and their inhibition by cortisol. * As in Fig. 1; † difference between experimental and 'cortisol alone' mean values is significant

the presence of cortisol, however, testosterone at 0.001 and 0.01 $\mu\text{g/ml}$ caused a striking reversal of cortisol-induced inhibition of DNA synthesis ($P < 0.01$); inhibition of cell proliferation and protein synthesis was partly reversed by testosterone at these concentrations.

The highest concentration of testosterone used 0.1 $\mu\text{g/ml}$, inhibited all three parameters of cell activity and did not reverse the effects of cortisol.

DIHYDROTESTOSTERONE (DHT)

The effects of DHT were essentially similar to those of testosterone: slight stimulation of DNA and protein synthesis at 0.01 $\mu\text{g/ml}$, reversal of the effects of cortisol on cell proliferation and DNA synthesis ($P < 0.05$, $P < 0.01$, respectively), and partial reversal of the inhibition of protein synthesis. DHT at 0.1 $\mu\text{g/ml}$ did not inhibit protein synthesis, but suppressed proliferation and DNA synthesis and failed to reverse cortisol-induced inhibition (Fig. 3).

ACTH

ACTH (not shown) was tested at 0.1, 1, and 10 mU/ml. At 10 mU/ml cell proliferation and DNA synthesis were decreased by 25% and 40% respectively; in the presence of cortisol there was further decrease which appeared additive, with no suggestion that ACTH reversed the inhibitory effects of cortisol.

Discussion

The stimulatory effect of testosterone and DHT on the fibroblasts and the absence of this effect with DHAS agree with the established biological activity

of these steroids. The formation of the 5 α -reduced derivative of testosterone (DHT) appears to be an integral part of testosterone action on target cells. DHT formation is limited to those tissues in which testosterone is known to exert major effects (Gloyne and Wilson, 1969) and in most bioassay systems DHT is as potent or more potent than testosterone (Wilson, 1972). Fibroblasts are able to convert testosterone to DHT (Wilson and Walker, 1969) and Jenkins and Ash (1970) found that DHT was the predominant metabolite of testosterone in human skin.

None of the steroids tested stimulated the rate of cell proliferation above control levels, although testosterone and DHT did antagonize the inhibition of cell proliferation by cortisol. As the inhibition of fibroblast activity by cortisol is unlikely to be due to a single mechanism, equally the antagonism by androgens is likely to be exerted through more than one mechanism. Antagonism of cortisol action by androgens can theoretically occur wherever a cellular system is capable of a steroid-specific response. Such specificity is known to occur—for instance in the binding of the steroid at its receptor sites, in the functional modification of subcellular membrane structures, and in the determination of the fate of hydrogen in cellular oxidation reduction pathways (Chayen and others, 1974). The antagonism by androgens is unlikely to be due to direct competition for steroid receptor sites because the disparity in concentration between androgens and cortisol and the specificity of steroid binding demonstrated in fibroblasts by Hackney and others (1970). Naturally-occurring androgens and synthetic anabolic steroids can reverse the inhibitory effects of

FIG. 3 Effect of dihydrotestosterone (DHT) on cell proliferation, DNA, and protein synthesis, and their inhibition by cortisol. * and † as in Figs. 1 and 2

anti-inflammatory steroids *in vivo* (Little, 1970; Little and Munuera, 1970). Raised levels of circulating androgens have also been implicated in increased dermal fibroblast activity, reflected in greater skin thickness and collagen content in women with hirsutes (Shuster and Black, 1970), and in patients with osteoporosis treated with a variety of androgens (Black, Shuster, and Bottoms, 1970). The evident responsiveness of fibroblasts to androgens and the antagonism of glucocorticoid effect shown by our results suggest that increased circulating levels of adrenal androgens occurring after administration of ACTH (Nieschlag and others, 1972) may protect dermal fibroblasts from the inhibitory effects of corticosteroids: this may be the mechanism responsible for the comparative absence of 'steroid bruising' and 'skin-thinning' during ACTH therapy.

Inherent in this proposal is the probability that the balance between androgens and glucocorticoids exerts a regulatory effect on cellular activity in steroid-sensitive tissues. Kirschner and Bardin (1972) showed that administration of corticosteroids depressed circulating levels of testosterone and androstenedione, thus accentuating the increase in the ratio of corticosteroids to androgens. If connective tissue is influenced by the ratio of 'anabolic' to 'catabolic' steroids, the maintenance of a near-normal ratio during ACTH therapy may be the important factor in the prevention of fibroblastic inhibition and loss of dermal collagen.

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