EFFECTS OF DEXAMETHASONE ON THE METABOLISM OF TESTOSTERONE IN HUMAN GINGIVAL FIBROBLASTS

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ABSTRACT

Dexamethasone has been known to have synergistic interactions with growth factors to induce proliferation of human diploid periodontal ligament and gingival fibroblasts. It can also stimulate the formation of new cementum and alveolar bone. The anabolic effects of androgens especially 5α-dihydrotestosterone (DHT) are well established. The aim of this investigation was to study the effects of dexamethasone (Dex) on the metabolism of testosterone in human gingival fibroblasts (HGF).

Confluent monolayer cultures of HGF of 5^{th}-9^{th} passage derived from chronically inflamed gingival tissues were established in Eagle's Minimum Essential medium. Duplicate incubations were performed with 14C-testosterone (14C-T) as substrate and serial concentrations of Dex ranging from 0.5-50 μg/ml for 24 hours. At the end of the incubation period, the steroid metabolites were analysed and quantified using a radioisotope scanner.

Most of the lower concentrations of Dex showed a stimulatory effect on DHT synthesis from 14C-T, with a maximum increase of 44% at 1.0 μg/ml, decreasing to 14% at 10 μg/ml and reaching control values at higher concentrations. The increases in DHT in response to the lower concentrations of Dex (ranging from 0.5-8.0 μg/ml) were statistically significant ($n=6$; $p<0.05$; ANOVA). Synthesis of the other major metabolite, 4-androstenedione from 14C-T showed 20-36% increases in response to the range of 0.5-5.0 μg/ml of Dex ($n=6$; $p<0.05$; ANOVA).

Dexamethasone at lower concentrations could induce the metabolism of testosterone in cultured human gingival fibroblasts resulting in the formation of anabolic metabolites. This could enhance connective tissue and bone matrix synthesis and play an important role in tissue regeneration in inflammatory periodontal lesions.

INTRODUCTION

Glucocorticoids modulate the effects of other hormones and are mediators of cell function. They enhance the mitogenic activity of fibroblast growth factor (Holley & Kiernan, 1974) and insulin-like growth factor-1 (Conover, Rosenfeld & Hintz, 1986).

Dexamethasone is a potent synthetic glucocorticoid and has been shown to act synergistically with cartilage derived growth factor to stimulate DNA synthesis in Swiss mouse 3T3 cells (Levenson et al, 1985). It also has shown synergistic interaction with platelet derived growth factor (PDGF) to show induction of human diploid periodontal ligament and

gingival fibroblast proliferation in vitro (Rutherford et al, 1992). A single exposure of fibroblasts to physiological levels of dexamethasone and pharmacological concentrations of PDGF synergistically enhanced the proliferation of the cells for up to 7 days. This may have implications in wound healing and tissue regeneration in vivo.

Studies show that dexamethasone has good potential for osteogenesis. Dexamethasone could stimulate proliferation of osteoprogenitor cells (Bellows, Heersche & Aubin, 1990), and induce adult bone marrow cells to differentiate into osteoblasts (Kasugai et al, 1991). When ostegenic cells derived from the foetal calvaria and bone marrow were treated with dexamethasone in vitro, there were increases in alkaline phosphatase activity and elevated bone-specific marker proteins such as osteopontin and osteocalcin (Kasugai et al, 1991: Leboy et al, 1991; Nagata et al, 1991). Treatment with dexamethasone also increased the number of mineralised bone nodules (Bellows, Aubin & Heersche, 1987). When dexamethasone was omitted from osteogenic cell cultures there was only a moderate increase in alkaline phosphatase mRNA, low levels of type-1 collagen mRNA and little osteopontin mRNA produced (Leboy et al, 1991). It was suggested that dexamethasone plays an important role not only in early but also in late stages of osteogenic cell differentiation (MaCulloch & Tenenbaum, 1986; Leboy et al, 1991). Thus there is well-documented evidence of dexamethasone inducing mineralised, bone-like tissue in vitro (Bellows et al, 1987; Grigoriadis, Heersche & Aubin, 1988; Maniatopoulos, Sodek & Melcher, 1988). Cultured fibroblasts from periodontal ligament exhibited a relatively high level of spontaneous alkaline phosphatase activity which is one of the known bone cell-like characteristics of these fibroblasts in vitro (Kawase et al, 1988; Somerman et al, 1988; Nojima et al, 1990).

Dexamethasone has been shown to inhibit PGE_2 and IL-1 production strongly in lipopolysaccharide (LPS)-stimulated mouse calvaria organ culture; it also inhibited LPS-induced bone resorption (Ishihara et al, 1991). Matsuda et al (1993) showed that when periodontal ligament fibroblasts were treated with dexamethasone, there was an increase in alkaline phosphatase activity in a dose-dependent manner. In pulp cell cultures of Wistar rats, dexamethasone, in the presence of beta-glycerophosphate has been shown to enhance the formation of mineralised tissue (Kasugai et al, 1993). The therapeutic combination comprising PDGF, dexamethasone and a carrier matrix has shown to induce the formation of more new cementum, periodontal ligament and supra crestal bone in periodontal lesions in monkeys (Rutherford et al, 1993). Thus dexamethasone singly or in combination with other agents which can enhance regeneration could play an important role in the regeneration of tissues in inflammatory periodontal lesions. In human bone cell cultures, dexamethasone stimulated insulin-like growth factor binding protein-1 (IGFBP-1), which can modulate bone formation (Conover et al, 1996).

Many tissues of humans and animals are capable of metabolising sex steroidal hormones. The salivary glands of humans and animals too contain enzymes involved in steroid metabolism (Gower, 1984). Metabolic studies have shown that androgens are actively metabolised in gingival tissue of human and animals; the presence of inflammation can increase the level of activity of the enzymes concerned for the metabolic conversion of these hormones. When testosterone was incubated with homogenate, mitochondrial, microsomal and soluble fractions of healthy and inflamed gingiva from humans of both sexes, the metabolic activity was higher in the preparation from inflamed tissue, than in the samples from healthy gingiva (Ojanotko, Neinstedt & Harri, 1980; Sooriyamoorthy & Gower, 1989b). In both types of

tissue, testosterone was converted to 5α-dihydrotestosterone, suggesting that gingivae might be a target tissue for androgens. It has been reported that 5α-dihydrotestosterone (DHT) and 4-androstenedione (4-A) are formed from testosterone metabolism by human gingival fibroblasts in culture (Sooriyamoorthy, Harvey & Gower, 1988). DHT is the most biologically active metabolite found in tissues when testosterone is metabolised, and it can contribute to growth and development. For example DHT could stimulate matrix synthesis in connective tissue and bone (Colvard et al, 1989; Kasperk et al, 1989; Normington & Russell, 1992; Dassouli et al, 1994). Such anabolic effects are more obvious when the normal synthesising capacity of tissues is reduced. Thus, when a reparatory response is required, DHT can contribute to synthetic activity in fibroblasts and osteoblasts (Vittek et al, 1979; Kasperk et al, 1989; Sooriyamoorthy & Gower, 1989a). The expression of androgen receptors has been detected in a high proportion of periodontal and gingival tissues and also in fibroblasts derived from the same source (Parkar, Newman & Olsen, 1996).

The present study aimed to investigate the modulatory effects of dexamethasone on testosterone metabolic pathway.

MATERIALS AND METHODS

Chemicals

Radiolabelled androgen, 14C-testosterone (14C-T), with its specific radioactivity of 58 μCi/μmol was obtained from Amersham International, Amersham, Bucks., UK. Organic solvents (benzene, acetone) for thin layer chromatography (TLC), ethyl acetate for extraction of metabolites and chloroform to re-dissolve the dried extract were all provided by Merck Ltd., Dagenham, Essex.

The incubation medium used was Eagle's Minimum Essential Medium (MEM) with 10% foetal bovine serum, L-glutamine, antibiotic solution (penicillin and streptomycin) and sodium bicarbonate which were all provided by Gibco Ltd., Paisley, Scotland. Dexamethasone used in the incubations was obtained from Sigma chemicals Co., Poole, Dorset, UK.

Cell culture techniques and analysis for androgen metabolites

Chronically inflamed gingival tissues were obtained from periodontal patients undergoing pocket elimination surgical procedures attending the Department of Periodontology, King's College Dental Institute, London, UK. All these patients had completed initial phase periodontal treatment comprising scaling and root planing before these procedures and subsequent isolation of the gingival tissues from the sites with pocket depths of 6-8 mm. Their ages ranged from 20 to 50 years. Previous workers have shown that although gingival tissues from healthy males metabolised testosterone better than those from healthy females, chronically inflamed gingiva from both sexes did not show any difference in testosterone metabolism (Ojanotko et al, 1980; Sooriyamoorthy & Gower, 1989b). Based on this evidence, the present study sample was not categorised for the sexes. However, the samples were not pooled, maintaining individual cultures; sample numbers include males and females.

The gingival tissue was minced into small fragments, approximately $1mm^3$ and gingival fibroblasts were established in primary culture in $25cm^2$ tissue culture flasks. Serial passaging of primary cultures was carried out by partial digestion with 0.25% trypsin solution.

Fibroblasts of the 5th-9th passage in confluent monolayer culture were used in the experiments. The contents of a fully confluent 25 cm^2 flask ($2.2x10^6$ cells) were distributed into 24 wells of a multi-well dish. The cells were allowed to become fully confluent in the multi-well dishes prior to experimentation to overcome mitogenic effects (Kahari, Heino & Vuorio, 1987). Duplicate incubations were performed for each individual cell line in Eagle's MEM, using 14C-testosterone as substrate (0.025 μCi), and serial concentrations of dexamethasone (0.5-50 μg/ml). After an incubation period of 24 hours, in a humidified cell culture incubator at 37°C, the medium was solvent extracted with ethyl acetate (2ml x 2) with added cold steroid standards. The extract was evaporated to dryness, re-dissolved in chloroform, spotted on TLC plates and the metabolites were separated in a benzene acetone solvent system (4:1 v/v). The separated metabolites were then quantified using a radioisotope scanner.

Confirmation of the identity of metabolites

The identity of the formed metabolites was confirmed using the mobility of cold standards added to the samples and disclosing them in iodine. The TLC plate was placed in a covered tank containing iodine crystals, and the iodine-stained steroids were marked for comparison with the position and pattern of separated metabolites identified by the radioisotope scanner.

RESULTS

Effects of serial concentrations of dexamethasone on the metabolism of testosterone by gingival fibroblasts.

All 11 concentrations of dexamethasone studied showed a stimulatory effect on the synthesis of DHT from 14C-T in gingival fibroblasts (Fig. 1). The increases were more marked in response to the lower concentrations. The maximum increase in DHT synthesis was seen in relation to a concentration of 1.0 μg/ml (44% increase from the control value). The increases in DHT in response to the lower concentrations of dexamethasone (ranging from 0.5-8.0 μg/ml) were statistically significant (n=6; $p<0.05$ as tested by ANOVA). The smaller increases in DHT in response to the higher concentrations of dexamethasone (10-50 μg/ml) ranged from 0-14%, and were not significant statistically.

Fig. 1 also shows the synthesis of 4-A from 14C-testosterone in gingival fibroblasts in response to the same concentrations of dexamethasone. There were 20%-36% increases in 4-A synthesis (with less stimulation at 3.0 and 5.0 μg/ml) in response to the range of 0.5-5.0 μg/ml dexamethasone compared to the control value (n=6, $p<0.05$; ANOVA). All the other higher concentrations, ranging from 8.0 to 50 μg/ml showed decreases in 4-A synthesis compared to the control value. The maximum reduction in 4-A synthesis was 47%, in response to 30 and 50 μg/ml.

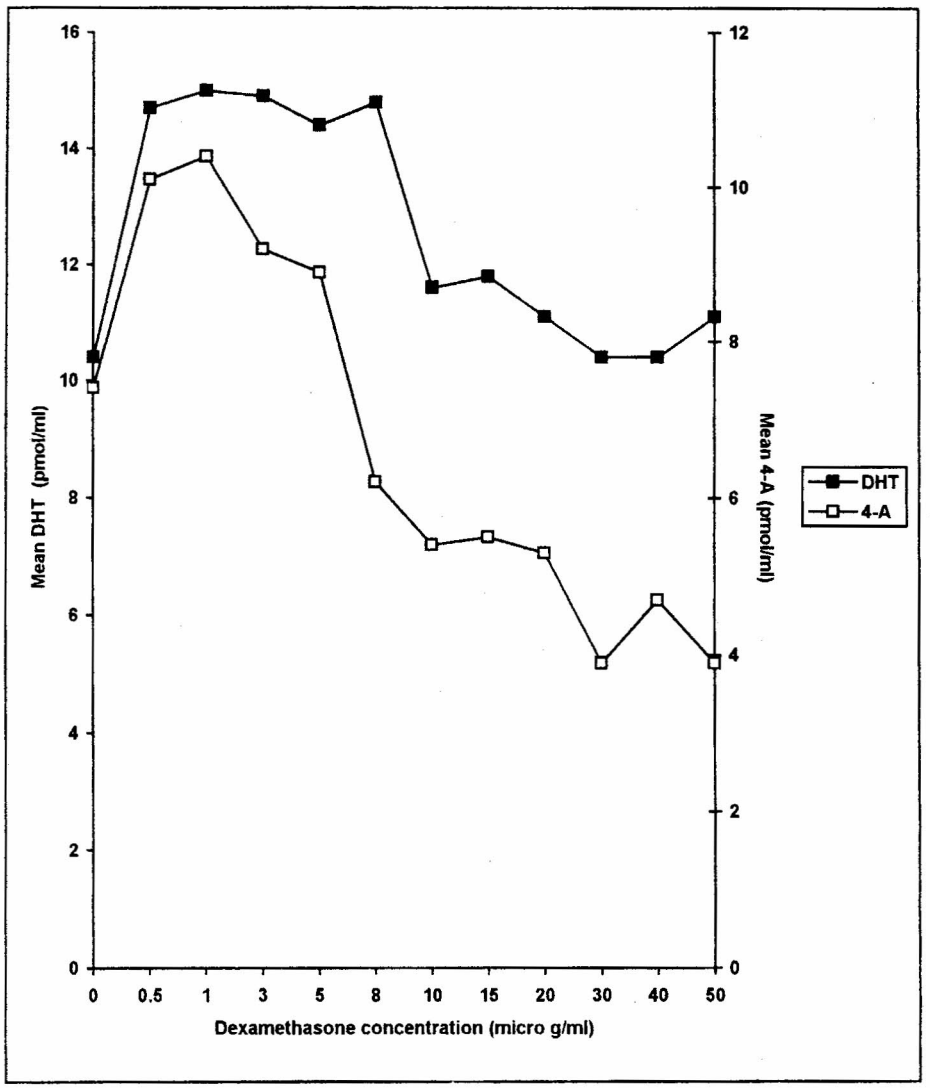

Fig. 1: Effects of 11 stimulatory / inhibitory concentrations of dexamethasone on the metabolic conversion of 14C-testosterone to DHT and 4-A. Duplicate incubations were performed with radio-labelled testosterone in multi-well plates for 24 hr, when the reaction was terminated by the addition of ethyl acetate. The metabolites were extracted, analysed and quantified. The mean values derived from duplicate control and test incubations of 6 gingival cell lines are shown.

DISCUSSION

The general effect of dexamethasone on 5α-reductase activity in testosterone metabolism was shown to be stimulatory, mostly in response to the lower concentrations studied but the higher concentrations of dexamethasone showed only a little or no significant differences in this activity compared to the controls. The effect of dexamethasone on 17β hydroxysteroid dehydrogenase (17β-HSD) activity in gingival fibroblasts is indicated by the yields obtained for 4-A. This enzyme activity appeared to be stimulatory in relation to lower concentrations of dexamethasone, ranging from 0.5-5.0 μg/ml.

Thus the role of dexamethasone on the androgen metabolic pathway appeared to contribute towards the formation of important metabolites with anabolic effects. However, it could be suggested that the effect of dexamethasone on both 5α-reductase and 17β-HSD activity is dose dependent. This finding is in agreement with the facts reviewed by Sooriyamoorthy & Gower (1989a) based on earlier studies showing that corticosteroids exerted dichotomous effects on connective tissue in monolayer cultures. Cell growth was stimulated at low corticosteroid dosages while higher levels were inhibitory. Similar results have been reported for prednisolone, another important synthetic corticosteroid (Sooriyamoorthy & Gower, 1989a).

Dexamethasone has been found to be a novel potent inducer of connective tissue growth factor expression (Dammeier et al, 1998). In cultured skin fibroblasts from mice, a striking induction of connective tissue growth factor expression was observed after dexamethasone treatment, and this occurred in a dose dependent manner. Connective tissue growth factor (CTGF) is known to have potent effects on fibroblast proliferation and extracellular matrix deposition, which could play an important role in tissue repair and wound healing. It may be suggested that in the androgen metabolic pathway, the induction of 5α-reduction and 17β-HSD activity by dexamethasone could partly have been mediated via induction of CTGF.

However, certain catabolic effects of dexamethasone observed in some studies, such as in decreasing newly synthesised endothelial cell fibronectin, resulting in weak endothelial cell substratum adhesion (Romer & Polin, 1995), could have been attributed to the difference in concentrations of dexamethasone used. Dexamethasone in cultured fibroblasts showed reduced collagen production in the absence of, or with low concentrations of insulin-like growth factor-1, but has potentiated collagen production in cells stimulated with higher concentrations of IGF-1 (Bird & Tyler, 1994). This also shows the influence of other factors and their concentrations on the effects of dexamethasone. Dexamethasone effectively reduced interleukin-6 (IL-6) secretion by human gingival fibroblasts. This down regulation of IL-6 production could render the gingiva less efficient at resisting the inflammatory challenges (Lapp, Thomas & Lewis, 1995). This effect of dexamethasone appeared to be dose related in giving rise to such effects.

Thus dexamethasone, at least in a dose dependent manner could give rise to important anabolic functions in tissues, resulting from the androgen metabolites formed.

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