Glucocorticoid effects on chondrogenesis, differentiation and apoptosis in the murine ATDC5 chondrocyte cell line

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Abstract

Glucocorticoids (GC) are used extensively in children and may cause growth retardation, which is in part due to the direct effects of GC on the growth plate. We characterised the ATDC5 chondrocyte cell line, which mimics the *in vivo* process of longitudinal bone growth, to examine the effects of dexamethasone (Dex) and prednisolone (Pred) during two key time points in the chondrocyte life cycle – chondrogenesis and terminal differentiation. Additionally, we studied the potential for recovery following Dex exposure. During chondrogenesis, Dex and Pred exposure at 10^{-8} M, 10^{-7} M and 10^{-6} M resulted in a significant mean reduction in cell number (28% vs 20%), cell proliferation (27% vs 24%) and proteoglycan synthesis (47% vs 43%) and increased alkaline phosphatase (ALP) activity (106% vs 62%), whereas the incidence of apoptosis was unaltered. Minimal effects were noted during terminal differentiation with both GC although all concentrations

of Dex lowered apoptotic cell number. To assess catch-up growth the cells were incubated for a total of 14 days which included 1, 3, 7, 10 or 14 days exposure to 10^{-6} M Dex, prior to the recovery period. Recovery of proteoglycan synthesis was irreversibly impaired following just one day exposure to Dex. Although cell number showed a similar pattern, significant impairment was only achieved following 14 days exposure. Irreversible changes in ALP activity were only noticed following 10 days exposure to Dex.

In conclusion, GC have maximal effects during chondrogenesis; Dex is more potent than Pred and cells exposed to Dex recover but this may be restricted due to differential effects of GC on specific chondrocyte phenotypes.

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Introduction

Glucocorticoids (GC) are commonly used as anti-inflammatory therapy and in immunosuppressive regimens and it is estimated that 5–10% of children may require some form of GC therapy at some time in childhood (Warner 1995). The functional effects of steroids on target tissues are difficult to predict and their use is hampered in some individuals more than others because of side-effects such as growth retardation and osteoporosis. Impairment of childhood growth with long-term GC was described almost 50 years ago but more recent studies have shown that altered growth and bone turnover also occur during relatively short periods of GC therapy and that these effects may vary depending on the type of corticosteroid used (Blodgett *et al.* 1956, Crofton *et al.* 1998, Ahmed *et al.* 1999, 2002).

At the level of the growth plate, local and systemic factors regulate longitudinal bone growth which involves the differentiation of committed stem cells into proliferating chondrocytes; after a finite number of cell divisions

these cells terminally differentiate into the hypertrophic phenotype that deposit a matrix which is mineralised and eventually replaced by bone (Green *et al.* 1985, Isaksson *et al.* 1991). GC-induced growth failure may be due to a combination of factors such as a disruption of the growth hormone–insulin-like growth factor-I (GH–IGF-I) axis, a defect in sex steroid action, a disturbance in calcium and phosphate homeostasis as well as direct effects on the growth plate (Crilly *et al.* 1978, Unterman & Phillips 1985, Baron *et al.* 1992, Jux *et al.* 1998).

Studies in children suggest that growth retardation following a short period of systemic exposure to GC may be followed by a period of catch-up growth and that alternate day therapy may be less adverse for growth (Jabs *et al.* 1996, Ahmed *et al.* 1999). Catch-up growth has also been observed following direct injection of GC into the growth plate of rabbits (Baron *et al.* 1994).

The direct effects of GC on chondrocytes are not clearly understood and previous studies that have examined the effect of GC on primary growth plate chondrocytes have

Table 1 Primer pairs used for specific gene analysis

Gene	Primer sequence	Cycles	Product size (bp)
18S	Unknown, purchased commercially from Ambion	15	488
Collagen type II	TTAGAAAGGGGAGCACAGTCC TACACTGCCATGAAGCATGG	35	323
Collagen type X	CAGAGGAAGCCAGGAAAGC GGTGTCCAGGACTTCCATAGC	32	330

Ambion, Huntingdon, Cambs, UK.

been unable to examine the effect of GC on the different stages of the chondrocyte life cycle due to the heterogeneous mixture of maturational phenotypes (Robson *et al.* 1998, Koedam *et al.* 2000). Recently, the murine ATDC5 chondrocyte cell line has been shown to undergo the temporal sequence of events that occur during longitudinal bone growth *in vivo* and thereby provide a good model to study the molecular mechanisms underlying regulation of endochondral bone formation (Atsumi *et al.* 1990, Shukunami *et al.* 1997). In this study, this cell line was used to explore the effects of two commonly used glucocorticoids, dexamethasone (Dex) and prednisolone (Pred), on cell number, proliferation, differentiation and apoptosis at key maturational time points (chondrogenesis and terminal differentiation), within the chondrocyte life cycle. The effect of the GC on the ability of chondrocytes to recover following GC exposure was also studied to assess the potential for catch-up growth.

Materials and Methods

Chondrocyte cell culture

The ATDC5 chondrocyte line was obtained from the RIKEN cell bank (Ibaraki, Japan) and maintained as described by Atsumi *et al.* (1990). Cells were cultured at a density of 12 000 cells per cm² in multi-well plates (Costar, High Wycombe, Bucks, UK) in a maintenance medium of DMEM/Ham's F12 (Invitrogen, Paisley, Strathclyde, UK) supplemented with 5% FCS (Invitrogen), 10 µg/ml human transferrin and 3 × 10⁻⁸ M sodium selenite (Sigma, Poole, Dorset, UK) until confluent (day 6). Thereafter, differentiation was induced by the addition of insulin (10 µg/ml; Sigma) and ascorbic acid (20 µg/ml) to the maintenance medium (differentiation medium). Incubation was at 37 °C in a humidified atmosphere of 95% air/5% CO₂ and the medium was changed every second day.

Gene expression

For the determination of chondrocyte phenotype, cells were grown for up to 20 days as above and RNA was

extracted, reverse transcribed and analysed for collagen type II and collagen type X expression at days 6, 8, 10, 13, 15, 17 and 20 by semi-quantitative RT-PCR.

RNA extraction

Total RNA was extracted from chondrocytes by repeated aspiration through a 25-gauge syringe needle in 1.5 ml Ultraspec (Biotecx, Houston, TX, USA). After extraction with chloroform, RNA in the aqueous phase was precipitated with isopropanol and bound to RNA Tack resin (Biotecx) following the manufacturer's protocol. After washing with 75% ethanol, the RNA was eluted in 100 µl ribonuclease-free water (Houston *et al.* 1999). In each case the 260/280 ratio was 1.9–2.0, confirming the purity of the RNA. All preparations were diluted to a concentration of 50 ng/µl and stored at –70 °C.

Semiquantitative RT-PCR

Gene expression was analysed by semiquantitative RT-PCR (Farquharson *et al.* 1999, Houston *et al.* 1999, Jefferies *et al.* 2000). Aliquots of 500 ng RNA (or an equivalent volume of water as a control) were reverse transcribed in 20-µl reactions with 200 ng random hexamers and 200 U Superscript II reverse transcriptase using the Superscript preamplification protocol (Invitrogen). PCR was performed in 20-µl reactions containing cDNA equivalent to 10 ng RNA and 200 nM gene-specific primers in 11.1 × PCR buffer (Jefferies *et al.* 1998) (Table 1). The cycling profile was 1 min at 92 °C (first cycle, 2 min), 1 min at 55 °C, and 1 min at 70 °C. The number of cycles performed was carefully titrated to ensure that the reactions were in the exponential phase. Reaction products were analysed on 1.5% agarose gels in the presence of ethidium bromide (250 µg/l), and a digital image of each gel was captured using a gel documentation system (Bio-Rad Laboratories, Inc., Hemel Hempstead, Herts, UK).

Chondrocyte number, proliferation, differentiation and matrix production

Dex and Pred (Sigma) were added to the cells at a final concentration of 10⁻⁸ M, 10⁻⁷ M and 10⁻⁶ M, in 0.01% ethanol and compared with control cultures which

contained 0.01% ethanol only. Collagen type II and collagen type X expression was first noted at 10 and 15 days respectively. The GC were added from day 6 or day 11 for the 4 days leading up to the expression of these two chondrocyte phenotypic markers.

Cell layers were rinsed with phosphate buffered saline (PBS) and lysed with 0.9% NaCl and 0.2% Triton X-100 and centrifuged at 12 000 *g* for 15 min at 4 °C. The supernatant was assayed for protein content and alkaline phosphatase (ALP) activity as a measure of cell number and chondrocyte differentiation respectively. The protein content of the supernatant was measured using the Bio-Rad protein assay reagent (Bio-Rad Laboratories) based on the Bradford dye binding procedure, and gamma globulin was used as standard (Farquharson *et al.* 1995). Enzyme activity was determined by measuring the cleavage of 10 mM *p*-nitrophenyl phosphate (pNPP) at 410 nm. Total ALP activity was expressed as nmoles pNPP hydrolysed/min/mg protein (Farquharson *et al.* 1999). The rate of chondrocyte proliferation was assessed by incubating the chondrocytes with 0.2 µCi/ml [³H]thymidine (37 MBq/ml; Amersham Pharmacia Biotech, Little Chalfont, Bucks, UK) for the last 18 h of the culture period and the amount of radioactivity incorporated into trichloroacetic acid-insoluble precipitates measured (Farquharson *et al.* 1999). Proteoglycan synthesis was evaluated by staining with Alcian Blue as previously described (Shukunami *et al.* 1997). In brief, cells were washed twice with PBS, fixed in 95% methanol for 20 min and stained with 1% Alcian Blue 8 GX (Sigma) in 0.1 M HCl overnight and rinsed with distilled water. Alcian Blue-stained cultures were extracted with 1 ml 6 M guanidine-HCl for 6 h at room temperature and the optical density (O.D.) was measured at 630 nm using a Jenway 6105 spectrophotometer.

Apoptosis

Apoptosis of the cells was measured by two complementary methods: (1) APOPercentage Apoptosis assay, (Biocolor Ltd, Belfast, N Ireland), which quantifies dye uptake by apoptotic cells only after the translocation of phosphatidylserine to the outer surface of the cell membrane (Fadok *et al.* 1992) and (2) Nucleosome ELISA kit (Oncogene Research Products, Nottingham, Notts, UK), which allows the quantification of apoptotic cells *in vitro* by DNA affinity-mediated capture of free nucleosomes followed by their anti-histone-facilitated detection. Both kits were used according to the manufacturers' instructions. Dex and Pred at concentrations of 10⁻⁸ M, 10⁻⁷ M and 10⁻⁶ M were added to the cell cultures on day 6 or day 13 for a period of 24 h. As a positive control, cells were incubated as above with 5% ethanol.

Recovery following GC exposure

For these experiments, a single concentration of Dex at 10⁻⁶ M was used as it was noted to have the most potent

Figure 1 Semiquantitative RT-PCR analysis of the expression of chondrocyte marker genes. Collagen type II is expressed from day 10 and collagen type X from day 15. B, blank.

effects in the above experiments. This dose was added to all cells at confluency (day 6) and subsequently replaced with differentiation medium without Dex after 1, 3, 7 and 10 days. All cultures were maintained for a total of 14 days along with a group that was exposed to Dex for the whole 14 days duration (no recovery period). Additional culture plates of Dex (10⁻⁶ M)-treated cells and their respective controls (containing 0.01% ethanol) were stopped at the allocated time points (days 1, 3, 7 and 10) to assess the impact of Dex prior to the period of recovery. Cell number, ALP activity and proteoglycan content were determined as described above.

Statistical analysis

All experiments were performed at least twice. Data were analysed by one way analysis of variance. All data are expressed as the mean ± S.E.M. of four observations within each experiment and statistical analysis was performed using Statview (SAS Institute Inc., Cary, NC, USA; version 5.0.1). *P* < 0.05 was considered to be significant.

Results

Temporal expression of chondrocyte phenotype specific markers

Using gene-specific primers, collagen type II expression by the ATDC5 cells was first noted after 10 days in culture indicating that the differentiation of mesenchymal cells to the chondrocyte phenotype (chondrogenesis) had occurred. Similarly, collagen type X expression was noted from day 15 onwards indicating that terminal differentiation of the chondrocytes occurred from day 10 to day 15 (Fig. 1). This information was used to study the effects of

Table 2 Effect of Dex and Pred on cellular protein, proteoglycans and alkaline phosphatase activity during chondrogenesis and terminal differentiation. All data are expressed as means ± S.E.M.

		10 ⁻⁸ M		10 ⁻⁷ M		10 ⁻⁶ M	
	Control	Dex	Pred	Dex	Pred	Dex	Pred
Chondrogenesis							
Cellular protein (mg)	0.66 ± 0.01	0.54 ± 0.05*	0.59 ± 0.04	0.44 ± 0.01*	0.52 ± 0.01*	0.45 ± 0.03*	0.46 ± 0.02*
Proteoglycan (O.D.)	0.26 ± 0.02	0.15 ± 0.01*	0.17 ± 0.005*	0.13 ± 0.003*†	0.15 ± 0.01*	0.13 ± 0.003*	0.12 ± 0.002*
ALP (nmoles pNPP hydroly/min/mg protein)	331 ± 8	660 ± 32*†	440 ± 15*	723 ± 27*†	585 ± 10*	716 ± 36*†	586 ± 19*
Terminal Differentiation							
Cellular protein (mg)	0.92 ± 0.01	0.91 ± 0.02	0.85 ± 0.05	0.84 ± 0.03	0.79 ± 0.03*	0.92 ± 0.02†	0.82 ± 0.04*
Proteoglycan (O.D.)	0.82 ± 0.07	0.89 ± 0.05	0.82 ± 0.03	0.7 ± 0.02	0.77 ± 0.04	0.73 ± 0.05	0.72 ± 0.04
ALP (nmoles pNPP hydroly/min/mg protein)	262 ± 11	265 ± 17	244 ± 34	292 ± 21	270 ± 9	245 ± 10	253 ± 23

*P<0.05 compared with control cultures; †P<0.05 Dex cultures compared with equivalent dose of Pred.

Dex and Pred during the periods leading up to the expression of these maturation markers.

Effects of GC on cell number and proliferation

In comparison with control cultures the addition of Dex and Pred to cells during the chondrogenic period (days 6–10) caused a significant reduction in cell number as indicated by cell protein data (Table 2). The reduction in cell number from control values for the Dex concentrations tested were: 10⁻⁸ M, 18.2%; 10⁻⁷ M, 33.3%; and 10⁻⁶ M, 31.8% (P<0.05). The apparent plateau noted at 10⁻⁷ M for Dex was not seen with Pred, where a dose-dependent reduction was observed over the three concentrations tested: 10⁻⁸ M, 10.6% (not significant); 10⁻⁷ M, 21.2% (P<0.05); and 10⁻⁶ M, 30.3% (P<0.05). The mean reduction in cell number over the three concentrations was 28% with Dex and 20% with Pred.

The effect of Dex and Pred on [³H]thymidine uptake during the chondrogenesis period is shown in Fig. 2. Both GC caused a significant concentration-dependent decrease in cell proliferation from control values – Dex: 10⁻⁸ M, 11.7%; 10⁻⁷ M, 33.8%; and 10⁻⁶ M, 36.6%; Pred: 10⁻⁸ M, 9.6%; 10⁻⁷ M, 24.7%; and 10⁻⁶ M, 37% (P<0.05). As was noted for cell number, the apparent plateau noted at 10⁻⁷ M for Dex was not seen with Pred, where a dose-dependent decrease was observed over the three concentrations tested. The mean reduction over the three concentrations for Dex and Pred was 27% and 24% respectively. Dex at 10⁻⁷ M was significantly more antiproliferative than Pred at 10⁻⁷ M (P<0.05).

During the terminal differentiation phase (days 10–15) Dex did not significantly alter cell numbers when compared with control values (Table 2), whereas Pred caused a significant reduction (P<0.05) at both 10⁻⁷ M (14.1%) and 10⁻⁶ M (10.9%). The cell proliferation rate in control cultures was sixfold less during the terminal differentiation phase than during the chondrogenic stage and the addition

of GC led to a significant suppression of proliferation with Dex at 10⁻⁸ M (40.9%), 10⁻⁷ M (24.1%) and 10⁻⁶ M (40.3%) whereas a reduction in proliferation by Pred was noted at 10⁻⁸ M (26.3%), with a rise in proliferation at 10⁻⁶ M (21.6%, P<0.05) (Fig. 2).

Effects of GC on proteoglycan production

In comparison with control cultures during the chondrogenesis period, there was a concentration-dependent reduction in proteoglycan synthesis ranging from 42 to 50% with Dex and 35 to 54% with Pred (Table 2). An apparent plateau was noted at 10⁻⁷ M for Dex which was not seen with Pred, where a dose-dependent reduction was observed over the three concentrations tested.

Figure 2 Effect of Dex and Pred on cell proliferation as assessed by [³H]thymidine uptake during chondrogenesis and terminal differentiation phases. Effect of Dex on chondrogenesis (▲ and long dashed lines); effect of Dex on terminal differentiation (▲ and short dashed lines); effect of Pred on chondrogenesis (■ and solid line); effect of Pred on terminal differentiation (◆ and solid line). All data are expressed as means ± S.E.M. *P<0.05 compared with control (Cont); †P<0.05 significance level between Dex and equivalent dose of Pred.

Comparing Dex and Pred at equivalent concentrations, Dex at 10^{-7} M caused a significantly greater fall in proteoglycans than Pred at 10^{-7} M ($P<0.05$) (Table 2). Over the three concentrations, Dex caused a mean reduction in proteoglycan synthesis of 47% compared with 43% with Pred. No significant differences were noted during terminal differentiation.

Effect of GC on chondrocyte differentiation

The effect of GC on terminal chondrocyte differentiation as assessed by ALP activity is shown in Table 2. During chondrogenesis, enzyme activity in comparison with control values was significantly increased with both Dex: 10^{-8} M, 83%; 10^{-7} M, 118%; and 10^{-6} M, 116% and Pred: 10^{-8} M, 39%; 10^{-7} M, 77%; and 10^{-6} M, 77% ($P<0.05$). The mean elevations in ALP with all concentrations of Dex and Pred were 106% and 62% respectively and at equimolar concentrations of GC, Dex caused significantly larger increases in ALP than Pred. No significant differences in ALP activity were noted during the terminal differentiation phase.

Effects on apoptosis

Using the APOPercentage Apoptosis assay the number of apoptotic cells was higher in the terminally differentiating chondrocytes in comparison with cultures in the chondrogenesis phase. No evidence was detected for an effect of Dex and Pred on apoptosis during the chondrogenesis phase (Fig. 3a), however during terminal differentiation all Dex concentrations, and Pred at 10^{-6} M caused a significant decrease in apoptotic cell numbers ($P<0.05$) (Fig. 3b). Ethanol acted as a positive control and caused an elevation in apoptosis at both developmental phases ($P<0.05$). These data were confirmed with the use of the nucleosome ELISA kit (results not shown).

Recovery following GC exposure

Exposure of the ATDC5 cells to Dex for one or more days resulted in lower cell numbers on day 14. These differences, however, did not reach statistical significance unless the cells were exposed to Dex for all 14 days ($P<0.05$) (Fig. 4a and b). There was a significant reduction in proteoglycan content after 7, 10 and 14 days of GC exposure (Fig. 4c). After the recovery period (Fig. 4d), all Dex-exposed cells showed a significant reduction in proteoglycan content ($P<0.05$). ALP activity was increased after Dex treatment at days 7, 10 and 14 compared with control cultures but this increase was statistically significant only after 7 (65%) and 14 (148%) days of exposure (Fig. 4e). After the recovery period (Fig. 4f) ALP remained significantly elevated from day 10 ($P<0.05$).

Discussion

GC are known to exert effects on many physiological systems and can retard growth in children (Loeb 1976). While they may do this by altering GH secretion or GH sensitivity (Luo & Murphy 1989, Lima *et al.* 1993, Devesa *et al.* 1995), it is very likely that they may also exert direct effects on growth plate chondrocytes (Baron *et al.* 1992). *In vivo* studies in rats and *in vitro* studies using primary cultured rat epiphyseal chondrocytes show a down-regulation of GH receptor mRNA expression after GC treatment as well as an inhibition of IGF-I production and secretion into the culture medium (Gabriellsson *et al.* 1995, Jux *et al.* 1998). However, Heinrichs *et al.* (1994) reported an increase in GH receptor gene expression levels after treatment of rabbits with Dex. A reduction in rat growth cartilage width after GC treatment has also been observed and these authors have suggested that this is a likely consequence of the lower chondrocyte proliferation rate and increased hypertrophic chondrocytes apoptosis (Silvestrini *et al.* 2000).

Studies using rat chondrocyte cultures showed that Dex and Pred reduced both cell proliferation and colony formation and also that Dex was more potent than Pred at equimolar concentrations (Dearden *et al.* 1986, Robson *et al.* 1998). This culture data is in accord with *in vivo* observations where Dex appears to be more potent than Pred at causing impairment of normal bone growth (Strauss *et al.* 2001, Ahmed *et al.* 2002).

Our present experiments used the ATDC5 chondrocyte cell line, which has less phenotypic diversity than cultures containing a heterogeneous population of primary chondrocytes (Robson *et al.* 1998, Koedam *et al.* 2000). Furthermore, it allows the study of two critical events during cartilage formation: the early differentiation of committed mesenchymal cells into chondrocytes (chondrogenesis) and the terminal differentiation of proliferating to hypertrophic chondrocytes (Cancedda *et al.* 1995). Cell numbers were reduced by both Dex and Pred during the chondrogenesis period, but little effect of either GC was noted during the terminal differentiation period. Cell numbers may be reduced by GC by mechanisms such as loss of proliferative activity, increased apoptosis and cyto-stasis. Our present data strongly support the proposal that loss of proliferative activity is, at least in part, responsible for the decrease in chondrocyte numbers by GC treatment. We found no evidence of increased apoptosis, which is in accordance with Mehls *et al.* (2001). In the growth plate it is well recognised that apoptotic chondrocytes are most prevalent in the terminally differentiated zone (Ohyama *et al.* 1997) and this is also reflected in the ATDC5 cell line as shown in the present study. Interestingly, GC reduced apoptosis in the terminally differentiated cells whilst having no effect on the chondrogenesis phenotype, suggesting that GC control of chondrocyte apoptosis is phenotype dependent. This observation

Figure 3 Effect of Dex and Pred treatment for 24 h on the incidence of apoptosis during (a) chondrogenesis and (b) terminal differentiation. All data are expressed as means $\pm$ S.E.M. * $P < 0.05$ compared with control (Cont). No effects of the GC were observed during the chondrogenesis phase whereas all concentrations of Dex, and Pred at 10^{-6} M, caused a significant reduction in apoptosis during terminal differentiation. Ethanol (5%) caused a significant elevation in apoptosis at both developmental time points.

requires further study. Cell proliferation rates and cell numbers were more greatly affected by Dex and Pred during the chondrogenesis period when the chondrocytes were rapidly proliferating. These results extend the data from cultures containing chondrocytes of various maturational phenotypes (Robson *et al.* 1998) and are also in agreement with studies on other bone cell types which indicate that Dex was more potent than Pred in reducing osteoblast cell number and DNA synthesis (Kasperk *et al.* 1995, Davies *et al.* 2002). Davies and colleagues (2002)

also reported that osteoblast precursor cells (HCC1) were more chemosensitive to Dex than fully differentiated osteoblasts and together with our present data suggest that in bone cells, GC exert their maximum effect at the cell precursor stage. Over the three concentrations, Dex was also more potent than Pred as it caused a 44% greater increase in ALP activity and greater reductions in proteoglycan synthesis, cell number and cell proliferation. Annefeld (1992) also showed that Dex treatment in rats results in inhibition of both chondrocyte proliferation and

Figure 4 Effect of 10^{-6} M Dex on protein (a and b), proteoglycans (c and d) and ALP activity (e and f) either after a period of no recovery (a, c, e) or where the Dex-treated cells were allowed to recover and were assayed at 14 days (b, d, f). (a) The cell number increased with time in culture. The only significant reduction was after 14 days Dex treatment ($P < 0.05$). (b) Protein was reduced at all time points, but this was only significant at day 14 ($P < 0.05$). (c) Proteoglycan levels were elevated with time in the control group, but there was a significant reduction in proteoglycans ($P < 0.05$) from day 7 to day 14 in the Dex-treated group as compared with their controls. (d) A significant reduction in proteoglycan content occurred after 1 day Dex treatment ($P < 0.05$) and this was more pronounced with longer periods of treatment. (e) There was a gradual elevation in ALP activity (nmoles pNPP hydrolysed/min/mg protein) at each time point with a significant elevation above the respective control at days 7 and 14. (f) After the recovery period, ALP activity remained elevated and reached significance at days 10 and 14 ($P < 0.05$). Cont, control.

cartilage matrix production. The pro-differentiating effects of Dex are in agreement with studies using costochondral cultures, in which Dex promoted ALP activity (Schwartz *et al.* 1995). However, the results are at variance with other studies where Dex lowered enzyme activity in

prehypertrophic chondrocytes but had minimal effects on hypertrophic or mineralising chondrocytes (Robson *et al.* 2001). Although these results are in contrast to those presented here, they do substantiate our own observations and those of others that the response of chondrocytes to

Dex is dependent on their stage of differentiation (Yasuda *et al.* 1995).

In growth failure, amelioration of the growth retarding insult results in a period of supranormal linear growth described as catch-up growth (Prader *et al.* 1963). Two underlying mechanisms for this phenomenon have been suggested – a neuroendocrine model which adjusts the growth rate to an age appropriate set point, and a local mechanism intrinsic to the growth plate (Prader *et al.* 1963, Baron *et al.* 1994). Baron *et al.* (1994) demonstrated a 77% reduction in the growth rate of 5-week-old rabbit limbs that had Dex infused into the proximal tibial growth plate. Following cessation of Dex, catch-up growth was observed in the affected growth plate and not in the contralateral tibia. It is postulated that this is due partly to a delay in growth plate senescence by Dex (Gafni *et al.* 2001).

The ATDC5 cell line allowed us to study this recovery phenomenon in greater detail. No differences were observed in proteoglycan content in the culture plates stopped prior to recovery at 24 and 72 h of treatment. However, after the recovery period a reduction in proteoglycans was apparent at all treatment lengths. This would indicate that Dex-induced suppression may take some time to manifest itself even though the stimulus for suppression has been removed. Longer treatments with Dex of 7 and 10 days duration showed unchanged levels of proteoglycans compared with the 14-day treated group and indicates that within the timescale of this experiment the ability to recover after 7 days of Dex treatment was irreversible. Longer periods of recovery allowed the ALP activity to be suppressed towards control levels, although after 10 days there was no further recovery noted as compared with the 14-day Dex-treated group. It cannot be ruled out, however, that longer periods of recovery could result in suppression of ALP activity back to control levels. It is possible that catch-up growth is never complete and merely falls below the statistical detection limit of a study (Silverstein *et al.* 1997).

In conclusion, Dex and Pred reduce cell number, cell proliferation and proteoglycan content whilst stimulating chondrocyte differentiation. The GC have maximal effects during chondrogenesis with minimal effects during terminal differentiation. The ability to recover is related to the length of Dex exposure and possibly the chondrocyte phenotype. Our studies suggest that the potential for recovery of different events of the chondrocyte life cycle may vary. Our findings in the ATDC5 cell line will allow a more focussed approach towards studying the mechanisms underlying GC-induced growth retardation as well as investigating the potential benefit of growth-promoting therapy.

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