Effect of the synthetic glucocorticoid, deflazacort, on body growth, pulsatile secretion of GH and thymolysis in the rat

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Abstract

Design: Deflazacort (DFZ) is a relatively new glucocorticoid that has been reported to exhibit fewer side-effects than other commonly used corticosteroids. The present study was designed to test the effects of DFZ on thymus gland involution (thymolysis), as compared with body growth and the secretory pattern of GH in the rat. Beginning at 38 days of age, male animals were treated for 8 consecutive days by s.c. injection of DFZ (0.15 mg/day), cortisone (CORT) (5 mg/day) or vehicle (control, CTRL).
Results: Both glucocorticoids had a similar thymolytic effect and caused growth failure, but the growth rate for the DFZ group was significantly higher than that of the CORT group. On day 46, pulsatile GH secretion was quantitated by blood sampling via an indwelling catheter at 10 min intervals for 6 h. GH was assayed by RIA and analyzed by multiparameter deconvolution. CORT caused an increase in pulse frequency (5.8 ± 0.4 (S.E.M.)) in comparison to DFZ (4.4 ± 0.4) and CTRL (3.8 ± 0.3). Both glucocorticoids significantly shortened the interval between secretory bursts. In CTRL animals the interval between bursts was 69.3 ± 4.5 min. In DFZ animals this was reduced to 58.5 ± 7.1 min, and in CORT rats it was further reduced to 47.0 ± 2.6 min. The mass of GH secreted per burst was reduced in CORT animals (52% of CTRL), while DFZ did not alter this parameter. A similar trend was observed for total GH production, with CORT causing a reduction and DFZ not affecting the secretion.
Conclusion: Rats treated with glucocorticoid show a profound thymolytic effect, as well as important changes in growth. While CORT suppresses GH secretion and alters its pulsatile mode of release, DFZ causes a less significant alteration in the pattern of GH secretion and does not negatively affect the overall amount of GH secreted.

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Introduction

In children, daily treatment with corticosteroids inhibits growth in a dose-dependent fashion when used for a prolonged period of time (1–3). Growth inhibition by corticosteroids has also been demonstrated in rats (4–6).

In the complete absence of glucocorticoids, growth hormone (GH) production is low (7, 8). It increases with the addition of thyroxine and low doses of glucocorticoids *in vivo* and *in vitro* (9–11). While short-term application of glucocorticoids stimulates GH secretion *in vitro* (12–14) and *in vivo* (15), as measured by standardized stimulation tests (16, 17), prolonged exposure to glucocorticoids has negative effects on GH secretion *in vivo* (16, 18). After cessation of cortisone (CORT) treatment, increased secretion of GH with concomitant failure of catch-up growth has been observed in rats (19).

In 1967 Nathansohn *et al.* (20) synthesized a novel corticosteroid, deflazacort (DFZ), which is an oxazoline derivative of CORT. In rats, DFZ has a plasma half-life of 1.1 h for the alpha-phase and 11.6 h for the beta-phase (21–23). DFZ is metabolized in the liver to at least five different compounds, although the oxazoline group is not known to undergo degradation or metabolism in any species (22). DFZ is less lipophilic than other glucocorticoids (24), and therefore less likely to permeate into the brain (25). Assuming that glucocorticoids act in part upon central nervous system control mechanisms, it might be expected that DFZ would exert less influence on GH secretion by having less access to the brain. However, no data exist regarding the effects of DFZ on pulsatile GH secretion.

Immunosuppressive and anti-inflammatory properties of DFZ have been demonstrated in animals (26). At comparable dosages, DFZ was associated with less growth retardation than other corticosteroids in children with different diseases (27–30) and in rats (31). Studies on comparable dosages show a ratio of 1:31–1:33 for DFZ to CORT *in vivo* in rats (31–35). These bioequivalence studies are based on carrageenin- and nystatin-induced edema, thymolytic effects, and the *ex vivo* binding to glucocorticoid receptors of various glucocorticoids. Humans are less sensitive to DFZ with a

dosage ratio of 1:1.2 for prednisone to DFZ (equivalent to about 1:3.3 for DFZ to CORT (36, 37)). The present studies were designed to test the effects of CORT and DFZ on thymolysis, growth and the pulsatile mode of GH secretion in animals receiving this treatment.

Materials and methods

Thirty-seven male Long Evans rats (Charles River Laboratories, Wilmington, MA, USA) at 32 days of age were assigned to three weight-matched groups as follows: CORT (5.0 mg/day, $n = 12$), DFZ (0.15 mg/day, $n = 12$) and control (CTRL, vehicle, $n = 12$). The rats were placed in single cages. Animals were housed in a 12 h light:12 h darkness cycle with the lights on at 0700 h and off at 1900 h.

Starting at 38 days of age the animals received the respective glucocorticoid or vehicle for 8 consecutive days. CORT was supplied as cortisone-acetate in a 0.9% benzyl alcohol solution. Generic crystalline DFZ (a gift of Marion Merrell Dow, Cincinnati, OH, USA) was dissolved in ethanol and then brought up to a solution of 0.9% ethanol with isotonic saline. The vehicle solution was saline containing 0.9% ethanol. Injections were given s.c. in the scapular region at 1700 h. Standard powdered laboratory chow (Prolab, Purina, St Louis, MO, USA) and water were freely available. Animals and food containers were weighed daily.

On day 42 a polyvinyl catheter (Dural Plastics Ltd, Auburn, Australia) was implanted under brevital (sodium methohexital, Eli Lilly, Indianapolis, IN, USA) anesthesia into the right atrium through the right external jugular vein. The heparin-filled catheter was tunnelled under the skin to exit dorsally, between the shoulder blades. The catheter was plugged and the animals kept on a heating pad until being returned to their cages. Blood samples for determination of GH pulsatility were taken via an intra-atrial catheter on day 46. The indwelling catheter was attached to a 50 cm polyvinyl extension by a piece of stainless steel tubing. The animals were allowed to adapt to the polyvinyl extensions for approximately 30 min. Beginning at 1030 h, samples were collected every 10 min for 6 h. Blood was withdrawn into heparin-treated 1.0 ml syringes. Plasma was immediately collected after centrifugation, the red blood cells resuspended in saline, and returned to the animal after the subsequent sample. The initial blood sample was replaced with saline. This protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University. All samples were stored at $-20\,°C$ until assay for GH by RIA.

The assays were performed using RIA kits provided by the National Hormone and Pituitary Program of the National Institute of Diabetes, Digestive and Kidney Diseases (Bethesda, MD, USA) and Dr A F Parlow. The reference preparation was rat GH RP-2, and intra- and interassay coefficients of variation were 6.9 and 9.8% at 1.0 and 0.625 ng per tube respectively.

Mean half-life, mean frequency of GH secretory pulses, mean GH secretory pulse amplitude (maximal rate attained per burst), mean GH secretory burst area (mass) and mean total GH secretion were calculated and analyzed by multi-parameter deconvolution of the plasma hormone concentrations according to the method of Veldhuis and Johnson (38, 39). Small pulses contributing less than 5% to the total secretion of a respective animal were excluded from further analyses. Intergroup comparisons were done by a Kruskal–Wallis test with a Mann–Whitney test for further testing in case of significance (JMP/SAS Institute, Cary, NC, USA).

Results

During the control period preceding treatment, all animals showed comparable weight gain (Table 1). During the treatment period, weight gain was slightly lower in the CTRL group in comparison to the preceding period of observation. This was mainly due to lack of weight gain peri-operatively. The two glucocorticoid groups showed significant reductions in weight gain. The DFZ group maintained about 28% of the CTRL mean daily weight gain (1.3 ± 0.4 g/day and 4.8 ± 0.3 g/day respectively (S.E.M.)). The CORT group gained

Table 1 Weight gain, food intake, food efficiency and final weight of thymus for CORT-treated (5 mg/day), DFZ-treated (0.15 mg/day) and CTRL rats. Values are means $\pm$ S.E.M.

	CTRL	CORT	DFZ
Number of animals	12	9	9
Daily weight gain (g/day)			
Control period	7.8 ± 0.5	8.5 ± 0.7	8.3 ± 0.4
Treatment period	4.8 ± 0.3^a	0.1 ± 0.5^b	1.3 ± 0.4^c
Food intake (g/day/100 g BW)	10.1 ± 0.30	9.8 ± 0.28	9.8 ± 0.29
Food efficiency (g weight gain/g food intake)	0.31 ± 0.02^a	0.02 ± 0.05^b	0.06 ± 0.05^b
Thymus/BW (g/100 g)	275 ± 10^a	62 ± 12^b	47 ± 11^b

Superscripts denote significant differences ($P < 0.05$) by Kruskal–Wallis/Mann–Whitney test between groups not sharing the same letter in any one row.

Table 2 Deconvolution analysis for all derived parameters (mean $\pm$ s.e.m.)

	CTRL	CORT	DFZ
Number of animals	12	9	9
GH half-life (min)	12.9 ± 1.2	14.5 ± 1.9	11.7 ± 1.2
GH production rate (ng/ml/6 h)	265 ± 24	207 ± 46	296 ± 43
Number of pulses (n/6 h)	3.8 ± 0.3^a	5.8 ± 0.3^b	4.2 ± 0.5^a
Interval (min)	69 ± 4.5^a	47 ± 2.6^b	53 ± 4.5^b
Mass secretion per burst (ng/ml/burst)	73 ± 7.3^a	38 ± 9.5^b	96 ± 31^a
Amplitude (ng/ml/min)	19 ± 6.6	9.8 ± 2.4	31 ± 15.5

Superscripts denote significant differences ($P < 0.05$) by Kruskal–Wallis/Mann–Whitney test between groups not sharing the same letter in any one row.

weight at a rate of only 2.5% of that of the CTRL (0.1 ± 0.5 g/day). As shown in Table 1, daily weight gain was significantly different among all three groups during the treatment period. At the same time, food intake did not differ among groups during the control or treatment periods. In this respect, food efficiency (g body weight gained/g food consumed) was reduced in the two treatment groups compared with CTRL animals. Although mean food efficiency was three times higher in the DFZ group in comparison to CORT animals, no statistical difference could be proven due to the high variability (Table 1).

Thymus weights are also shown in Table 1. The weight of the thymus, adjusted to 100 g body weight (BW), was significantly and equally reduced in both glucocorticoid groups. The lack of difference between thymus weights of the CORT- and the DFZ-treated animals supported the previous finding (31) that 5 mg CORT were essentially equal to 0.15 mg DFZ with respect to thymolytic activity. Side-effects of treatment other than growth disturbances, especially polyuria, were not observed.

During the blood collection experiments, three animals of each glucocorticoid group were excluded because of catheter malfunction. In the remaining successful collections, mean GH concentrations were 15.7 ± 1.8 ng/ml in CTRL, 12.6 ± 2.1 in CORT and 17.8 ± 2.0 in the DFZ group. There were no differences among the three groups. Exclusion of minor pulses (less than 5% of total secretion) reduced the percentage of total secretion remaining in the analyses only to a minor extent. The precise remaining percentages of total secretion were: CTRL, $90.8 \pm 2.4\%$; CORT, $94.9 \pm 1.4\%$; DFZ, $91.9 \pm 2.9\%$ (no significant differences among the groups).

Analyses of the specific parameters of pulsatile GH secretion by deconvolution revealed that significant changes had occurred during CORT treatment, while changes during DFZ were relatively mild. The half-life of plasma GH was not affected by glucocorticoid treatment (Table 2). Likewise, there were only marginal changes in total GH production among the groups. The absolute value for the CORT group was lower in comparison to the DFZ and CTRL groups, but the differences were not significant ($P = 0.07$, Table 2).

Probably more important than the above data, the pattern of GH pulsatility was significantly altered by the glucocorticoids. Significantly more detectable GH pulses per 6 h occurred in the CORT group than in the DFZ or CTRL group (Fig. 1, Table 2). Both glucocorticoids shortened the intervals between secretory bursts. In CTRL animals, the interval between bursts was 69.3 ± 4.5 min. In DFZ rats this was reduced to 53.0 ± 4.5 min and in CORT rats it was lowest with 47.0 ± 2.6 min (Fig. 1). The reduction in interpulse intervals in both treatment groups in comparison to CTRL was significant ($P = 0.0006$ for CORT and $P = 0.048$ for DFZ). There was no difference between DFZ and CORT in regard to interval of the secretory bursts. The mass of GH secreted per burst was reduced in CORT to 52% of the respective control value (Table 2). DFZ did not significantly alter the mass of GH secreted per burst in comparison to CTRL. In this respect, CORT led to a marked and significant reduction in the mass per secretory burst in comparison to that of both of the other groups.
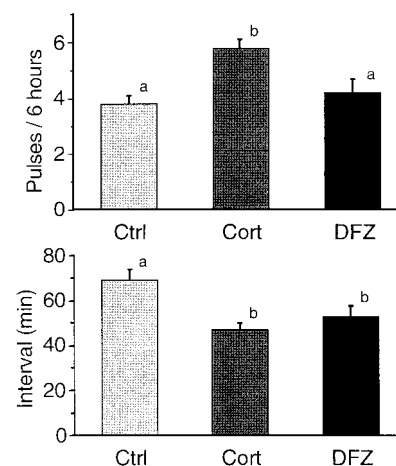

Figure 1 The mean number of secretory pulses ($\pm$ s.e.m.) occurring per 6 h and the interval between these pulses as derived by deconvolution analysis. Different superscripts denote significant differences ($P < 0.05$) by Kruskal–Wallis/Mann–Whitney test between groups not sharing the same letter. Thus, for pulses, Ctrl and DFZ are not different, because they share the letter 'a'. Cort is different from the other two groups, because they do not bear the letter 'b'.

A similar, although not significant, pattern was observed for the pulse amplitude, which was highest in the DFZ animals and lowest in the CORT group. The CTRL group showed an intermediate pulse amplitude (Table 2). Due to high variability, however, these data failed to reach statistical significance.

Discussion

Glucocorticoids penetrate the brain and pituitary gland, and have been shown to affect DNA transcription of the GH gene (40). DFZ is assumed to penetrate less into the brain because it is more hydrophilic (24, 25). Autoradiographic findings in rats after DFZ injection, where no DFZ could be detected in the central nervous system support this concept (26).

DFZ is a relatively new glucocorticoid with presumably fewer side-effects. Preliminary human studies, and at least one rat study, demonstrate beneficial effects of DFZ in terms of growth (29, 31, 41, 42). In the present study, growth rate was higher in the DFZ group than in the CORT group.

Pulsatile GH secretion was markedly affected in glucocorticoid-treated animals. The number of detectable GH pulses during the 6 h period was significantly increased in the CORT group, and only slightly, but not significantly, elevated in the DFZ animals. Most studies report intervals between major secretory events of 3 h for male rats after 30 days of age (43–45). Those studies were not based on deconvolution analyses, and included 'volleys' (multiple medium-sized peaks) in each of the major secretory events. By counting all individual secretory events as separate episodes, Martin *et al.* (46) described intervals of 68 min for normal rats. This is in very close agreement with the present data for CTRL animals, where a mean interval of 69 min was found. In both treatment groups the interval between pulses was decreased.

The mass of GH secreted per burst was significantly reduced, and total GH production was lower in the CORT but not in the DFZ group in comparison with CTRL. The intergroup pattern of the mean amplitudes of the secretory bursts correlated with the above changes, but varied widely, raising some concern in interpreting this aspect of the data. In general, amplitudes of pulses were relatively low. The peak heights of GH secretion are known to be affected by the pubertal status of the animals (44), with peaks getting higher the more puberty advances. This may have influenced our results, although pubertal status was not assessed. Thus, maximal GH secretory activity was suppressed by CORT, while DFZ did not exhibit this effect.

However, the marked growth failure in the DFZ group is in sharp contrast to the relatively minor change observed in the total GH secretion of these animals. This suggests that total GH secretion is only one aspect in the pathophysiology of growth failure during glucocorticoid treatment, and that attenuated GH pulse height in combination with increased pulse frequency may contribute to the growth failure. This interpretation would agree with other studies that indicate the critical importance of the pulsatile pattern of GH secretion to the typically accentuated growth rate in males of many species. Further studies will be necessary to determine the importance of GH pulsatility to actual growth in glucocorticoid-treated animals, as well as the importance of potential changes in GH receptor abundance and activity, and insulin-like growth factor-I responsiveness to GH.

Schäfer *et al.* (47) demonstrated comparable changes in GH secretion in children after kidney transplantation under glucocorticoid treatment. Total GH secretion and secretory mass per burst were reduced, while the number of pulses was increased in prepubertal boys. These parameters correlated with the applied dose of glucocorticoid. Extrapolation of our animal data to humans should be done with the utmost caution. Nevertheless, the similarities to the cited results and our findings with a conventional glucocorticoid, and the striking difference between DFZ and CORT, all point towards a similar potential benefit in GH secretion for humans under DFZ therapy.

In summary, the present data support the possibility that DFZ affects central nervous regulation of GH secretion less than does CORT. While CORT was thymolytic, suppressed growth, and altered the pulsatile mode of GH secretion, DFZ was thymolytic to the same degree, but inhibited growth and modified the pattern of GH secretion to a lesser degree. In this respect, DFZ may be considered to have a potent immunosuppressive effect with somewhat less growth inhibitory capacity.

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