Hypophyseal-Adrenocortical Function in Experimental Iatrogenic Canine Cushing's Syndrome

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Iatrogenic Cushing's syndrome (ICS) is caused clinically by long-term administration of glucocorticoids (GC) and its clinical findings are essentially the same as those of spontaneously induced hyperadrenocorticism [14]. The plasma temporal levels of cortisol and ACTH are lower in dogs with ICS and higher in dogs with spontaneously Cushing's syndrome than in normal dogs [10, 14]. However, change of plasma cortisol and ACTH levels was not clear in relation to monitoring the ICS development and recovery processes while treating with short-acting GC in dog.

In the present study, the hypophyseal-adrenocortical function in dog with ICS was examined chronologically during dexamethasone (DM) administration and prednisolone (PS) regimen.

Eight (3 males, 5 females) Beagle dogs, 3–10 years of age, weighing 8–10 kg were housed under alternating 12-hr lighting and 12-hr darkness. All dogs were injected intramuscularly with DM at 0.4 mg/kg/day for 63 days to induce ICS. They were treated with prednisolone orally for 4 weeks after termination of DM administration. PS was given 1 mg/kg, twice a day at 9:00 and 21:00 hr for the first week and 2 mg/kg, once a day at 9:00 hr for the next 3 weeks. Clinical signs, blood eosinophil count, plasma cortisol and ACTH levels were taken as indicators of the progress and recovery. Blood collection was carried out before, day 3 after initiating DM adminstration (iDM day 3) and at 5-day intervals thereafter until 98 days.

Sampling for circadian variation was carried out between 9:00 and 9:00 hr (24 hr period) at 6-hr intervals before and iDM day 30. Eosinophil count was carried out by the method of Doe *et al.* [2]. Plasma cortisol levels were determined by the radioimmunoassay described by Makino [7]. Antisera against cortisol-21-succ-BSA were purchased (Teikoku Hormone Mfg. Co., Ltd, Tokyo).

Crossreactivities of cortisol antisera to cortisone, prednisolone and corticosterone were 28.1, 10.9 and 2.87%, respectively. Tritiated cortisol (specific activity 55.8 Ci/mM) was used a product of New England Nuclear Co. (Boston, U.S.A.). The minimum sensitivity of the assay was 15 pg/tube. The coefficients of variation for intra- and inter-assays were 7.3 and 13.8%, respectively. Plasma ACTH was determined by an ACTH radioimmunoassay kit (Compagnie Oris Industrioe, France). The minimum sensitivity of this kit was 10 pg/tube. The coefficients of variation for intra- and inter-assays were 10.2 and 15.4%, respectively. Statistical significance was verified with the Student's t test, and the correlation coefficients were evaluated.

Circadian changes: In normal intact dogs, blood eosinophil count was the lowest level (225±39 cells/mm^3) at 9:00 hr and the highest (320±75 cells/mm^3) at 21:00 hr, showing significant difference ($p<0.05$) at each indicated time. Plasma cortisol and ACTH levels showed the highest (27.5±5.2 ng/ml and 47.3±15.7 pg/ml, respectively) at 9:00 hr and the lowest (17.9±8.1 ng/ml and 22.6±5.2 pg/ml, respectively) at 21:00 hr, showing significant difference ($p<0.01$) at each indicated time. This result confirmed previous findings [11, 12] (Fig. 1). On the contrary, there are some [6, 15] who can not observe circadian cortisol variation. When compared with our results, this discrepancy may be attributed to exercise, sleep and other factors such as blood collection frequency and other procedural differences [12]. Among the 3 parameters, a significant negative correlation ($p<0.01$) was seen between blood eosinophil counts and plasma ACTH levels and between blood eosinophil counts and plasma cortisol levels, and a significant positive correlation ($p<0.01$) between cortisol and ACTH levels was observed. However, no circadian changes were found in blood eosinophil counts, plasma cortisol and ACTH levels in any dogs on iDM day 33, where the 3 parameters registered less than 25 cells/mm^3, 1 ng/ml and 20 pg/ml, respectivley.

Clinical findings of ICS: All animals treated with DM showed polyuria, compensatory polydipsia and diarrhea until iDM day 10. In addition, the distention of abdomen and muscular weakness were also presented from iDM day 15 thereafter, whereas alopecia was not seen. Symptoms of polyuria and polydipsia disappeared within 7 days followed by the other symptoms almost within 14 days after initiating prednisolone regimen (iPS 14 days).

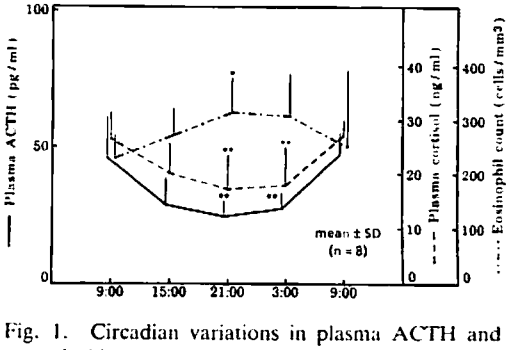

Fig. 1. Circadian variations in plasma ACTH and cortisol levels and eosinophil count in normal dogs. The difference between 9:00 and 21:00 hr in the 3 parameters were statistically significant (*: $p<0.05$, **: $p<0.01$).

The common clinical signs observed in dogs with Cushing's syndrome are plyuria, polydipsia, hair loss, pendulous abdomen and muscle atrophy [14]. These symptoms, except hair loss, were coincide with those showed in the ICS dogs.

Chronological changes: Blood eosinophil counts decreased significantly ($p<0.01$) from 550±145 cells/mm^3 on iDM day 0 to 21±5 cells/mm^3 on iDM day 3 and remained at very low levels until iDM day 63. It started to increase gradually to significant ($p<0.01$) levels on iPS day 5 (Fig. 2). However, blood eosinophil counts were inconsistent on recovery from the induced-ICS, corresponding closely to other observations [3]. Further studies to account for such an inconsistancy during the PS regimen were warranted. Plasma cortisol levls also decreased significantly ($p<0.01$) from 24.2±6.4 ng/m*l* to 2.2±0.5 ng/m*l* on iDM day 3 and then remained very low levels of 0.2 to 4.0 ng/m*l* until iDM day 63. Cortisol showed clearly a significant ($p<0.01$) increase on iPS day 15 (Fig. 3). Similarly plasma ACTH levels decreased significantly

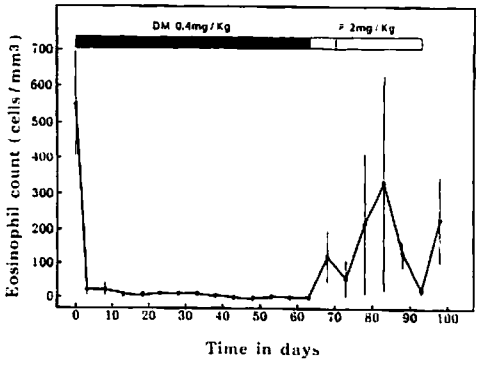

Fig. 2. Changes in blood eosinophil count during DM treatment and PS regimen in dogs. Closed-bar represents duration of IM administration of DM from day 0 to day 63. Open-bar indicates duration of oral administration of PS at 1 mg/kg twice a day (at 9:00 and 21:00 hr) for the first week and 2 mg/kg once a day (at 9:00 hr) for the following 3 weeks. Each point represents the mean value of 8 dogs.

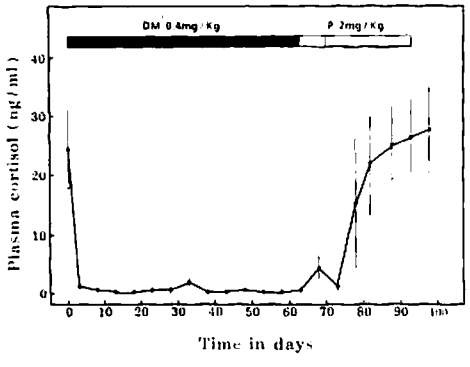

Fig. 3. Changes in plasma cortisol level during DM treatment and PS regimen in dogs.

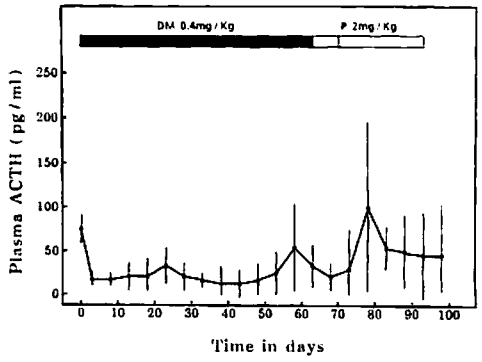

Fig. 4. Changes in plasma ACTH level during DM treatment and PS regimen in dogs.

($p<0.01$) from 75.8±16.1 pg/m*l* to 18.6±5.9 pg/m*l* on iDM day 3 but varied in an undulating manner even during DM administration. On iPS day 15, ACTH levels increased significant ($p<0.01$) to 105.4±85.7 ng/m*l*, and showed inconsistent changes thereafter (Fig. 4). The above results indicate that plasma cortisol levels and eosinophil counts were potently suppressed by DM treatment whereas suppression of plasma ACTH was of a lesser degree. In human, plasma ACTH levels decrease significantly after DM administration [1] because long-term application of GC induces a negative feedback to the hypothalamic-pituitary-adrenal axis. Therefore, our results might explain that functional suppression of hypothalamic-pituitary in dog was relatively less potent than that in human during GC treatment. Additionally, the decrease of ACTH levels on iPS day 20 probably might result from the increase of sensitivity of adrenal gland against ACTH [4] or the insufficient revival of pituitary function [14].

With respect to the regimen after GC treatment, one [6] suggests that ICS dogs need to be maintained on small, near physiological dose with a short-acting GC in order to supply the daily cortisol requirement. Others [8, 13] believe alternate-day administration of a short-acting GC reduces both adrenocortical atrophy and extra-adrenal adverse effects. In this similarly designed experiment, though the use of rather high doses of PS [10] was indicated, and satisfactory recovery of hypophyseal-adrenocortical function, especially in plasma cortisol levels, to normality on iPS day 15 was achieved. These results may indicate that recovery from induced-ICS in dog was faster than that in human [9].

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