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Cushing's disease in dogs: Cabergoline treatment

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Abstract

The treatment of pituitary-dependent hyperadrenocorticism (PDH) in dogs has for a long time been focused on inhibiting the adrenal gland using drugs such as *o–p′*-DDD, Ketoconazole and Trilostane, without attacking the primary cause: the corticotrophinoma. Corticotroph cells can express the D2 dopaminergic receptor; therefore cabergoline (Cbg) could be effective as a treatment. Follow-up over 4 years was carried out in 40 dogs with PDH that were treated with Cbg (0.07 mg/kg/week. Out of the 40 dogs, 17 responded to Cbg (42.5%). A year after the treatment, there was a significant decrease in ACTH ($p < 0.0001$), α-MSH ($p < 0.01$), urinary cortisol/creatinine ratio ($p < 0.001$), and of the tumor size ($p < 0.0001$) evaluated by nuclear magnetic resonance. Dogs responding to Cbg lived significantly longer ($p < 0.001$) than those in the control group. To conclude, Cbg is useful in 42.5% of dogs with PDH, justifying its use as a treatment.
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Keywords: Hyperadrenocorticism; Cushing's disease; Corticotrophinoma; Cabergoline; D2 dopamine receptor

1. Introduction

The treatment of the pituitary-dependent Cushing's disease (PDH) or Cushing's disease (CD) in dogs has for a long time been mainly focused on the inhibition of the secretion of cortisol by means of drugs that act on the adrenal gland, either by causing its necrosis or by inhibiting the synthesis of steroids (Castillo et al., 1996; Feldman et al., 1990, Kintzer and Peterson, 1991; Komanicky et al., 1978; Sieber-Ruckstuhl et al., 2006). Although the solution would be the removal of the pituitary tumor, the problem in veterinary cases lies in the fact that the whole pituitary gland is removed and not only the diseased area (Meij et al., 2002). Radiotherapy has also been proposed as a therapeutic method, the problem being not only the cost, but also the high risk of damaging the optical chiasm or the surrounding brain tissue (Goossens et al., 1998; Gittoes, 2005). With regard to drugs that act upon the synthesis and release of ACTH and also control the corticotroph adenoma, we have recently shown that 9-*cis* retinoic acid is effective to control PDH in dogs, as it had been previously reported in mice (Páez-Pareda et al., 2001), causing a reduction of the tumour and the inhibition of the synthesis of ACTH (Castillo et al., 2006).

The presence and inhibitory action of dopaminergic receptors in the anterior pituitary lobe was discovered some time ago (Antakly et al., 1987; Caron et al., 1978; Herder et al., 1995). Dopaminergic drugs (such as bromocriptine) have been tested for the treatment of PDH with varying success (Bevan et al., 1992; Labeur et al., 2004; Mercado-Asis et al., 1992; Rijnberk et al., 1988a; Sonino and Boscaro, 1999). The debate as to the use of these types of drugs arises due to the different opinions on the genesis of the corticotroph adenoma (Dahia and Grossman, 1999; Melmed, 2003), as well as the results obtained. It has been widely accepted that the origin of the corticotroph adenoma is monoclonal, but the theory that an over stimulation of the corticotroph area of the hypophysis by the hypothalamus (increase in ACTH [CRH] releasing factor or reduction of the dopaminergic tone) may bring about the development of neoplasia, after a first stage of

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hyperplasia, has not been ruled out (Asa and Ezzat, 1998; Dahia and Grossman, 1999; Kemppainen et al., 1989). It has been described that both the degeneration of dopaminergic neurons and the loss of D2 receptors due to age, affects the actions of dopamine (DA), both in the central nervous system as well as in the negative regulation on the hypophysis, especially on the lactotroph and corticotroph cells of the pars intermedia (PI) (Saiardi et al., 1997; Hereñú et al., 2006a). This age-related neurodegeneration has also been described in dogs therefore, the reduction of DA would lead to the lifting of the hypothalamic inhibition and chronic stimulation of the CRH over the corticotroph area, causing its hyperplasia (Rothulzen, 1991). On the other hand, both Young et al. (2004) and Van Craenenbroeck et al. (2005) describe a reduction in the synthesis and actions of dopamine in cases of chronic stress, resulting in a greater activation of the hypothalamus-pituitary-adrenal axis (HPA) and in hyperplasia of the corticotroph cells. It is known that the pars intermedia (PI) of the pituitary is negatively neuro-regulated by DA, inhibiting in this way the synthesis of the melanocyte-stimulating hormone (α-MSH), and it has been described in the dog that the corticotroph cells of the pars distalis (PD) may also be inhibited by DA (Kemppainen et al., 1989; Munemura et al., 1980). In dogs the corticotropinoma's origin may be either from the PD, the PI or indeed be of mixed origin (Peterson et al., 1982). PI tumours are resistant to the suppressive effects of dexamethazone (Kemppainen and Zenoble, 1985; Kooistra et al., 1997).

Cabergoline (Cbg) is a dopamine D2 receptor agonist with a higher affinity and a longer half-life than bromocriptine (Colao et al., 2000). In humans, its effectiveness and tolerance in the treatment of prolactinomas (Verhelst et al., 1999) and growth-hormone secreting adenomas (Abs et al., 1998) have been demonstrated. Pivonello et al. (1999) describe reduction in size of the aggressive corticotroph adenoma which causes Nelson's Syndrome, while the anti-neoplasic effects of Cabergoline have been reported to be effective in the treatment of non-functional tumours of corticotroph cell (Petrossians et al., 2001). Casulari et al. (2004) describe the presence of D2 receptors in a corticotroph adenomas in a patient with Nelson's Syndrome.

As the usefulness of Cbg in the treatment of PDH in dogs had not been studied, and taking into account the neurophysiological characteristics of the canine hypophysis (Kemppainen et al., 1989; Middleton et al., 1987; Peterson et al., 1986), we decided to treat a group of dogs with PDH with Cbg with the purpose of evaluating the effectiveness of this drug in the therapy of the disease.

2. Materials and methods

2.1. Population under study

A single-blind, longitudinal study, with a 4 year follow-up, was performed in 63 dogs with PDH, diagnosed according to the following criteria: presence of at least 4 clinical signs characteristic of the disease (Ling et al., 1979), cortisol: creatinine ratio in urine greater than 70 nmol/L with elevated plasma ACTH and evidence of a pituitary tumour or abnormal PI appearance detected by nuclear magnetic resonance imaging (NMRI).

The dogs' average age was 9 (range 3–14 years old), 40% of them being mixed-breed and the rest of several breeds: Poodle, German Shepherd, Beagle, Boxer, Doberman, Daschund, Syberian Husky, Samoyedo, Shetland Sheep dog, Standard Schnauzer. All cases were sent to the Endocrinology Unit of the Veterinary School Hospital of the Faculty of Veterinary Sciences of the University of Buenos Aires and had the written consent of their owners to participate in the study.

Dogs were divided into two groups in the following manner: every 3 cases, the first one was incorporated to the control group (up until 23 cases) and the following 2 cases into the Cbg group (up to 40 cases). This was done so as to end up with the same amount of cases in both control and Cbg groups, based on a previous experience in nude mice with an implanted ACTH secreting adenoma where approximately 50% did not respond to Cbg. Thus, the resulting groups were:

Control group (Ketoconazole group): 23 dogs (13 female [9 non-castrated] and 10 male) which received ketoconazole (Ktz, 20 mg/kg/day), this being the conventional therapy for canine Cushing's syndrome in Argentina. This was used as a control because of ethical and legal reasons that do not allow us to leave animals without a proven treatment.

The Cbg group: 40 dogs (26 female [15 non-castrated] and 14 male) which received a total of 0.07 mg/kg Cbg per week, dividing the dose into 3 and giving one every 48 hours. Cases where there was no response to Cbg within 3 months after starting the treatment or when there were side effects ascribed to the therapy that put the animal's life at risk were withdrawn from the study and transferred to the conventional therapy used in Argentina (in accordance with the ethics committee's indications) and therefore were not evaluated statistically nor incorporated in the Ktz group. The group with those dogs that continued with Cbg was renamed: Cbg responding treatment (CbgRT).

As a clinical follow-up, the following parameters of objective evaluation, as well as those highly frequent in dogs with PDH (Ling et al., 1979), were controlled: presence or absence of polydipsia–polyuria (Pd–Pu), urine density, polyphagia (Pf), size of abdomen (prominent or normal), weight (kg) and return to oestrous cycle in females. The appearance of the skin (elasticity, thickness and pigmentation) and of the hair (alopecia and signs such as brightness and greasiness) were also evaluated. Response to the treatment was considered positive if within 3 months the following improvements were observed: suspension or reduction of Pd–Pu (liquid intake <100 ml/kg/day), increase of urine density, and at least a 5% reduction in weight, compared to the initial weight. It was deemed that there had been a full response to the treatment if besides continuing with the normalization of the above

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mentioned parameters, there was evidence of normalization of the endocrine-biochemistry and a reduction of the size of the adenoma or of the aspect of the PI in the images after one year of treatment.

2.2. Diagnosis of Cushing's disease and follow-up of groups

The PDH diagnosis protocol (Endocrinology Unit, Faculty of Veterinary Sciences of the University of Buenos Aires) was based on: (1) an increase of urine clearance of cortisol (evaluated through the cortisol/creatinine ratio in urine, C/CR) with inadequate high concentrations of plasma ACTH, and (2) a reduction of the C/CR to more than 50% of the basal value of C/CR previously obtained according to Rijnberk et al. (1988b) and Galac et al. (1997) after oral administration of 0.1 mg/kg of dexamethazone every 8 h. Confirmation of the pituitary adenoma was obtained through nuclear magnetic resonance imaging (NMRI), with sections every 2 mm. To evaluate the appearance of the adenoma, the sagittal section was taken through the middle line, classifying the tumours into intrasellar ($\leqslant$5 mm) and extra-sellar ($\geqslant$5.5 mm) according to the phases described by Asa and Ezzat (1998) and validated by our previous observations in normal dogs and dogs with PDH. With this same sagital section, the appearance of the PI was studied (abnormal: image of more than 0.5 mm of thickness at the sagittal section, without an openly visible signal to the gadolinium enhancing, according to our observations in healthy dogs). The size of the adenoma was measured through the transversal section, calculating the greatest diameter or height (greatest height from the base to the highest point of the section) and the tumour surface by multiplying the height (greatest diameter of the tumour in the transversal section) by the width (height $\times$ width $=$ mm^2). A pituitary with a normal appearance in a NMRI is equivalent to "0" mm. The images obtained by NMRI were repeated after one year of treatment with Cbg and after 4 years, and were performed by the same operator. The coefficient of variation between studies was of $\pm$0.5 mm.

2.3. Hormone measurements

Plasma ACTH concentration was measured by means of the immunoradiometric assay (IRMA) using an available commercial kit (Nichols Advantage ACTH Assay, Nichols Institute Diagnostics, Bad Vilbel, Germany). The α-MSH (Euro-Diagnostica AB, Malmö, Sweden) was obtained with the same sample as the ACTH and measured by means of the radioimmuno assay (RIA), the plasma being frozen at -80 °C until its processing. The intra-assay and inter-assay coefficients of variation for ACTH were 3% and 6.8% respectively; and for α-MSH were 2.9% and 4.0%, respectively.

Urine cortisol was measured by means of RIA, using a commercial kit (DPC Corporation, San Diego, California, USA). The urine cortisol was expressed as a ratio of urine cortisol to creatinine. (measured in Metrolab Autoanalizer Merck, Germany, according to the manufacturer's indications). The inter- and intra-assay coefficients of variation for cortisol were 8% and 5%, respectively.

Evaluations of ACTH and C/CR were repeated every year for four years, while the α-MSH was only repeated after one year of treatment.

2.4. Statistical analysis

Student's *t*-test was used for the analysis of the same group and the unpaired t test was used to compare both groups, both before the treatment and after 1 year of treatment. The Wilcoxon's test and the Mann–Whitney's test were used in those cases deemed necessary, according to results of the normality test of the variables under study. The survival curve was performed and evaluated according to the log-rank χ^2 test. The Odds ratio (OR) was calculated by means of the χ^2 test (contingency table and Fisher's exact test), as well as if the clinical improvement was due to the use of Cbg. Values are expressed as means $\pm$ standard deviation (SD), median and range, as applicable, with a significance level of 0.05.

2.5. Ethical approval

The study was approved by the Ethics Committee of the Faculty of Veterinary Sciences of the University of Buenos Aires and by the Secretaría de Ciencia y Técnica (Secretariat of Science and Techniques) of the said University (UBACyT; V045 project) in fulfilment of the national laws on experiments with animals. Dog owners gave their signed consent for the participation of their animals in this study.

3. Results

3.1. Response to treatment and survival

The only side effect in the dogs receiving Cbg was vomiting, which occurred one hour after taking the first dose in 90% (36/40) of the animals. Only 10% repeated the vomiting with the second dose, and there was no report of emesis with the third dose.

Out of the 40 dogs treated with Cbg, 24 (60%) responded after the first month and 16 (40%) did not respond after 2 months of having begun the study and hence were switched to receive Ktz at the above mentioned dose. Of the dogs that responded to the treatment, 7/24 (29%) showed a favourable initial response (improvement of the clinical signs) which lasted between 4 and 6 months depending on the dog, to afterwards become non-respondent (with a return of the clinical signs and persistence of a high C/CR), being switched to treatment with Ktz. These dogs were removed from the study. Therefore, dogs with a full response to the treatment with Cbg (CbgRT) ended up being 17/40 (42.5%) composed of 6 non-castrated females and four castrated.

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When looking at the clinical signs evaluated (Table 1), the most conclusive response to the treatment with Cbg was the normalization of the water intake, already mentioned by the owners within two months of having started the treatment, and consequently, a decrease in the frequency of urination, accompanied by the significant increase in urinary density after one year of treatment when compared to the pre-treatment baseline value (1010 ± 4.57 vs. 1019 ± 4.98; $p < 0.0001$). Weight reduction was also significant after one year of treatment ($p < 0.01$). These improvements were predictably also present in the Ktz group, and no significant differences were found between both groups.

The oestrous cycle returned in five of the non-castrated females in the CbgRT group. On the other hand, only one female dog of the 10 non-castrated females of the other group returned to oestrus, this difference being significant ($p < 0.01$) when comparing both groups, with an OR = 50.

Regarding dermatological signs, 3 dogs in the CbgRT group that showed hyperpigmentation when the disease was diagnosed, returned to their normal skin colour after 3 months of treatment (2 of them had an increased PI).

In the following years, the clinical signs evaluated remained unchanged when compared to the ones observed at clinical diagnosis of the disease (data not shown).

Survival after initiation of treatment was significantly longer in the CbgRT compared with Ktz group (Fig. 1). Seven out of the 17 dogs in the CbgRT group completed the 4 years of study and follow-up. After one year of treatment, the survival rate was already different between the two groups ($p < 0.01$) with an OR = 19.2.

3.2. NMRI pituitary evaluation pre- and post-treatment

Of the dogs treated with Cbg, 55% (22/40) had tumours with extra-sellar projection, 27.5% (11/40) with intra-sellar projection, and 17.5% (7/40) showed an increase in the size of the PI in the sagittal section of the NMRI but without evidence of tumour (Fig. 2). It is necessary to stress that the average age of the 7 dogs with the affected PI was 10.8 years (range 8–14), this being significant ($p < 0.05$) when compared to dogs (33/40) with evident pituitary tumours in PD (mean age 8.7; range 3–13 years). In the

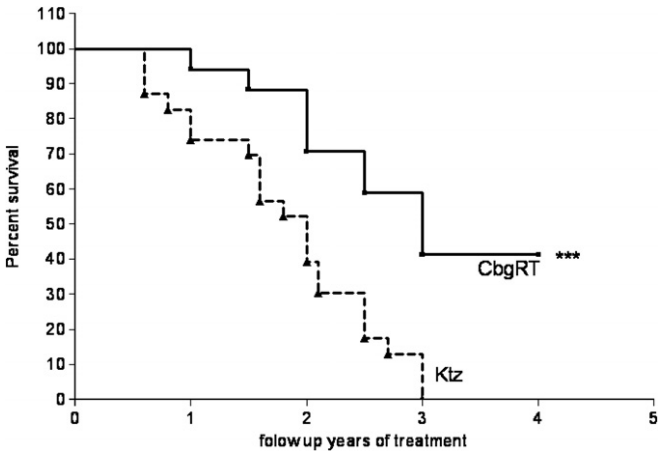

Fig. 1. Survival curve in dogs with pituitary-dependent Cushing's syndrome: comparison of those CbgRT vs. those unresponsive and treated with Ktz. After diagnosis, 50% of the dogs in the Ktz. Group die approximately 1.8–2 years (range 0.6–3) vs. animals in the CbgRT group (***, $p < 0.001$), where the average survival time was 3 years (range 1–4). In this last group, 41% (7/17) of the dogs live 4 years after having been diagnosed and started treatment with Cabergoline. As opposed to the Ktz. Group, none of these dogs died from complications of Cushing's Disease, but due to age-related causes (>13 years; log-rank test χ^2).

Ktz group 52.2% (12/23) had tumours with extra-sellar projection, 30.4% (7/23) intra-sellar and the others showed an affected PI, with the range of age being similar to that in the CbgRT group.

In the CbgRT group, diameter and surface of the tumours were significantly reduced ($p < 0.0001$) compared to the baseline values (Fig. 3) and compared to the Ktz group after one year of treatment ($p < 0.0001$). This implies an average reduction of the diameter and of the surface of 31.4% and 38.6%, respectively. In the Ktz group, there were no differences in the analyzed values (diameter and surface). Animals with altered PI images presented similar characteristics to the normal pituitary.

When analyzing the response or lack of response to Cbg (Fig. 3) in connection with the size of the tumour or its intra- or extra-sellar projection, dogs that did not respond to Cbg (16/40 dogs) presented a significantly greater tumour size than those which were responsive (diameter $p < 0.01$ and surface $p < 0.01$), with only 2 out of 16 unre-

Table 1
Changes in clinical signs in dogs with PDH treated with 0.07 mg/kg/week of cabergoline (CbgRT) compared with 20 mg/kg/day of ketoconazole (Ktz group)

Clinical signs	Cabergoline		Ketoconazole		OR
	Pre-treatment	Post-treatment (1 year)	Pre-treatment	Post-treatment (1 year)	
Water intake (Pd–Py/normal)*	17/17	0/17	23/23	5/17	15.4
Polyphagia (with/without)*	17/17	0/17	23/23	5/17	15.4
Skin and hair $^{\delta}$ (abnormal/normal)	13/17	2/17	18/23	9/17	8.4
Abdomen (prominent/normal)	17/17	4/17	23/23	10/17	4.6

Pre-treatment and post-treatment CbgRT $n = 17$, pre-treatment Ktz $n = 23$, post-treatment Ktz $n = 17$ (see text). All data are expressed as number of cases (with alteration in sign/improved or normal sign). Contingency table and Fisher's exact test were used for statistical analyses. OR represents odds ratio for post-treatment CbgRT vs. Ktz group.

* $P < 0.05$ post Cbg vs. post Ktz; $^{\delta}P < 0.05$ post Cbg vs. post Ktz, NS: not statistic differences ($P = 0.08$) post Cbg vs. post Ktz.

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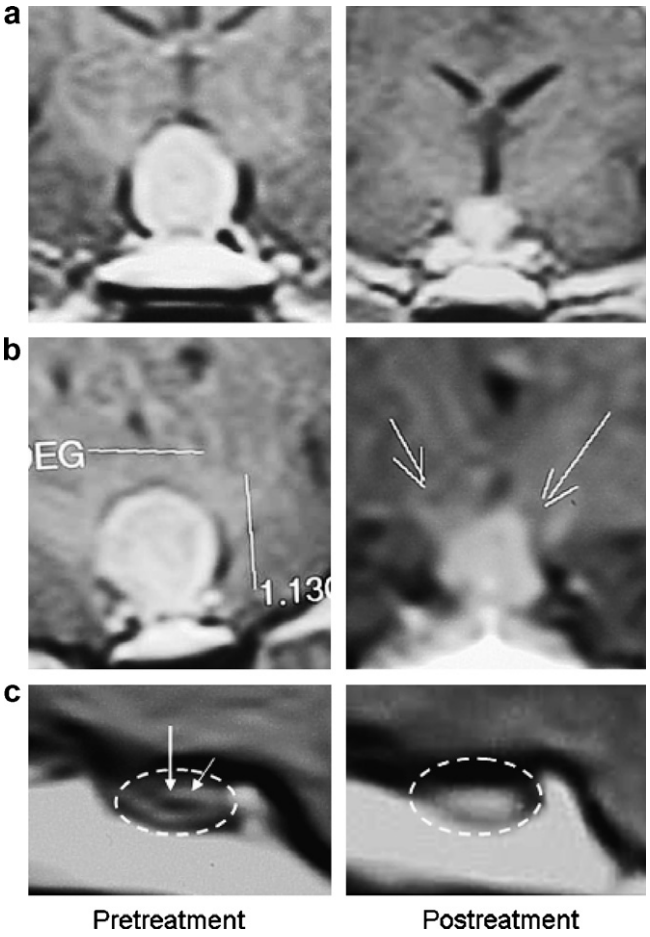

Fig. 2. Nuclear magnetic resonance imaging (gadolinium enhanced) in dogs before and after 1 year of treatment with Cbg. a–b supra-sellar adenoma (both transverse sections).The reduction in the tumour size can be appreciated in both cases. c, image of the increased PI (doted circle around PI; arrows point to dark thick line which indicates the lack of signal on MNRI) compared to a normal one (right and post-treatment) visible in the sagital section. These images did not show changes on the fourth year of evaluation in dogs in the CbgRT group that continue alive.

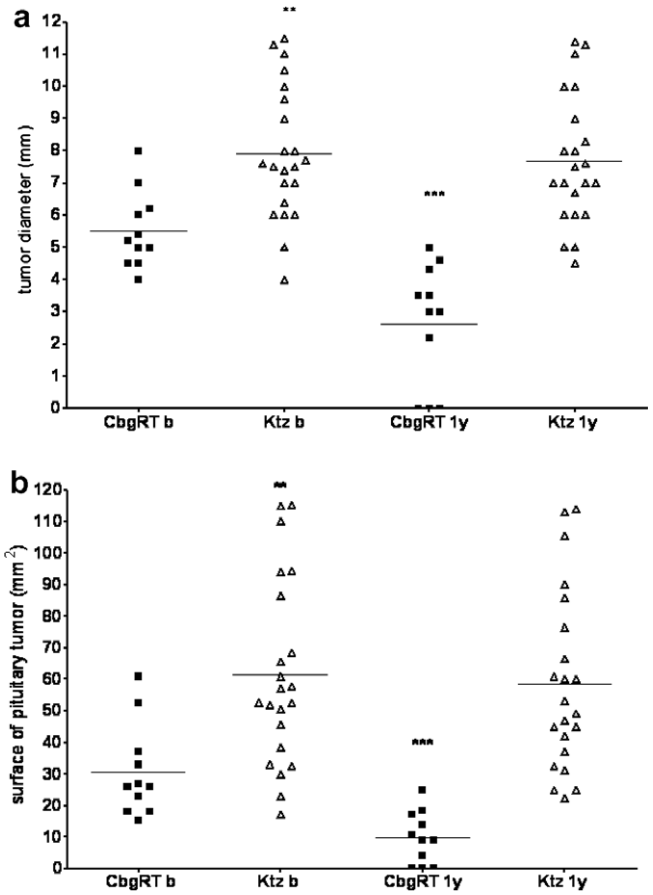

Fig. 3. Size of the ACTH producing pituitary tumour: diameter (a) and surface (b). It is evident that both measurements are greater at the time of the diagnosis and beginning of the treatment in the Ktz. Group (diameter, ** $p < 0.01$; surface, ** $p < 0.01$). After one year of treatment, tumour size is reduced in dogs in the CbgRT group vs. their baseline values (both measurements, *** $p < 0.0001$) as well as compared to those in the Ktz. Group (both measurements, *** $p < 0.0001$). Values are expressed as mean ± SD. Unpaired *t*-test (CbgRT vs. Ktz comparison) and Student's *t*- test (same group). $CbgRT_b$, Ktz_b = responsive and ketoconazole groups respectively, basal values at the time of diagnosis and beginning of therapy; CbgRT 1y, Ktz 1y = 1 year of follow-up of the treatment. Shaded squares = CbgRT, open triangles = Ktz.

sponsive tumours being intra-sellar. All dogs whose PI was affected showed a full response to Cbg. After 4 years of follow-up, the 7 dogs in the CbgRT group presented a normal pituitary image (data not shown).

3.3. Hormone analysis

Concentration of ACTH and α-MSH of the 17 dogs in the CbgRT group (Fig. 5) was significantly reduced after one year of treatment compared to the baseline values (ACTH, $p < 0.0001$; α-MSH, $p < 0.01$) and compared to dogs in the Ktz group (ACTH, $p < 0.0001$, α-MSH $p < 0.01$). ACTH in the CbgRT group remained within the normal range in the subsequent re-evaluations (data not shown), without significant differences after 4 years vs. 1 year of therapy. The Ktz group evidenced a significant increase ($p < 0.0001$) of ACTH after one year of receiving the drug, but no significant differences in α-MSH (Fig. 4).

Once the dogs in the CbgRT group were separated into those with a tumour and those with a PI with increased size in the NMRI, it became evident that the α-MSH had significantly higher values in the 7 dogs with altered PI image compared to the rest of the animals of that group (97.34 ± 42.5 pmol/L vs. 25.1 ± 10 pmol/L; $p < 0.0001$) before starting the treatment.

The C/CR in the CbgRT group (Fig. 5) showed significant differences ($p < 0.001$) with the baseline value, with no significant changes in the subsequent evaluations (data not shown). The C/CR was also reduced in the Ktz. group ($p < 0.0001$) after one year, also showing differences with the values of the CbgRT group after one year of treatment ($p < 0.05$). As with ACTH, the C/CR did not show significant variations in successive evaluations, remaining within the normal values with no significant differences after 4

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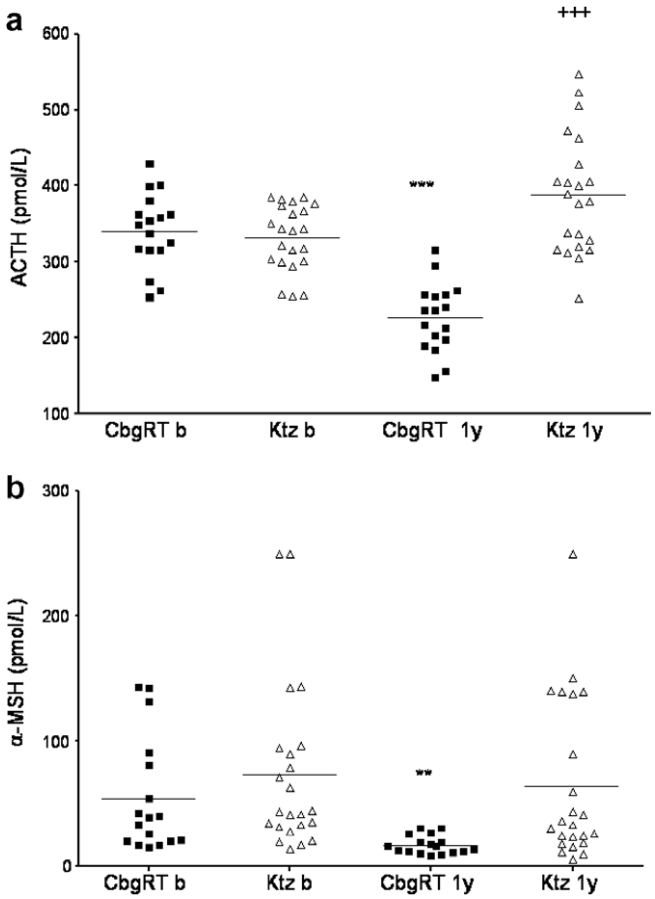

Fig. 4. Changes in ACTH and α- MSH in dogs CbgRT vs. Ktz group. a, ACTH after one year of treatment (CbgRT 1 y) vs. $CbgRT_b$ and vs. Ktz 1y, *** $p < 0.0001$. Using Ktz, after one year of treatment (Ktz 1y) ACTH increases $^{+++}$ $p < 0.0001$ vs. baseline values (Ktz_b). b, α-MSH in CbgRT 1y vs. $CbgRT_b$, ** $p < 0.01$, CbgRT 1y vs. Ktz 1y, ** $p < 0.01$. This hormone did not present significant changes after one year in the Ktz group vs. its baseline values. Values are expressed as means ± SD. Unpaired *t*-test (comparison CbgRT vs. Ktz) and Student's *t*-test (intra-group). $CbgRT_b$, Ktz_b = responsive and ketoconazole groups respectively, basal values at the time of diagnosis and beginning of therapy; CbgRT 1y, Ktz 1y = 1 year of follow-up of the treatment. Shaded squares = CbgRT, open triangles = Ktz.

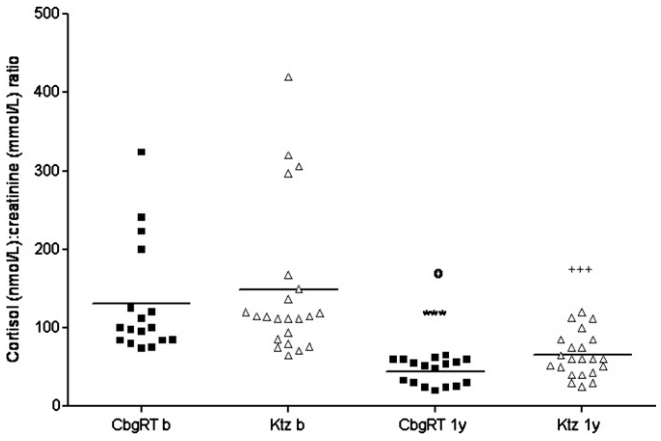

Fig. 5. Changes in the urine cortisol/creatinine ratio in dogs that responded to treatment with cabergoline (CbgRT) vs. the unresponsive group treated with Ketokonazol (Ktz). Reduction is significant in both groups after one year of treatment ($CbgRT_b$ vs. CbgRT 1y, *** $p < 0.0001$ and Ktz_b vs. Ktz 1y, $^{+++}$ $p < 0.0001$). However the reduction is most evident in CbgRT 1y vs. Ktz 1y, $^{\delta}$ $p < 0.05$. Values are expressed as median and ranges, Mann–Whitney's Test (CbgRT vs. Ktz comparison) and Wilcoxon's Test (intra-group). $CbgRT_b$, Ktz_b = responsive and ketoconazole groups, basal values at the time of diagnosis and beginning of therapy respectively; CbgRT 1y, Ktz 1y = 1 year of follow-up of treatment. Shaded squares = CbgRT, open triangles = Ktz.

years of follow-up vs. 1 year of therapy in the CbgRT group (data not shown).

4. Discussion

The functional corticotroph adenoma that causes PDH, has been and is currently under study as a therapeutic target for different drugs both in dogs and humans (Castillo et al., 2006; Labeur et al., 2004; Hofmann and Fahlbusch, 2006; Paez-Pereda et al., 2002). Although control of cortisol is achieved with the drugs that act upon the adrenal, the tumour that produces ACTH continues synthesizing the hormone, resulting in an increase of ACTH secretion and the risk of growth of the tumour (Peterson et al., 1986; Sieber-Ruckstuhl et al., 2006). This is similar to what happens when an adrenalectomy is performed, bringing about the so-called Nelson's Syndrome (Boscaro et al., 2001; Karl et al., 1996).

Cbg has been shown to have a positive effect in humans with those tumours that express the D2 dopaminergic receptor, especially prolactinomas and somatotrophinomas (Bevan et al., 1992; Colao et al., 2002). Pivonello et al. (2004) show that in the corticotroph tumour cells, the D2 receptor is expressed in 80% of them, being functional in 60%, and they conclude that the therapy with Cbg would be effective in 40% of the cases, and therefore it would be acceptable to use dopaminergic drugs (such as Cbg) for the treatment of PDH. This finding is confirmed in our study on dogs. Although there was an initial favourable response in 60% of the cases, Cbg was ultimately useful in 42.5% of the treated dogs, agreeing with the above mentioned authors. Improvement in the clinical signs evaluated occurred without side effects, normalization of the ACTH and α-MSH concentration and C/CR, reduction in the tumour size or PI image and prolonged survival time were achieved with Cbg.

From a clinical point of view, it was possible to see a favourable evolution, especially concerning recovery of the oestrous cycle. This event marked the difference in the clinical response of the CbgRT group compared to the other group. The inhibiting action of cortisol over the reproductive function is known (Lado-Abeal et al., 1998; Gore et al., 2006). Meij et al. (1997) describe that the dog with PDH has hypogonadism–hypogonadotrophism and an increase of prolactin (PRL). In this sense Cbg, as the negative action of cortisol over the gonadal axis ceases, besides normalizing the PRL as is widely known, shows its positive action by normalizing the adrenal axis.

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These events are decisive for the return of the female's reproductive function; and possibly of the male's too, where the sexual dimorphism observed in the corticotroph cells must be taken into account (Speert et al., 2002). The other clinical signs which should be emphasized are the decrease in water intake and frequency of urination, events that occur early on during the treatment with Cbg, indicating control of cortisol secretion and its action on the renal function (Ling et al., 1979). With respect to the others signs evaluated, their improvement was greater in the CbgRT group than in the Ktz group, already evident after 1 year of treatment.

Regarding the effects on the synthesis of ACTH and α-MSH, the inhibition exercised by Cbg over these hormones (with the corresponding reduction of the C/CR) is clear. This makes it evident that in animals responding favourably, the D2 dopaminergic receptor must be expressed; otherwise there would not be any response. On the contrary, and as expected, ACTH increased in the other group. This increase is attributable to the reduction of cortisol (reflected by the C/CR) due to the effect of Ktz, altering the negative regulating action that it exercises over the corticotroph area, even in individuals with corticotroph adenoma (Dahia and Grossman, 1999; Thapar et al., 1993). It is important to point out that the reduction of the C/CR in the Ktz group was not as evident as in the group treated with Cbg. This is caused by the persistent stimulation of the adrenal due to the increase in ACTH in this group, as has also been previously mentioned (Castillo et al., 2006). Evidently, by inhibiting the synthesis of ACTH and the adenoma that produces this hormone, the result of administering Cbg will be a physiological regulation of the adrenal steroidogenesis.

Full therapeutic response is less than expected, which could be due to how the D2 receptors are expressed in dogs (and maybe in humans too). The evidence that Cbg has a different effect to other dopaminergic drugs such as bromocriptine over the corticotroph adenoma that expresses D2 receptors, could be due to their different molecular structures and pharmacological actions, especially the greater affinity for the D2 receptors and the longer action of Cbg compared to bromocriptine (Colao et al., 2000; Colao et al., 2002). This may possibly be explained in connection with the tumour size, the origin of the corticotroph adenoma or the molecular characteristics of the D2 receptor (Herder et al., 1995; Lamberts and McLoad, 1990). The greater tumour size in dogs that did not respond to Cbg leads one to believe that in these cases Cbg is perhaps less effective, because these macroadenomas present a different behaviour and molecular origin to the tumours in intra-sellar locations (Bosje et al., 2002; Levy and Lightman, 2003). It is probable that they have lost the expression of the D2 receptor, or that these receptors are expressed in a lower quantity or that they are not functional because they express another isoform (Giros et al., 1989; Missale et al., 1998; Ooi et al., 2004), or then again that they never expressed the receptors, taking into account the genesis of the corticotroph adenoma (Dahia and Grossman, 1999; Melmed, 2003). On the other hand, resistance to the inhibiting action of glucocorticoids observed in macroadenomas (Bosje et al., 2002; Kemppainen and Zenoble, 1985) is accompanied by high levels of cortisol, bringing about a greater reduction in DA and its receptors (Antakly et al., 1987; Dong and Day, 2002; Van Craenenbroeck et al., 2005). In this way, Cbg would have no effect or its effect would be partial. On the other hand, the existence in dogs of tumours of mixed origin (Peterson et al., 1982) may explain the initial favourable response to afterwards escape from Cbg. Conversely, the favourable response in the intrasellars (except for two cases) leads one to think of a greater functional expression of the D2 receptor or that one is in the presence of a hyperplasia of the corticotroph area with an adenomatous aspect. These concepts are strengthened by the observed tumour reduction (both in those of extrasellar as well as intra-sellar projections that responded) and are indicative of a regulatory and proapoptotic action for Cbg (Bevan et al., 1992; Petrossians et al., 2001) as was described by Laurent et al. (2002) and Saiardi et al. (1997). It is important to emphasize the conclusive effect in the 7 dogs that presented the altered PI image, and whose average age was 10.8 years. This is a clear indicator that Cbg normalizes the dopaminergic system and the regulation of this lobe. This positive action on the PDH originating in the PI is perfectly reflected, not only by the clinical and endocrine-biochemical progress observed, but also by the normalization of the image observed in the NMRI. In these dogs, the cause of the disease would be a consequence of the degeneration of dopaminergic neurons caused by age, with a reduction of DA and the deregulation of the corticotroph area of the PI (Bruyette, 1995), as well as of the PD (Hereñú et al., 2006a,b). When DA decreases, the action of the proconvertase 2 (PC2) is reduced, and therefore, the cleavage of ACTH in α-MSH (Tanaka, 2003). This brings about an increase in the first hormone and the development of hyperadrenocorticism (Dong and Day, 2002; Laurent et al., 2002; Westphal et al., 1999). Therefore, by normalizing the DA, the action of PC2 and the concentrations of ACTH and α-MSH are normalized. One then obtains a double beneficial effect of Cbg in treating the PDH: its antiproliferative and proapoptotic action and its effect over the dopaminergic system and the subsequent normalization of the enzymatic system of the proconvertases. Reduction of the tumour size and improvement of the PI aspect, evidenced in CbgRT but not Ktz group, is a clear demonstration of the proapoptotic and inhibitory effects of Cbg on the corticotroph adenoma and DA system.

The lack of serious side effects, without alteration of the hepatic enzymogram, or of the glucose or the lipid profiles (data not shown), make Cbg a safe long-term drug. The dogs' survival has been encouraging, because the average expected life-time with classical treatments is of approximately 2 years (Feldman et al., 1990; Kintzer and Peterson, 1991). Unfortunately, this happened with those animals treated with ketoconazole.

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The fact that after 4 years none of the dogs in the CbgRT group presented clinical signs again and that the endocrine-biochemistry remained normal, is an indication that Cbg exercises a long lasting control over the disease, as was observed by Pivonello et al. (1999) and by Shraga-Slutzky et al. (2006).

Thus, one can conclude that with 42.5% of the cases showing a favourable response the use of Cbg as a treatment for PDH is justified, especially if we take into account the actions of this drug both over the tumour as well as over the function of the dopaminergic system. In the case of intrasellar tumours (no bigger than 5 mm) or when the PI is affected, this would be the primary drug indicated. In cases of neoplasias of extrasellar projection, it could be effective or not, depending on the expression, isoform and/or function of the D2 dopaminergic receptors. In this case, a correct therapeutic management would be to suspend the treatment if there is no response in 3 months, or if the clinical signs and the endocrine-biochemical alterations typical of the disease return.

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